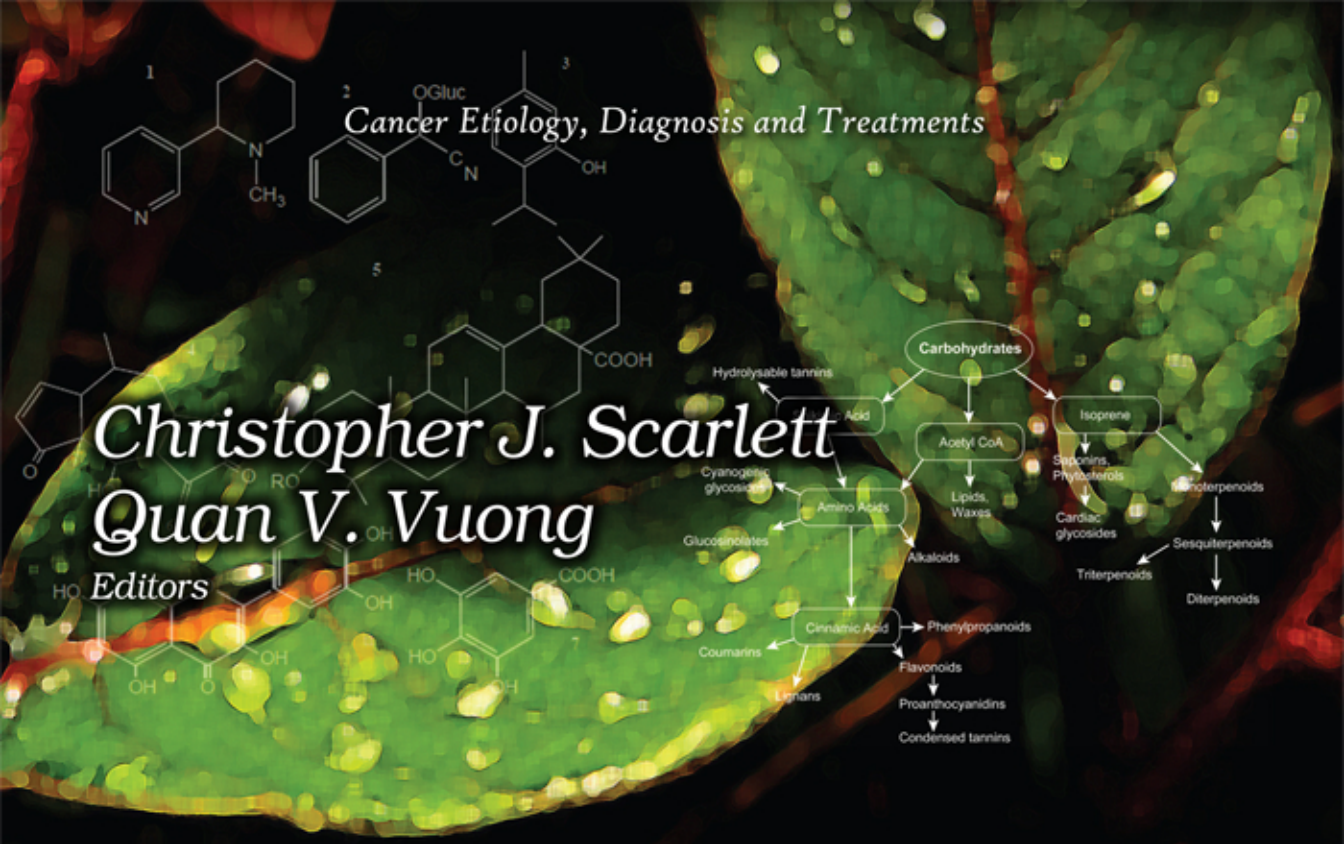

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Editors

Plant Bioactive Compounds for Pancreatic Cancer Prevention and Treatment

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PLANT BIOACTIVE COMPOUNDS FOR PANCREATIC CANCER PREVENTION AND TREATMENT

CANCER ETIOLOGY, DIAGNOSIS AND TREATMENTS

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CANCER ETIOLOGY, DIAGNOSIS AND TREATMENTS

PLANT BIOACTIVE COMPOUNDS FOR PANCREATIC CANCER PREVENTION AND TREATMENT

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nova publishers
New York

Library of Congress Cataloging-in-Publication Data

ISBN: 978-1-63463-357-4 (eBook)

Library of Congress Control Number: 2014953242

Published by Nova Science Publishers, Inc. † New York

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PREFACE

With the rapid advancements in medical research, diagnostic technology and increased public health initiative and awareness, overall cancer death rates in western societies are declining each year with the number of deaths from major cancers such as breast, colorectal and lung following this trend. However, the survival rate for those with pancreatic cancer has been at a standstill for over four decades and there are concerns that pancreatic cancer may become the second deadliest cancer in the US by the year 2030. The diagnosis of pancreatic cancer has dire consequences as it presents late in its course and is rapidly progressive. This is clearly one of the most devastating of human cancers, and as there are very few treatment options for those with the disease, new approaches and novel therapeutic strategies are urgently required. For thousands of years, plants have been used as traditional indigenous remedies for a variety of ailments in many parts of the world. It is thought that ~80% of the rural population worldwide still relies on plants as medicines. Plants have assumed the greatest prominence as a source of medicinal compounds with thousands of species associated with the treatment of cancers or conditions with cancer-like symptoms. Scientific evaluation of a range of traditional medicines has lead to the development of highly effective cancer therapeutic agents, and it is estimated that ~50% of all pharmaceuticals currently available for administration are still derived from natural origins. With this in mind, within the plant kingdom there remains great potential for the development of novel therapeutic agents with significant efficacy against pancreatic cancer. With our increasing understanding of the molecular pathology of pancreatic cancer and the rapid advancement of DNA sequencing technology to understand the structure of the genome and infer biology, pancreatic cancer is one of the most appropriate diseases to test multiple novel plant derived therapeutics in a molecular phenotype driven personalized approach.

In this book we aim to highlight the challenges of therapeutic efficacy facing pancreatic cancer patients as well as providing up-to-date information concerning the heterogeneity of pancreatic cancer and the consequent hurdles for therapeutic development (Dr's Jamieson, Grimmond, Biankin and Chang). We describe the development of plant derived compounds into clinically used anti-cancer agents (Dr Colvin); a concise history of plant phytochemicals as traditional medicines (Dr Pengelly), as well as their numerous health benefits (Dr's Street and McGaw); a summary of the bioactive composition of plants and plant foods (Dr Naumovski); their extraction and isolation methods (Dr's Kha and Nguyen); the synthetic complexities and strategies for selected chemotherapeutic agents for pancreatic cancer (Dr's Scarlett, Vuong, McCluskey and Bowyer); as well as an overview of medicinal plants with

anti-cancer properties from selected regions around the world (Australia - Dr's Vuong and Scarlett; Vietnam - Dr's Thuong, Khoi, Scarlett and Ito; The Subcontinent - Dr's Bharat and Patel; and Africa - Dr Abubakar). It is clear that the plant-derived compounds described in this book represent a mere *tip of the iceberg* when it comes to the thousands of plant species with potential medicinal efficacy. However we hope to enlighten our readers on the issues and complexities concerning the effective treatment of pancreatic cancer and identify the realistic potential for the development of novel therapeutic agents derived from plants.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 1

PANCREATIC CANCER: CHALLENGES FOR THERAPEUTIC DEVELOPMENT

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is the fifth leading cause of cancer related death in western societies. It is one of the most deadly cancers with an overall 5-year survival of less than 5%, a figure that has not changed in decades. The reasons for such high mortality are likely multi-factorial. In this book chapter, we will present the epidemiology and the aetiology of PDAC, then outline the challenges in early diagnosis

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and screening. We will then present the key improvements in perioperative care, multi-modality therapy, and discuss molecular heterogeneity as a potential hurdle for therapeutic development. Finally, we will outline key findings from landmark genomic studies of PDAC, and explore the possibility of molecular phenotype guided personalized therapy as a strategy for novel therapeutic development.

1. EPIDEMIOLOGY AND AETIOLOGY OF PDAC

Epidemiology

PDAC is the 10th most common cancer diagnosed in the UK, with 8,773 new cases in 2011, and approximately equal distribution between men and women (CancerStats, Accessed August 2014). Comparison between the 1997-1999 and 2006-2008 periods demonstrated that the incidence of PDAC in UK men appears to have risen, from 10.5 to 10.6 cases/100,000. For women this rate appears static at 8.2 cases/100,000. It ranks as the fifth most common cause of cancer related mortality in 2011 (8,320 deaths), with a 5-year overall survival rate of 3.7% in men and 3.8% in women, the poorest survival figures for any cancer. PDAC represents a substantial health burden, and is growing, as it is projected to be the second leading cause of cancer related death by 2030 (Rahib et al., 2014). The reasons are likely multi-factorial, however, most patients present late with locally advanced or metastatic disease that are not amenable to surgical resection, which is the only chance of cure.

Aetiology and Risk Factors

As the majority of patients present with incurable disease, identification and evasion of modifiable risk factors may be important for those at greatest risk. These risk factors can be divided into those that are potentially modifiable and those that are not. Although most do not directly cause the disease, the level of exposure has influence on cancer development.

Non-modifiable Risk Factors

1. Diabetes mellitus: the association between PDAC and diabetes mellitus has been speculated for a long period of time, however, the linkage is complicated as while long-term diabetes is considered a risk factor, newly developed diabetes may be an early manifestation of PDAC (Chari et al., 2008). Meta-analysis data suggests an overall two-fold relative risk for developing PDAC (Huxley et al., 2005). Of note metformin potentially may decrease PDAC risk, and it is hypothesised that this effect was due to the amelioration of hyper-insulin state, in turn blocking the mitogenic effects of insulin and insulin like growth factor 1 (Li et al., 2009).
2. A number of studies have associated previous gastric surgery with PDAC risk, with the mechanism postulated to result from hypoacidity leading to excess N-nitroso-carcinogens in gastric juice (Caygill et al., 1987).
3. *H. pylori* infection has been proposed as a risk factor, but the evidence is not strong (de Martel et al., 2008).

4. Further non-modifiable risk factors include old age, male gender, non-O blood group (Amundadottir et al., 2009) and African-American ethnicity. Evidence for the latter appears to be conflicting, and potentially confounded by environmental factors, incidence of diabetes and differing $Kras^{G12V}$ mutation rates (Pernick et al., 2003).

Modifiable Risk Factors

1. Cigarette smoking: while multi-factorial interactions appear to underlie this disease, cigarette smoking dominates and remains the most consistently reported modifiable risk factor (Coughlin et al., 2000). Tobacco exerts its carcinogenic effect on pancreatic tissue by the direct action of N-nitrosamines or their secretion into bile and subsequent reflux into the pancreatic duct. The relative risk of PDAC development was shown to be 2.5 fold in current smokers, and 1.6 fold for previous smokers, when compared to those with no history, with a dose dependent increase in risk (Fuchs et al., 1996). However, the risk of former smokers decreases precipitously, approaching that of those with no smoking history after 10 years (Fuchs et al., 1996). It is estimated that up to 20% of PDACs are attributable to cigarette smoking, with such cancers harbouring more genetic aberrations (Blackford et al., 2009).
2. Alcohol consumption: the evidence for alcohol consumption resulting in PDAC development is confounded as alcohol excess is often accompanied by cigarette smoking. A retrospective cohort study of 200,000 patients with heavy alcohol intake demonstrated only a modest 40% increased risk of PDAC development when compared to a reference population (Ye et al., 2002). Unfortunately, smoking data was deficient, although following adjustment for the population-smoking rate it was felt that excess risk among alcoholics could conceivably be attributed to confounding by smoking.
3. Chronic pancreatitis: the role of alcohol is made more complex as it contributes risk to the development of chronic pancreatitis. Two large retrospective analyses of patients with chronic pancreatitis suggest an increased relative risk of between 2.0 and 18.5. However, both were limited by poor definition of chronic pancreatitis and reliance on patient registry data (Talamini et al., 1999, Bansal and Sonnenberg, 1995). A prospective, single centre trial observing 373 patients with stringent chronic pancreatitis diagnostic criteria, demonstrated an increased risk of developing PDAC (Malka et al., 2002). However, this study was limited by only four cases of PDAC therefore limiting how the conclusions are drawn.
4. Further modifiable risks include obesity, (Berrington de Gonzalez et al., 2003) a diet high in saturated fat and red meat, (Nothlings et al., 2005) while low in folate and methiathione (Larsson et al., 2006).

Familial Predisposition

It is estimated that about 10% of PDAC cases are associated with an inherited predisposition based on familial clustering (Petersen and Hruban, 2003). An afflicted first-degree relative doubles the risk of developing PDAC with the risk increasing with the number of affected relatives, implicating a hereditary component. Some cases arise in the setting of a familial cancer syndrome; however, for most the genetic basis of the familial aggregation is

not apparent (Shirts et al., 2010). Five hereditary syndromes are described which increase the risk of PDAC development.

1. The Familial Atypical Multiple Mole Syndrome (FAMMs) is the result of a *CDKN2A* germline mutation, carrying a 20–34 fold risk (Begg et al., 2005), especially those with a specific 19-base pair deletion (de vos tot Nederveen Cappel et al., 2003).
2. Hereditary pancreatitis is an autosomal dominant disorder accounting for 5% of pancreatitis resulting from a mutation in the cationic trypsinogen gene *PRSS1* (Howes et al., 2004) or the serine peptidase inhibitor kazal-type 1 (*SPINK1*) (Threadgold et al., 2002) and carries a lifetime risk of 25-40% by age 60 of PDAC development, increasing to 75% with paternal transmission of hereditary pancreatitis.
3. Peutz-Jeghers Syndrome (PJS) is the result of mutation in the *STK11/ LKB1* gene, (Esteller et al., 2000) a serine threonine kinase affecting multiple pathways in particular cell polarity and metabolism. It is associated with a 132-fold increased risk of PDAC development with a 30–60% lifetime risk by the age of 70 (Giardiello et al., 2000). LKB1 has been shown to be involved in apoptosis, regulation of cell growth and cell cycles (Qanungo et al., 2003),(Karuman et al., 2001).
4. PDAC is notably over-represented in families with a clustering of breast and ovarian cancers (Friedenson, 2005). In subgroups of these high risk cancer families, germline mutations in either the *BRCA1* or *BRCA2* genes are found, conferring a significantly higher lifetime risk for breast (50-85%) and ovarian (up to 64%) cancers (King et al., 2003). Germline *BRCA2* gene mutations are accountable for approximately 10% of familial PDAC; yet even in sporadic cases its frequency is up to 7% (Couch et al., 2007). Recently, a near doubling of risk for PDAC among *BRCA1* and *BRCA2* mutation carriers was reported (Iqbal et al., 2012). The BRCA1 and BRCA2 proteins are integral to response to DNA damage, in particular to cross-linking DNA repair via homologous recombination (Schutte et al., 1995). BRCA1/2-deficient cells that lack homologous recombination activity accumulate DNA double-strand breaks, resulting in genomic instability and an increased predisposition to malignant transformation and progression. The BRCA2 protein interacts with different genes in the Fanconi Anaemia pathway, including FANCC, FANCG and PALB2 (Slater et al., 2010). In an analysis of nearly 100 families with familial PDAC, four families had evidence of protein-truncating mutations in *PALB2*, (Jones et al., 2009) ranking second in prevalence after *BRCA2*, a finding confirmed in another study (Tischkowitz et al., 2009). The therapeutic implication of tumours bearing homologous recombination defects is discussed later in the chapter.
5. Lynch syndrome resulting from mutation in the DNA mismatch repair (MMR) gene family (*hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2*), resulting in micro- satellite instability (MSI) in colon cancers and is associated extra-colonic cancers. However, its exact role in PDAC development along with risk, requires further elucidation (Wilentz et al., 2000). In a study of 147 families with germline MMR gene mutations, the cumulative risk of PDAC was 1.31% up to age of 50 years old, and 3.68% up to age of 70, an 8.6-fold increase compared with the general population. However, MSI is not common in sporadic PDAC (Kastrinos et al., 2009).

2. DIAGNOSIS AND CHALLENGES IN EARLY DIAGNOSIS

Diagnosis

Unfortunately, most patients with PDAC present with non-specific symptoms, and are not diagnosed until late in the course of the disease. Common symptoms include pain, particularly epigastric pain that radiates to the back, unexplained weight loss, jaundice, abnormal stools, nausea, and in greater than 10% of the patients, migratory thrombophlebitis. As discussed earlier in the chapter, patients with PDAC sometimes present with new-onset diabetes mellitus or with signs and symptoms of chronic pancreatitis. Of interest, depression is common in patients with PDAC, and in some instances the diagnosis of depression is established before the patient is found to have the cancer.

Challenges in Early Diagnosis and Screening

Screening, early detection and management of adenomatous polyps, in situ lesions, and other premalignant or potentially malignant entities of the colon, oesophagus, cervix and breast have reduced mortality. PDAC rarely presents early, however, with the increasing use of cross-sectional imaging in recent years, increasing number of pre-malignant lesions such as intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN) are being diagnosed. It is also known that the survival of patients with disease detected and resected early is excellent compared to patients with more advanced disease at diagnosis (Wasif et al., 2010). Therefore, pancreatic surgery can be curative for early stage PDAC and premalignant lesion should be treated if detected. However, the best approach for PDAC screening is yet to be established, as prevalence of PDAC is relative low in the general population, and a screening test with reasonable sensitivity, specificity, and high positive and negative predictive values is yet to be identified. Multi-disciplinary PDAC screening programs such as that of American Cancer of the Pancreas Screening (CAPS) Consortium have screened high-risk individuals with one or more imaging modalities. Family history has been used to enrich for high-risk individuals (Klein et al., 2004). Several CAPS studies have been performed to date with CAPS 4 and 5 still recruiting. In the CAPS 2 study, 10% of the patients (8/78) screened had pancreatic neoplasms detected by EUS, in addition three patients had five extrapancreatic neoplasms. Of the eight patients, six had benign IPMNs, one had IPMN with invasion and one had pancreatic intraepithelial neoplasia (PanIN) (Canto et al., 2006). The study demonstrated the possibility in diagnosing significant pancreatic and extrapancreatic neoplasms in high-risk individuals. In the CAPS 3 study, the investigators also included MRI, and found that CT, MRI and EUS detected a pancreatic abnormality in 11%, 33% and 43% of high-risk individuals respectively. The majority (82/85) of the proven or suspected neoplasms identified were IPMNs. The conclusion echoes the CAPS 2 study, that screening is able to detect small pancreatic cysts, including curable, non-invasive high-grade lesions. Authors also concluded that EUS and MRI were better at detecting pancreatic lesions than CT (Canto et al., 2012). CAPS 4 and 5 studies have further expanded the inclusion criteria to include individuals with germline mutations in *BRCA1*, *BRCA2*, *PALB2*, *p16*/*CDKN2A* or have hereditary non-polyposis colorectal cancer (HNPCC). CAPS 5 will

also evaluate the utility of mutation analysis in pancreatic fluid post secretin stimulation, pancreatic cyst fluid and circulating pancreatic epithelial cells with sporadic disease and normal healthy controls. Despite multi-institutional collaborations with large study population, it will still require extended follow up to demonstrate the impact of screening on PDAC survival (Canto et al., 2013, Ludwig et al., 2011).

3. CHALLENGES IN THERAPEUTIC DEVELOPMENT

Surgery Is Safer, but Still Under-Utilised

Only approximately 15% of patients with PDAC are eligible for surgery at the time of presentation, and surgical resection remains the only potential for cure. However, 80% still recur and succumb to disease within 5 years. Minimising perioperative morbidity and mortality is of paramount importance. Over the last decades, the safety of pancreatic surgery has improved dramatically with perioperative mortality rates decreasing from 25% in the 1960s, to less than 5% in the 2000s. Much of these improvements for both surgical and oncological outcomes have been associated with hospital volume. Pancreatic surgery has been shown to be the most acknowledged high-risk, low volume surgical specialty in the relationship between hospital volume and outcome (Birkmeyer et al., 2002). Low volume centres have perioperative mortality rates of up to 16.3% compared to 3.8% in high volume centres. Furthermore, long-term survival appears to be better in high volume centres, even after adjusting for perioperative mortality (Gooiker et al., 2011, Birkmeyer et al., 2007). This relationship is likely multifactorial, however can be summarised into improved technical experience, earlier recognition and better management of complications, (Ghaferi et al., 2009, Joseph et al., 2009) and the higher completion rate of multi-modality therapy associated with high volume centres (Bilimoria et al., 2007b).

Despite surgery being the only chance of cure, there is still a general nihilistic attitude among clinicians with alarming reports from the US outlining the under-utilisation of surgery in clearly operable PDAC (Bilimoria et al., 2007a). Bilimoria *et al.* reviewed the National Cancer Database in the USA and identified 9,559 patients with early and potentially resectable, clinical stage I PDAC (T1/2N0M0) between 1995 and 2004. They found that 71% of patients did not undergo surgery and only 6.4% of these cases were excluded due to co-morbidity, 4.2% refused surgery, and 9.1% were excluded due to age. However, 38% of patients were ‘not offered surgery’, and a further 14% of patients did not undergo surgery with reasons not reported in the record. Therefore, a total of 52% of patients with resectable and potentially curable PDAC without any identifiable contraindications failed to undergo surgery. These data indicate that there is still room to improve the awareness of PDAC among clinicians and support the arguments that all PDACs should be treated in high volume centres.

Advances and Challenges in Multimodality Therapy

PDAC is characterised by a high metastatic potential with possible systemic dissemination early in the disease course. In order to improve the outcome of operable PDAC,

several adjuvant therapy randomised-controlled trials (RCT) have been carried out. The European Study Group of Pancreatic Cancer (ESPAC) was the first to report a statistical significant survival benefit with adjuvant fluorouracil plus folinic acid compared to surgery alone in the ESPAC-1 trial (5-year survival 21% Vs 8%, P = 0.009) (Neoptolemos et al., 2004). More recently, the CONKO-001 trial also reported the survival benefit associated with adjuvant gemcitabine compared to surgery alone (5-year survival 20.7% Vs 10.4%) (Oettle et al., 2013, Oettle et al., 2007). Overall, the addition of adjuvant chemotherapy has shown a survival benefit over surgery alone. However, single agent gemcitabine seems to have similar efficacy to that of 5-FU in unselected patients as demonstrated in the ESPAC-3 and RTOG-9704 trials (Neoptolemos et al., 2010, Regine et al., 2008). The role of neoadjuvant therapy for PDAC remains poorly understood with current evidence supporting its benefit originating from Phase I and II trials and retrospective analyses. Single centre experience is growing with a variety of agents, (Gillen et al., 2010) with further prospective RCTs planned by the ESPAC consortium to specifically address this question. Unlike chemotherapy, currently adjuvant radiotherapy is used sporadically, as evidence from RCTs is controversial despite its widespread use in North America.

Despite improvements in peri-operative multimodality therapy, some key questions remaining to be addressed are: (1) do all patients require and benefit from adjuvant or neoadjuvant chemotherapy, and (2) what is the most effective chemotherapy regimen for each individual patient?

4. PDAC AS A SYSTEMIC DISEASE

Despite surgery being the only chance of cure for PDAC, more than half of patients recur within 12 months after surgical resection (Chang et al., 2009, Jamieson et al., 2013). Ultimately, the majority of patients succumb to PDAC despite multi-modality therapy. The pattern of failure post-resection is mainly in the form of distant metastatic disease with less than 30% of patients with local only recurrences (Iacobuzio-Donahue et al., 2009, Barugola et al., 2007). This suggests that occult metastatic disease was present at the time of resection despite modern staging procedures. This demonstrates that PDAC is most commonly metastatic even if it appears to be localised on cross-sectional imaging and laparoscopy. This may also explain why clinical trials assessing the efficacy of loco-regional therapy, such as adjuvant radiotherapy have failed, as these studies are grossly underpowered as only small proportion has truly localised disease. The efficacy of adjuvant radiotherapy was only demonstrated in margin positive patients using subgroup analysis in a meta-analysis (Stocken et al., 2005).

In more recent years, clinicians managing PDAC have come to the realisation that in a cancer type that is predominately metastatic, more emphasis should be placed in the use of systemic chemotherapy, even in clearly resectable cancers. This has led to several cancer centres adopting an all-comer neoadjuvant chemotherapy approach. The proponents argue that chemotherapy is better tolerated in a pre-operative setting with an intact immune system, and is more effective in a well-perfused tumour. It also allows for the treatment of micro-metastatic disease. Some also observe that subgroups of patients present with metastatic disease during neoadjuvant therapy, which spares the patients from a potentially morbid

operation that the patient would not have benefited from. However, neoadjuvant therapy is not without its associated morbidity and mortality, and the down-staging effect has not been as large as expected. Therapeutic development for PDAC has been slow and incremental at best despite decades of research. This raises several questions: (1) is PDAC generally a "chemoresistant" cancer type, or (2) are we just not treating PDAC with the right chemotherapeutics?

5. THERAPEUTIC DEVELOPMENT IN PDAC

Over the last few decades, numerous clinical trials have been performed in advanced PDAC (metastatic and locally advanced) with a high failure rate, particularly for targeted agents. Despite this, several RCTs have shown modest, but statistically significant improvement in overall survival.

In 1997, palliative gemcitabine was shown to be superior to 5-FU for overall survival (5.65 Vs 4.41 months, P = 0.0025) and progression-free survival (2.33 Vs 0.92 months, P = 0.0002). Gemcitabine was also associated with significant clinical benefit by alleviating disease-related symptoms (Burris et al., 1997). This landmark trial made gemcitabine the standard of care in advanced PDAC in most countries. It also made gemcitabine the backbone in most RCTs assessing experimental therapeutic regimens. A decade later, the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) in collaboration with Australasian Gastrointestinal Trials Group (AGITG) reported the PA.03 trial, which demonstrated a statistically significant, albeit modest survival benefit of gemcitabine plus erlotinib compared to gemcitabine alone (n = 569, 6.24 Vs 5.91 months, P = 0.023) (Moore et al., 2007). Erlotinib has remained the only targeted therapy that has demonstrated efficacy in a Phase III trial, but is associated with a Quality Adjusted Life Year (QALY) of greater than US $600,000, and is not often used. In 2009, the UK NCRI (National Cancer Research Institute) reported that combining gemcitabine and capecitabine was associated with a significantly better progression-free survival (n = 533, HR = 0.78, 95%CI = 0.66 – 0.93, P = 0.004) and a trend towards better overall survival (HR = 0.86, 95% CI = 0.72 – 1.02, P = 0.08) than gemcitabine alone. The authors then pooled two additional trials assessing the same regimen involving 935 patients to demonstrate that GEM-CAP was associated with a significant survival benefit over gemcitabine alone (HR = 0.86, 95% CI = 0.75 – 0.98, P = 0.02) (Cunningham et al., 2009).

More recently, two trials have significantly shaped the current management of advanced PDAC. In 2011, the French PRODIGE4 / ACCORD 11 trial demonstrated the survival benefit of a four drug combination FOLFIRINOX (oxaliplatin, irinotecan, flurouracil and leucovorin) over gemcitabine alone on overall survival (n = 342, 11.1 Vs 6.8 months, P < 0.001) (Conroy et al., 2011). However, this regimen was associated with significant toxicity and degradation in quality of life at six months. In 2013, the (Metastatic Pancreatic Adenocarcinoma Clinical Trial) MPACT trial showed that gemcitabine plus albumin-bound paclitaxel (*nab*-paclitaxel, Abraxane®) was superior to gemcitabine alone with a median overall survival of 8.5 Vs 6.7 months (n = 861, P < 0.001) and was associated with a more tolerable toxicity profile than FOLFIRINOX (Von Hoff et al., 2013). Numerous other trials assessing various chemotherapeutic and targeted agent combinations did not demonstrate

efficacy (Sultana et al., 2007, Gresham et al., 2014). However, meta-analyses do support the benefits of using gemcitabine combination therapy compared to gemcitabine alone. This benefit also extended to the three or four-drug combinations such as gemcitabine/erlotinib/bevacizumab and FOLFIRINOX (Gresham et al., 2014). This demonstrates that despite the majority of these trials failing to reach the primary endpoint, there was additional benefit with combination therapy over single agents alone. However, this was also associated with increased toxicity, a major hurdle in developing combination regimens when increasing the number of drugs.

What this has also demonstrated is that each drug is likely only benefiting a small proportion of patients in PDAC, and the statistical signal of survival benefit may fall below the detection threshold of conventional RCT design. Therefore, only when a therapeutic regimen testing multiple drugs in combination, such as FOLFIRINOX, it is likely that a large enough signal is appreciable, by producing a survival benefit in an increasing number of patients.

6. PDAC Is a Heterogeneous Disease

Until recently, each cancer type was considered to be a single entity, and classified into various subtypes by histopathology. However, with our increasing understanding of the molecular pathology of cancer, it is becoming apparent that any single cancer type is composed of multiple molecular subgroups with different prognosis and responses to therapy despite being indistinguishable histopathologically. With the rapid advancement of next-generation sequencing (NGS), (Hudson et al., 2010) large cancer sequencing initiatives such as International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) are revealing a high level of molecular heterogeneity. Some landmark genomic studies in PDAC are summarised below.

Pancreatic Cancer Genomes

Early work from Johns Hopkins University used capillary sequencing and SNP arrays to define mutations in protein coding regions and copy number alterations of 24 cell lines and xenografts derived from primary and metastatic PDACs (Jones et al., 2008). This study started to uncovered the vast molecular heterogeneity of PDAC, where apart from four highly prevalent mutations that are known for many years (*KRAS*, *TP53*, *CDKN2A* and *SMAD4*), a long tail of genes had a mutation prevalence of less than 5%. Despite the large number of mutations, the authors were able to classify these genomic aberrations into the 12 core signaling pathways (Jones et al., 2008). The authors concluded that therapeutic development by targeting the physiological effects of these altered pathways and processes may be preferred than targeting individual gene components. Apart from describing the mutational landscape of PDAC, this study also presents the possibility that genomic sequencing may assist therapeutic development.

In 2010, whole genome sequencing of a handful of PDACs defined structural rearrangements (Campbell et al., 2010) and explored clonal relationships between metastases

(Yachida et al., 2010). The authors found significant inter-tumoral heterogeneity in the pattern of genomic instability, with different prevalence (range 3 to 65 per patient) and type of rearrangements. A frequent distinctive pattern of structural rearrangement called "fold back inversions" was identified, and was found to be an early event in the development of PDAC that frequently underpinned amplifications of cancer genes. Analysis of clonal relationships among metastases in the same patient showed that genomic instability frequently persisted after cancer dissemination, resulting in ongoing, parallel and even convergent evolution among different metastases.

More recently in 2012, a collaborative effort as part of the ICGC, reported whole exome sequencing and copy-number analysis of a cohort of 142 early primary resectable PDACs (Biankin et al., 2012). As PDAC is characterized by intense desmoplastic stroma with an average stromal contents of 70%, the authors developed methods to perform full face frozen section and macrodissection to improve epithelial cellularity and as a consequence the sensitivity of mutation detection. The cellularity of the tumours was estimated by deep amplicon-based sequencing of exons 2 and 3 of *KRAS* at an average depth of 1,000X, and SNP array using a novel algorithm (qpure) to inform the sensitivity of mutation detection for each sample (Song et al., 2012). Detailed analysis of a cohort of 99 patients with an epithelial cellularity of >20%, identified 2,016 genes with non-silent mutations, and 1,628 copy-number variations. There were on average 26 mutations per patient (range 1 to 116). In the 79 mutated genes that were observed more than once, 38 (48%) were previously reported by Jones et al., (Jones et al., 2008) and 189 of all 998 (19%) mutated genes were also previously reported by the same study (Table 1). Significant Mutated Gene analysis identified 16 genes which included those known to be mutated in PDAC (*KRAS*, *TP53*, *CDKN2A*, *SMAD4*, *MLL3*, *TGFBR2*, *ARID1A*, *SF3B1)*, and identified novel genes involved in chromatin modification (*EPC1* and *ARID2*) and *ATM*. In addition, they also identified five other significantly mutated genes that were not previously reported (*ZIM2*, *MAP2K4*, *NALCN*, *SLC16A4* and *MAGEA6)*. This study once again demonstrated the significant molecular heterogeneity of PDAC, and further highlighted that apart from *KRAS*, *TP53*, *CDKN2A* and *SMAD4*, the majority of mutated genes had a prevalence of less than 2%. This poses significant challenges to differentiate "driver" from "passenger" mutations using existing computational tools. To overcome this, the authors incorporated data from two independent *sleeping beauty* transposon mutagenesis screens in a *Kras* transgenic model of PDAC, (Mann et al., 2012, Perez-Mancera et al., 2012) and *in-vitro* short hairpin RNA (shRNA) screens of 11,194 putative cancer genes in 102 cancer cell lines (Cheung et al., 2011). Data from these screening studies confirmed the functional importance of the four most frequently mutated genes and attributed potential functional importance in other genes. To further the functional analysis, the authors performed a series of pathway analyses and identified mechanisms known to be important in PDAC, (Samuel and Hudson, 2012) such as G1/S checkpoint machinery, apoptosis, regulation of angiogenesis, TGF-β signaling, and novel pathways including chromatin modification and axon-guidance, adding two additional core-signaling pathways to the original 12 (Yachida and Iacobuzio-Donahue, 2013). This was the first study in such scale to have used clinical samples as input material, and demonstrated the potential feasibility of clinical sequencing with minimal upfront processing.

Table 1. Single nucleotide variations and indels in pancreatic cancer

Gene	ABO + Jones (n=123)	ABO + Jones (%)
KRAS	118	96%
TP53	51	41%
SMAD4	24	20%
TTN	18	15%
MLL3	10	8%
PCDH15	8	7%
MUC16	7	6%
TGFBR2	7	6%
ARID1A	6	5%
CSMD1	6	5%
NEB	6	5%
SF3B1	6	5%
ATM	5	4%
DMD	5	4%
DNAH5	5	4%
LRP1B	5	4%
NALCN	5	4%
ZIM2	5	4%
ABCA12	4	3%
ADAMTS20	4	3%
AFF2	4	3%
CDH10	4	3%
CDKN2A	4	3%
DOCK2	4	3%
DPP6	4	3%
FMN2	4	3%
HMCN1	4	3%
PREX2	4	3%
PXDN	4	3%
RYR2	4	3%
RYR3	4	3%
SCN5A	4	3%
SYNE1	4	3%
XIRP2	4	3%

With the exponential increase in the number of cancer genomes sequenced through the efforts such as ICGC and TCGA, a large collaborative effort led by the Sanger Institute examined mutational signatures in the genomes of 30 different cancer types. Such mutational signatures can inform the underlying mutagenic processes during cancer development and progression (Alexandrov et al., 2013). This collaborative effort analysed 4,938,362 mutations from 7,042 cancers, and identified over 20 distinct mutational signatures. Some of these signatures are present in many cancer types such as older age, and some are more cancer specific, such as smoking in lung cancer and UV in melanoma. However, about half of the

signatures have an unknown origin. Based on 20 WGS and 100 WES of PDAC, four mutational signatures were defined. They included older age, APOBEC, *BRCA*-mediated DNA maintenance deficiency, and DNA mismatch repair deficiency. This study presents a novel approach to define mutagenic processes that may inform cancer prevention, early diagnosis and therapeutic development.

7. CHALLENGES MOVING FORWARD

These genomic studies have demonstrated the increasing ability of DNA sequencing technology understand the structure of the genome and infer biology. These studies have also demonstrated the possibility of utilising and implementing this technology in the clinic, with the aim of improving patient outcomes, by moving from cohort sequencing to a more personalised single patient sequencing effort. Indeed, the modern paradigm of clinical oncology is to better select patients for therapy based on predictive biomarkers of therapeutic responsiveness. Advances have been made in certain cancer types as demonstrated by the use of Herceptin® in *HER2* amplified breast and gastric cancer, (Brenton et al., 2005, Bang et al., 2010) Gleevec® in c-kit positive gastrointestinal stromal tumours (Tuveson et al., 2001) and the use of crizotinib in non-small cell lung cancers with EML-ALK fusion gene (Kwak et al., 2010). However, the clinical utilisation and implementation of this technology has been challenging in other cancer types particularly PDAC. The challenges are multi-factorial as the implementation of molecular phenotype driven medicine is paradigm changing, and requires a shift in approaches, not just for the medical community and patients, but also from multiple stakeholders such as government regulators, funders and patient advocates. There are also specific challenges in different cancer types, and in the case of PDAC, there are several inherent challenges in the approach, such as small candidate responsive subtypes in a relatively low prevalence cancer where many patients are too unwell to receive therapy.

8. ADVANCING MOLECULAR PHENOTYPE GUIDED THERAPY

Despite the challenges and difficulties in implementing molecular phenotype driven therapy in PDAC, some significant efforts in therapeutic development have been made to date with variable success. Novel therapeutic development does not always involve a new drug or a new target, but is also about how we better use existing approved therapeutics. The developmental strategies are broadly divided into four approaches: (1) to rationalise existing, (2) to rescue failed, (3) to repurpose currently approved therapeutics, and (4) novel/pre-clinical development. Some key examples are listed below and are also summarised in Table 2.

Table 2. Actionable molecular phenotypes in PDAC

Actionable Phenotype	**Therapeutic**	**Rationale**	**Molecular Characterization Proposed Biomarkers**	**Number of outliers per total examined (Methodology)**	**Overall Prevalence**
Gemcitabine Responsive	Gemcitabine	Phase 3 clinical trial data	High *hENT1*, *hCNT1*, *hCNT3* outliers	13/87 (mRNA array)	14%
DDR deficient	Platinum; MMC; PARPi	Case reports; Clinical Trial Signals FOLFIRINOX Trial	Pan-Genomic Instability BRCA2/ATM/PALB2 mutations	19/48 (WGS) 4/99 (NGS)	30% 4%
nab-paclitaxel responsive	*nab*-paclitaxel	Clinical Trial; preclinical models	SPARC expression	6/54 (IHC)	11%
5-FU Responsive	5-Fluorouracil; Capecitabine	Phase 3 Clinical Trials	Unknown	3% (Inferred)	3%
Anti-EGFR Responsive	Erlotinib	Phase 3 clinical trial data (PA3)	*KRAS* wt; Epithelial signature	6/121 (NGS)	5%
Irinotecan Responsive	Irinotecan	FOLFIRINOX trial	Topoisomerase 1 overexpression	2% (mRNA array)	2%
HER2 Amplified	Trastuzumab	Rescue	*HER2* amplification	10/469 (FISH)	2%
Hedgehog	SMO inhibitors	Rescue	HH pathway mutations	4/87 (NGS)	4%
PTEN null / *AKT* activated	mTOR inhibitor	Preclinical studies	Loss of *PTEN* expression	20/172 (IHC)	12%

Rationalise Existing

Significant improvements can be made by rationalising the use of existing therapeutics that are approved for PDAC by selecting the right drug for the right patient, and avoid the unnecessary adverse effects from the drugs that the patients are not otherwise going to respond to.

Gemcitabine

The putative biomarkers of gemcitabine responsiveness include the nucleoside transporters such as hENT1, hCNT1/3 and kinases involved in gemcitabine metabolism such as deoxycytadine kinase (dCK). Although there is a clear pre-clinical rationale, results from clinical trials have been mixed. Small cohort studies and retrospective analysis of large Phase III RCTs, such as RTOG 9704 and ESPAC 1 and 3 has supported a predictive role of hENT1 expression in adjuvant gemcitabine response (Farrell et al., 2009, Neoptolemos et al., 2013). However, it was not supportive in the CONKO-001 trial (Sinn et al., 2014) and the AIO-PK0104 trial (Ormanns et al., 2014). In addition, a recent Phase II RCT stratified by hENT1 expression, comparing gemcitabine versus CO-101 (lipophilic gemcitabine) in metastatic PDAC also failed to demonstrate its predictive role (Poplin et al., 2013). The discrepancy

may relate to methodological difference in hENT1 immunohistochemistry antibodies, and/or perhaps the role of hENT1 as a predictive biomarker varies in different stages of the disease.

DNA-Damaging Agents

Cancer cells with defects in homologous recombination DNA repair pathway are preferentially responsive to DNA damaging agents. Platinum based therapies in PDAC have mixed results in clinical trials of unselected patients, (Tabernero and Macarulla, 2009) although a recent meta-analysis of clinical trials (Ciliberto et al., 2013) and efficacy of the FOLFIRINOX regimen (Conroy et al., 2011) suggest activity in subgroups of patients. The efficacy of FOLFIRINOX on PDAC was demonstrated by the PRODIGE4 / ACCORD 11 study, (Conroy et al., 2011) however, this treatment can be associated with significant toxicity. Therefore predicting responders prior to therapy could significantly improve overall outcomes. Putative biomarkers of DNA-damaging agent responsiveness have not been well characterised due to the complex interactions between a large number of genes involved in the DNA maintenance machinery. Platinum agents and PARP inhibitors are currently in clinical trials for the treatment of hereditary breast and ovarian cancers, (Byrski et al., 2009, Clark-Knowles et al., 2010) recruited based on germline defects or variants in BRCA and Fanconi anaemia genes. However the responsive subgroup of patients maybe larger than just germline BRCA1/2 mutation carriers (Gelmon et al., 2011). Despite this enormous complexity, there is a significant opportunity to achieve impressive results if we can define a robust biomarker of platinum and/or PARP inhibitor responsiveness.

Abraxane®

Secreted Protein Acid and Rich in Cysteine (SPARC, also known as osteonectin) regulates extracellular matrix modeling and deposition and may act as a tumour suppressor or an oncogenic driver depending on differential expression in epithelial and stromal components in different cancer types (Neuzillet et al., 2013). High stromal and low epithelial expression of SPARC is a poor prognostic biomarker in PDAC (Infante et al., 2007, Mantoni et al., 2008) and due to its role as an albumin "sticker", it was developed as a therapeutic target for *nab*-paclitaxel (Abraxane®) to enable "stromal depletion", and in turn improve drug delivery. A Phase I/II study of gemcitabine plus *nab*-paclitaxel demonstrated that SPARC expression in the stroma, but not in the epithelium, co-segregated with improved survival, suggesting its potential utility as a predictive biomarker for *nab*-paclitaxel responsiveness (Von Hoff et al., 2011). However, the retrospective analysis of the Phase III MPACT trial did not demonstrate SPARC expression to be predictive of Abraxane® response (Hidalgo et al., 2014).

Erlotinib

In the NCIC CTG PA.3 study, the combination of erlotinib and gemcitabine demonstrated a modest, but statistically significant survival advantage over gemcitabine alone in advanced PDAC (Moore et al., 2007). However, for patients experiencing significant skin rash, the median survival was double that of those without (Moore et al., 2007). A retrospective molecular analysis of the trial failed to demonstrate either *KRAS* mutation status or *EGFR* gene copy number as predictive biomarkers of erlotinib responsiveness (da Cunha Santos et al., 2010). There were several limitations to the study as tissue was available for analysis in only 32% of the patients, and even less patients had *KRAS* and *EGFR* results

available (26% and 15% respectively). The proportion of *KRAS* wild type tumours was also much higher (21%) than that reported in large sequencing studies using NGS technology (7%) (Biankin et al., 2012). Whether KRAS mutation status can be used as a predictor of EGFR inhibition responsiveness in PDAC is yet to be determined.

Rescuing Failed Therapeutics

As discussed, there have been a large number of clinical trials performed with a high failure rate despite strong pre-clinical evidence, unfortunately quite often in late phase trials. This may be due to the molecular phenotype being targeted only being present in a small population of trial participants, leading to the survival signal falling below the threshold of detection. There is significant opportunity in the retrospective analysis of failed clinical trials to generate hypotheses and inform the design of future clinical trials.

HER2 Amplification/Herceptin®

HER2 amplification has been used as a predictive biomarker of trastuzumab responsiveness in breast and gastric cancers (Brenton et al., 2005, Bang et al., 2010). Preclinical data support the efficacy of anti-*HER2* therapy in HER2 over expressing PDAC (Kimura et al., 2006, Buchler et al., 2005). Activation of *HER2* also transforms HPDE ("normal" pancreatic cell line) with activated *KRAS* and inactivated *p16/p14* and *SMAD4*, suggesting that *HER2* may be an important oncogenic driver in PDAC (Chang et al., 2013). Results from trastuzumab clinical trials have been somewhat disappointing (Safran et al., 2004, Harder et al., 2012); however, this may be the consequence of nonstandardized assays that were used for patient selection leading to the overestimation of *HER2* amplified patients and thus the underpowering of the studies. The reported HER2 positive rates range between 0 and 80% in the literature due to different assays and cut-points. A recent large, study found that 2% of PDAC are *HER2* amplified (both IHC 2/3+ and FISH positive) in a cohort of 469 PDAC patients using standardized assays performed in a national reference laboratory (Chou et al., 2013). The authors also observed that *HER2*-amplified PDAC may have specific clinical features characterized by the lack of liver metastases and the preponderance of lung and brain metastases (Chou et al., 2013). Therefore, in addition to possible therapeutic implications, *HER2* amplification may also be important in disease staging and postoperative follow up.

Smoothened Inhibitor Responsiveness

A recent clinical trial of the smoothened inhibitor saridegib in PDAC was stopped before the recruitment target was reached due to poor survival in the experimental arm, despite promising efficacy in preclinical models (Olive et al., 2009). Activity of the hedgehog pathway was not used as a patient selection biomarker, and patients with tumours that harbor mutations in genes involved in hedgehog signaling may represent an appropriate target population. Mutations in *PTCH* are known to activate hedgehog signaling in experimental models, and are detected in 2% of PDAC (Biankin et al., 2012). In a recent study, Rhim *et al.* demonstrated that sonic hedgehog deficient mouse model of PDAC demonstrated a more aggressive phenotype with an undifferentiated histology, increased vascularity and heightened proliferation (Rhim et al., 2014). This is recapitulated in the treatment of standard KPC mice

with Smoothened inhibitor. However, the authors also demonstrated that the addition of VEGFR inhibitors selectively improves the survival of sonic hedgehog deficient mice by restraining angiogenesis. This may offer an alternative explanation to the worse outcome in the experimental arm of the clinical trial, and possibly the rationale of combining smoothened inhibitors with VEGFR blockade.

Repurposing Existing Therapeutics

One of the other ways to accelerate novel therapeutic development is to repurpose a therapeutic that has been approved by regulatory authorities for another cancer type or even for other medical conditions.

mTOR Inhibitor Responsiveness

Inhibitors of the mammalian target of rapamycin (mTOR) have been approved for several clinical indications. They may be used as immunosuppressants to prevent rejection after organ transplant, or as an anti-cancer agent for several tumour types including renal cell carcinoma, subependymal giant cell astrocytoma associated with tuberous sclerosis and more recently, advanced pancreatic neuroendocrine tumours (Yao et al., 2011). A recent study reported by Morran *et al.*, demonstrated the efficacy of targeting mTOR dependency in PDAC (Morran et al., 2014). The authors showed that genetically engineered mouse tumours driven by activated *KRAS* and PTEN deficiency preferentially responded to rapamycin as compared to the tumours driven by activated *KRAS* and mutant *p53*. The authors also found that ~20% of the human PDAC showed low PTEN expression and is a negative prognostic biomarker. This demonstrated the possibility of repurposing mTOR inhibitor as a novel therapeutic agent in the subgroup of PDAC that are mTOR dependent.

Novel/Pre-clinical

There are multiple novel therapeutics, target combinations in development currently for PDAC, including numerous plant-derived compounds (Chugh et al., 2012, Banerjee et al., 2014), which need to be tested and examined in well-characterised pre-clinical model systems to better define potential biomarkers of therapeutic responsiveness early in the drug development process. With our increasing understanding of the molecular pathology of PDAC and the rapid advancement of DNA sequencing technology to understand tumour biology, PDAC is one of the most appropriate diseases to test multiple novel plant derived therapeutics in a molecular phenotype driven personalized approach.

CONCLUSION

In this book chapter, we have presented some of the challenges that have contributed to the overall high health burden and poor outcome for PDAC. We have also highlighted some of the key challenges in therapeutic development and described the significant molecular

heterogeneity of PDAC. However, we feel that PDAC is one of the most appropriate diseases to test molecular phenotype driven personalized approach due to its molecular heterogeneity and the fact that the overall survival rate has changed little for decades despite significant research efforts.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 2

HISTORY AND DEVELOPMENT OF PLANT-DERIVED ANTI-CANCER AGENTS

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ABSTRACT

Plants have long been used in traditional medicine. Approximately one tenth of the more than 350,000 plant species identified have been used worldwide for medicinal purposes.

Numerous plant-derived bioactive compounds have been tested *in vitro* and *in vivo* for their anti-cancer properties and then further assessed in clinical trials. Up to now, several plant-derived compounds, such as taxol/paclitaxel, vinblastine, vincristine, topotecan, irinotecan, etoposide and teniposide, have been developed for clinical use for the treatment of many cancers, including pancreatic cancer. This chapter briefly outlines the history of discovery, development and clinical use of major anti-cancer plant-derived agents as well as highlighting a selection of agents that are proving effective in the treatment of pancreatic cancer.

1. INTRODUCTION

Traditional use of plants as medicines has a long history for the treatment of various aliments all over the world. In Eastern societies, plants were used for the treatment of various diseases for thousands of years (Newman and Cragg, 2012).

In China, a pharmacopoeia, the Pun-Tsao, with thousands of herbal cures was published around 1600, and in India, herbal medicine dates back several thousand years to the Rig-

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Veda, the collection of Hindu sacred verses, leading to a system of health care known as Ayurvedic medicine. In Western societies, plants were used as medicines by the Greek physician Hippocrates (460-377 BC), known as the Father of Medicine. In addition, a Roman military physician named Dioscorides wrote De Materia Medica, which contained an account of over 600 species of plants with medicinal value in the first Century (Kong et al., 2003). Presently, approximately 80% of the rural population worldwide still rely on plant medicines (Vuong et al., 2014), and of the 350,000 plant species identified, about 35,000 have been used for different medicinal purposes (Newman and Cragg, 2012, Kong et al., 2003).

Over the last 30 years, approximately 45% of all anti-cancer drugs have been derived directly or indirectly from plant compounds, of which 12% are natural products and 32% are semi-synthetic derivatives of such natural products (Newman and Cragg, 2012), revealing that plants are an excellent source for anti-cancer agents. The discovery of the vinca alkaloids and the isolation of the cytotoxic podophyllotoxins in the 1950s led to an increased interest in the potential of plants as a source of anti-cancer agents, with large scale screening programs established.

Currently, there are many plant-derived agents currently in clinical use for the treatment of multiple cancer types, with many more in development (Newman and Cragg, 2012). With the development of synthetically derived, molecular targeted therapies to treat cancer (eg. Imatinib, Trastuzumab), interest in plant-derived anti-cancer agents declined somewhat. However, recent advances in the development of new drugs from natural products has met with renewed success in treating cancer, illustrating that there is still much to be gained from continuing to identify and develop new anti-cancer agents derived from natural sources, including plants (Newman and Cragg, 2007).

This chapter seeks to provide a brief history of the major anti-cancer agents that have been derived from plant sources including their discovery, development and clinical use as well as highlight a selection of plant-derived agents that are proving effective in the treatment of pancreatic cancer.

2. Clinically Established Plant-Derived Anti-Cancer Agents

Numerous plant-derived bioactive compounds have been tested *in vitro* and *in vivo* and were reported to exhibit anti-tumour and anti-cancer activity (Table 1). These compounds were then introduced for testing at the clinical level, however a number of compounds were terminated at due to their low clinical efficacy. However, several compounds have now long been successfully used in the clinic to treat a variety of cancers. Currently, the plant alkaloids represent the most widely used class of plant-derived anti-cancer agents used in the clinic to treat a variety of cancers. These include the vinca alkaloids, taxanes, podophyllotoxin analogs and camptothecan analogs (Table 2).

Table 1. Plant-derived chemicals and their anti-cancer and anti-tumour activity (Kong et al., 2003)

Plant-derived Chemical	Plant Source	Action/Clinical Use
Betulinic acid	*Betula alba*	Anti-cancer agent
Colchiceine amide	*Colchicum autumnale*	Anti-tumour agent
Colchicine	*Colchicum autumnale*	Anti-tumour agent
Demecolcine	*Colchicum autumnale*	Anti-tumour agent
Etoposide	*Podophyllum peltatum*	Anti-tumour agent
Irinote	*Camptotheca acuminata*	Anti-cancer, anti-tumour agent
Lapachol	*Tabebuia sp.*	Anti-cancer, anti-tumour agent
Monocrotaline	*Crotalaria sessiliflora*	Anti-tumour agent
Podophyllotoxin	*Podophyllum peltatum*	Anti-tumour, anti-cancer agent
Taxol	*Taxus brevifolia*	Anti-tumour agent
Teniposide	*Podophyllum peltatum*	Anti-tumour agent
Topotecan	*Camptotheca acuminata*	Anti-tumour, anti-cancer agent
Vinblastine	*Catharanthus roseus*	Anti-tumour, Anti-leukemic agent
Vincristine	*Catharanthus roseus*	Anti-tumour, Anti-leukemic agent

Anti-tumour refers to assessment in pre-clinical animal models; Anti-cancer refers to human trials.

2.1. Vinca Alkaloids

The anti-cancer properties of the vinca alkaloids were first observed in the 1950s when scientists were investigating the effects of extracts from the leaves of the Madacascar periwinkle (*Catharanthus roseus*) for their purported hypotensive and hypoglycaemic properties. No effect on blood sugar in diabetic animals was observed when extracts were delivered orally, however when an intravenous injection was tried death due to infection was observed, and was due to a presence in the *C. roseus* extracts (Noble et al., 1958). Closer investigation found that *C. roseus* extract led to a decreased white blood cell count and depressed bone marrow, thereby sparking interest for vinca alkaloids as a potential anti-cancer therapy, particularly in cancers of white blood cells such as lymphoma. This extract is now known as vinblastine. Shortly after the discovery of vinblastine, another vinca alkaloid, vincristine, was also isolated from the leaves of *C. roseus* (Svoboda et al., 1959). Both of these agents were the subject of several clinical trials in the 1960s, which demonstrated their anti-tumour activity and they still remain widely used therapeutic agents that are used to treat a variety of cancer types. Since the introduction of vinblastine and vincristine into clinical use, several semi-synthetic derivatives of these drugs have been developed, including vinorelbine and vindesine, which are also approved for clinical use, and the much more recently developed vinflunine (Table 2).

Early investigations into the mechanism of action of vinblastine demonstrated both an *in vitro* and *in vivo* effect on cellular mitosis, with cells becoming arrested in metaphase upon treatment with drug (Cutts, 1961, Palmer et al., 1960). In addition, murine models of leukaemia, lymphoma and breast cancer all demonstrated an anti-tumour effect with vinblastine treatment (Johnson et al., 1960, Cutts et al., 1960). However, it wasn't until the 1970s that the ability of vinblastine to bind tubulin was discovered. Upon binding tubulin, at lower concentrations vinblastine is able to prevent tubulin polymerising to form microtubules

and at higher concentrations is able to cause the formation of spiral aggregates (Lee et al., 1975, Jordan et al., 1991). Formation of microtubules via the polymerisation of tubulin molecules is a dynamic process that is essential for the formation of the mitotic spindle during cell division. Therefore, vinblastine and the other members of the vinca alkaloids are cytotoxic to cells due to their ability to disrupt this process. The various vinca alkaloids all bind slightly differently to tubulin and therefore the effect on microtubule dynamics varies between the different agents, possibly accounting for the different efficacies seen across cancer types with this class of drug (Ngan et al., 2000). In addition to their role as anti-microtubule agents, the vinca alkaloids have also been shown to inhibit angiogenesis *in vitro* at non-cytotoxic doses (Vacca et al., 1999) and to act synergistically with vascular endothelial growth factor (VEGF) *in vivo* (Klement et al., 2000), highlighting another mechanism of action for these drugs.

Clinical trials for vinblastine and vincristine demonstrated the effectiveness of these drugs in leukaemia, lymphoma and breast cancer. Since these early trials, the vinca alkaloids have become an important therapy in the treatment of a wide variety of malignancies. Vinblastine is still used today primarily as part of combination therapy for Hodgkin's lymphoma, bladder and breast cancer. Vincristine is part of combination therapy for acute lymphoblastic leukaemia, lymphomas and nephroblastoma (Wilms' tumour). Vinorelbine can be used as a single agent or as part of combination therapy with cisplatin for the treatment of non-small cell lung cancer (NSCLC), advanced breast cancer and mesothelioma. Vindesine is used in the treatment of leukaemia and lung cancer.

Table 2. Summary of plant-derived anti-cancer agents and their clinical use

Class	Natural Source	Mechanism of action	Agents	Clinical use
Vinca alkaloids	*Catharanthus roseus*	Anti-microtubule	Vinblastine	Hodgkin's Lymphoma, bladder cancer, breast cancer
			Vincristine	Acute lymphoblastic leukaemia, lymphoma, nephroblastoma
			Vinorelbine	NSCLC, breast cancer, mesothelioma
			Vindesine	Leukaemia, lung cancer
			Vinflunine	Bladder cancer
Taxanes	*Taxus brevifolia* and other *taxus* species	Anti-microtubule	Paclitaxel	Ovarian cancer, breast cancer, lung cancer
			Docetaxel	Breast cancer, prostate cancer, NSCLC
			Nab-paclitaxel	Breast cancer, NSCLC, pancreatic cancer
Podophyllotoxin analogs	*Podophyllum* species	DNA Topoisomerase II inhibitor	Etoposide	SCLC, NSCLC, testicular cancer, lymphoma, leukaemia
			Teniposide	Acute lymphocytic leukaemia
Camptothecin analogs	*Camptotheca acuminata*	DNA Topoisomerase I inhibitor	Irinotecan	Colorectal cancer, pancreatic cancer
			Topotecan	Ovarian cancer, SCLC

Vinflunine is a much more recently developed vinca alkaloid derived from vinorelbine that has been approved for the treatment of bladder cancer. Studies show that vinflunine demonstrates increased bioavailability, decreased neurotoxicity and slower development of chemoresistance compared to the older vinca alkaloids (Hill et al., 1999), indicating that despite this class of agents being greater than fifty years old, there is still room for improvement in the development of new and improved vinca alkaloids.

2.2. Taxanes

The taxanes represent another major class of plant-derived anti-cancer agents that are still widely used in clinical practice. Paclitaxel (Taxol) was the first taxane to be isolated from the bark of the Pacific Yew tree (*Taxus brevifolia*) as part of a large-scale screening program of plant extracts run by the American National Cancer Institute (NCI). The chemical structure of paclitaxel was published in 1971 by Wani et. al. and was shown to demonstrate anti-leukaemic and cytotoxic properties (Wani et al., 1971). However, it was many more years before paclitaxel was approved for clinical use. There was initially little interest in the development of this drug for several reasons. Paclitaxel's complex structure, the difficulty associated with isolating sufficient quantities from the natural source and the lack of solubility in water all hindered its progress into the clinic. It wasn't until the mechanism of action of paclitaxel and preclinical studies demonstrating an impressive anti-tumour effect in colon and breast tumour xenografts was described that interest in the development of this agent was rekindled.

Like the vinca alkaloids, paclitaxel was found to be cytotoxic due to its interactions with tubulin and the formation of microtubules, although this was achieved through a completely different mechanism to that of the vinca alkaloids. Where the vinca alkaloids inhibit the polymerisation of tubulin, paclitaxel promotes the polymerisation, thereby stabilising microtubule formation (Schiff et al., 1979, Schiff and Horwitz, 1980, Rowinsky et al., 1990). This disturbs the normal microtubule dynamics required for cell division resulting in cell death. In addition, similar to the vinca alkaloids, taxanes have also been shown to display antiangiogenic properties at non-cytotoxic doses (Pasquier et al., 2005, Inoue et al., 2003, Grant et al., 2003).

Despite the excellent anti-tumour activity seen in preclinical studies as well as the unique mechanism of action, isolation of sufficient quantities of paclitaxel from the bark of the Pacific Yew tree remained a significant problem. Therefore alternative ways of isolating paclitaxel were investigated and it was found paclitaxel could be produced using a semi-synthetic process and a more abundant precursor, 10-deacetylbaccatin III, which can be derived from the needles of other, renewable Yew species (Samaranayake et al., 1993). The other widely used taxane, docetaxel, is also synthesised from 10-deacetlybaccatin III (Ringel and Horwitz, 1991).

Another obstacle in the development of paclitaxel for clinical use occurred during the first phase I trials in 1984, when paclitaxel was observed to cause severe allergic reactions in patients and led to one death. This was due to the formulation of paclitaxel as an emulstion with the polyethoxylated castor oil, Cremophor EL. Despite this, further trials using an increase in the infusion time overcame some of the toxicity issues initially seen. The first Phase II trial for paclitaxel was published in 1989 and showed promising results in patients

with drug-refractory ovarian cancer (McGuire et al., 1989). Good results were also seen in breast cancer patients treated with paclitaxel (Holmes et al., 1991). Paclitaxel was finally approved for clinical use in 1992 for treating refractory ovarian cancer and 1994 for refractory breast cancer, twenty-one years after it was initially discovered. Paclitaxel combined with cisplatin has now become the standard of care for patients with ovarian cancer, and is also used to treat metastatic breast cancer, NSCLC and AIDS-related Kaposi's sarcoma. Docetaxel is used to treat breast cancer and NSCLC, as well as metastatic prostate cancer.

2.3. Podophyllotoxin Analogs

Plants of the *Podophyllum* species have been used medicinally for centuries. The effective treatment of the skin condition condylomata acuminate, caused by the human papillomavirus, ignited interest in the potential use of *Podophyllum* plants to treat cancer. As early as the 1940s podophyllotoxin extracted from *Podophyllum* was shown to have cytotoxic effects due to its ability to inhibit mitotic spindle assembly and induce cell cycle arrest during mitosis (Sullivan and Wechsler, 1947, Hartwell, 1947). However, development of podophyllotoxin as an anti-cancer agent was severely limited due to high toxicity (Canel et al., 2000). Therefore, effort was put into developing podophyllotoxin derivatives with reduced toxicity. The compound that was found to be most effective in preclinical leukaemia models was 4'-demethylepipodophyllin benzylidene glucoside (DEPBG), from which two analogs were subsequently derived: etoposide and teniposide (Hande, 1998).

Podophyllotoxin exerts its cytotoxic effects by binding tubulin at the same site as colchicine, a well-known anti-microtubule agent, disrupting the formation of microtubules and causing cell cycle arrest (Shi et al., 1998). Interestingly, while the podophyllotoxin derivative etoposide also acts on the formation of microtubules, it does so at much higher concentration than can be achieved *in vivo* (Loike and Horwitz, 1976). At lower concentrations, etoposide was observed to induce single-strand and double-strand DNA breaks in cells, which was completely reversible upon withdrawal of drug. Interestingly, the DNA breaks were not a direct effect of etoposide; treatment of purified DNA with etoposide did not result in DNA breaks, whereas treatment of isolated nuclei did cause DNA breaks (Wozniak and Ross, 1983). This led to the discovery that etoposide exerted its actions through inhibition of the enzyme DNA Topoisomerase II (Ross et al., 1984). Topoisomerase II is responsible for preventing DNA tangles and supercoils during DNA replication by cutting the DNA during replication and religating the DNA breaks once replication has occurred (Zwelling, 1985). Etoposide and teniposide both function by stabilising the complex of DNA and Topoisomerase enzyme, thereby reducing the catalytic activity, inducing DNA breakage and causing cell death (Berger and Wang, 1996).

Early clinical trials of the podophyllotoxin analogs demonstrated activity in acute myelocytic leukaemia, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, NSCLC, small cell lung cancer (SCLC), gastric, breast and ovarian cancers. Currently, etoposide is used in combination with cisplatin for the treatment of SCLC, NSCLC and testicular cancer. It is also part of combination therapy for drug-resistant non-Hodgkin's lymphoma, other types of lymphoma and leukaemia. Teniposide is used in the treatment of acute lymphocytic leukemia. Limitations of etoposide and teniposide continue to be their poor water solubility and

acquired drug resistance, therefore there are continued efforts to develop better derivatives of these drugs, with several in preclinical and clinical development (Liu et al., 2014).

2.4. Camptothecin Analogs

Camptothecin was originally isolated from the Chinese Happy Tree (*Camptotheca acuminata*) in 1966 by Wall et. al. (Wall et al., 1976). The initial clinical results of camptothecin were largely disappointing, and Phase II trials were discontinued due to unpredictable adverse events. However, in 1985, camptothecin's mechanism of action was described and interest in its activity as an anti-cancer agent was renewed. Camptothecin was found to bind to and stabilise DNA Topoisomerase I (Hsiang et al., 1985, Hsiang and Liu, 1988), an essential cellular enzyme involved in DNA replication, transcription and repair. Similarly to the Podophyllotoxin analogs, stabilisation of the Topoisomerase I DNA complex results in an increase in Topoisomerase I-mediated DNA breaks and eventually cell death.

As with the several other plant-derived anti-cancer agents mentioned in this chapter, limitations of the original extract have led to the development of more efficacious derivatives. The major limitations of camptothecin were the extremely low aqueous solubility and a short half-life. The two camptothecin analogs currently approved for clinical use are irinotecan and topotecan.

Topotecan is a modified camptothecin analog with an increased water solubility. Preclinical studies demonstrated its effectiveness in killing leukaemia cell lines *in vitro*, albeit with less potency that camptothecin (Kingsbury et al., 1991). Importantly however, topotecan was superior to camptothecin in *in vivo* leukaemia models. Subsequent xenograft studies also showed effectiveness in models of ovarian, colon and osteosarcoma (Houghton et al., 1992, Pratesi et al., 1995). Topotecan was approved for clinical use in 2007 and is used to treat ovarian cancer and SCLC.

Irinotecan is another water-soluble agent derived from camptothecin shown to be effective in several *in vivo* xenograft models (Sawada et al., 1991). Clinical trials have demonstrated the efficacy of irinotecan as a single agent in treating drug refractory SCLC (Masuda et al., 1992a), chemo-naïve NSCLC (Fukuoka et al., 1992) and in combination with cisplatin in NSCLC (Masuda et al., 1992b). Irinotecan was approved for clinical use in 1998 and is most commonly used in the treatment of metastatic colorectal cancer.

3. Plant-Derived Anti-Cancer Agents Used in the Treatment of Pancreatic Cancer

The current standard of care for treating pancreatic ductal adenocarcinoma is the cytotoxic deoxycytidine analog gemcitabine. This is based on initial clinical trials indicating a modest survival benefit over 5-fluorouracil (5-FU), the previous standard of care therapy (Burris et al., 1997). Since then, many clinical trials have been initiated to identify other therapeutic regimens that are more effective than single-agent gemcitabine with very little success. This section of the chapter will introduce a selection of naturally-derived agents that show some promise in improving the survival of pancreatic cancer patients.

3.1. Irinotecan

Irinotecan has been evaluated as a single agent and in combination with gemcitabine in a number of Phase I and II clinical trials. As a single agent, irinotecan demonstrated a 9% response rate and median survival of 5.2 months in patients with metastatic pancreatic cancer (Wagener et al., 1995). A Phase II single-arm study of irinotecan in combination with gemcitabine demonstrated a median survival of 5.7 months and one-year survival of 27% (Rocha Lima et al., 2002).

Irinotecan as part of the FOLFIRINOX treatment regimen appears to have the most promising results to date. FOLFIRINOX consists of a bolus and infusional 5-FU combined with leucovorin, irinotecan and oxaliplatin. A phase II study in advanced pancreatic cancer patients who had not previously received any form of chemotherapy demonstrated a median progression-free survival of 8.2 months, a median overall survival of 10.2 months and a good safety profile (Conroy et al., 2005). Based on these results, FOLFIRINOX progressed to Phase III trials comparing it as a first-line therapy with gemcitabine. The results of this trial demonstrated a marked survival benefit over gemcitabine (Conroy et al., 2011). Median progression-free survival in the FOLFIRINOX arm was 6.4 months versus 3.3 months in the gemcitabine arm. Median overall survival in the FOLFIRINOX arm was 11.1 months versus 6.8 months in the gemcitabine arm. Objective response rates were also better in patients receiving FOLFIRINOX compared to gemcitabine (31.6% compared to 9.4%). However, there was an increased toxicity in patients receiving FOLFIRINOX. Although these results demonstrate a notable benefit for patients receiving FOLFIRINOX compared to gemcitabine, it is important to treat these results with caution due to the highly selected patient population that was included in the study, especially the requirement of an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (on a scale of 0 to 5 with a higher score indicative of worse disease). Despite this, FOLFIRINOX is an attractive chemotherapeutic option for patients with metastatic pancreatic cancer and good performance status.

3.2. Taxanes

Both docetaxel and paclitaxel have been evaluated as both single agents or in combination with other chemotherapeutic agents in pancreatic cancer. As a single agent, docetaxel demonstrated some activity in pancreatic cancer, particularly at higher doses (Rougier et al., 2000) and showed promising response rates in single arm studies when combined with gemcitabine (Schneider et al., 2003, Sherman and Fine, 2001, Stathopoulos et al., 2001, Ryan et al., 2002). However, a randomised Phase II study demonstrated no significant benefit for treatment of patients with combined docetaxel and gemcitabine compared to gemcitabine alone (Kulke et al., 2009). A Phase II trial of single-agent paclitaxel in patients with metastatic pancreatic cancer demonstrated minimal activity in patients (Whitehead et al., 1997). Despite these disappointing initial results, novel formulations of paclitaxel are showing more promising effectiveness in the treatment of pancreatic cancer. A formulation of paclitaxel embedded in cationic liposomes (EndoTAG^{TM}) was developed that binds to tumour vasculature, after which the liposomes are internalised to deliver paclitaxel to the tumour (Schmitt-Sody et al., 2003). In a phase II trial, EndoTAG^{TM} in combination with

gemcitabine showed promising results with increased median progression-free survival and overall survival compared to gemcitabine alone (Lohr et al., 2012).

Arguably the most exciting results of a modified taxane come from recent studies investigating the effectiveness of a cremophor-free, albumin-bound 130nm particle form of paclitaxel: nab-paclitaxel. Preclinical studies have shown an increased efficacy of nab-paclitaxel compared to equitoxic doses of paclitaxel in several mouse xenograft models (Desai et al., 2006). The major advantages of nab-paclitaxel include the circumvention of cremophor-related toxicity and the ability to increase the intratumoural concentration of paclitaxel. Interestingly, in a genetically-engineered mouse model of pancreatic cancer, nab-paclitaxel was also found to increase the intratumoural concentration of gemcitabine when administered in combination (Frese et al., 2012). This was found to be due to a decrease in the gemcitabine-metabolising enzyme cytidine deaminase. Phase III clinical trials in metastatic breast cancer and advanced NSCLC both demonstrated the superiority of nab-paclitaxel compared to standard paclitaxel and nab-paclitaxel is now an accepted treatment for these malignancies (Gradishar et al., 2005, Socinski et al., 2012). The Phase III trial results comparing combination therapy of nab-paclitaxel and gemcitabine versus gemcitabine alone in advanced pancreatic cancer were recently published by Von Hoff et. al. with promising results (Von Hoff et al., 2013). Combination treatment significantly increased median progression-free survival (5.5 versus 3.7 months), median overall survival (8.5 versus 6.7 months) and the one year survival rate (35% versus 22%). As such, combination of nab-paclitaxel and gemcitabine is now approved as a first-line therapy in the treatment of pancreatic cancer.

3.3. Minnelide

Triptolide is a bioactive extract from the Chinese plant, *Tripterygium wilfordii*, which has been used for centuries as part of traditional Chinese medicine to treat various ailments including rheumatoid arthritis, lupus and diseases of the central nervous system. Several preclinical studies have demonstrated an impressive anti-tumour activity *in vitro* and *in vivo* of triptolide in several cancer types, including pancreatic cancer (Phillips et al., 2007). The mechanism of action for triptolide is not fully understood. Triptolide has been shown to act on multiple target genes across multiple tumour types (Li et al., 2014). One identified mechanism of action is the inhibition of heat shock protein 70 (HSP70), which is upregulated in pancreatic cancer patients and causes inhibition of apoptosis (Ogata et al., 2000). Triptolide treatment in pancreatic cancer mouse models was shown to decrease expression of HSP70 (Phillips et al., 2007).

However, translation of triptolide into the clinic as a cancer treatment has been prevented by high toxicity, a narrow therapeutic window and very low water solubility. Therefore researchers have developed minnelide, a water soluble prodrug of triptolide that has shown promising results in preclinical models. In a study published in 2012, researchers extensively tested the effect of minnelide in several preclinical models of pancreatic cancer (Chugh et al., 2012). Minnelide was shown to decrease cell viability of multiple pancreatic cell lines *in vitro*, and demonstrated a dramatic increase in survival and a decrease in tumour burden and tumour metastasis in several orthotopic and a transgenic model of pancreatic cancer. Importantly, minnelide was shown to be markedly more effective than gemcitabine in

reducing tumour growth in mice. A recent paper published by the same group demonstrated that minnelide is also effective against tumour initiating cells, which are known to be refractory to many current chemotherapies (Banerjee et al., 2014). Confirmation of these encouraging results in humans remains to be seen, with minnelide currently undergoing Phase I trials.

CONCLUSION

Plant-derived products have proven to be an extremely valuable resource of anti-cancer agents. Despite the introduction of many new synthetically-derived anti-cancer agents over the last few decades, natural products, including plant-derived agents, have withstood the test of time and still play a substantial role in the treatment of many cancer types. While the plant-derived anti-tumour agents described in this chapter have limitations, recognition of their importance to cancer treatment has led to continued work to try and improve their effectiveness. This approach has met with considerable success with the development of newer, more effective derivatives such as vinflunine and nab-paclitaxel, with many more in various stages of preclinical testing. In the past, plant-derived products have been largely ineffective in treating pancreatic cancer, however new formulations of plant-derived products are proving to be more exciting and will no doubt lead to better treatment options for patients in the future.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
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Chapter 3

TRADITIONAL USE OF PLANTS AS FOLK MEDICINE

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ABSTRACT

Historically, different geographical and cultural regions base their traditional medicine systems around the vitalistic concept of a life force or *invisible intelligence* that regulates normal physiological processes. In the context of cancer, which represents a breakdown of this vitality, treatment measures have traditionally taken into account the imbalance of not only the body, but also the mind and soul. This chapter describes the treatment of cancer as practiced by different medicine systems around the world including Traditional Chinese Medicine, Ayurveda, Greco-Arabic Traditions, European Traditions and North American Traditions. Further, we describe anthrosophical medicine as well as physiomedical and eclectic traditions, naturopathic movements and integrative health, while exploring traditional plant medicines for bioactive leads and the role of herbal remedies as adjuvants in conventional cancer therapy.

1. INTRODUCTION

Global Perspective on Traditional Medicine and Approaches to Cancer Treatments

There is a common thread in traditional medicine systems. While the plants used and methods of treatment will vary in different geographical and cultural regions, all traditional systems are based on the vitalistic concept of a life force or "invisible intelligence" that regulates normal physiological processes (Yance, 1999). Cancer represents a disruption or breakdown of this natural order or vitality, and measures to treat cancer must take account of the need to address the imbalance - not only of the body but the mind and souls also. Most

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traditional practitioners, wherever they come from would agree that there is no one herb that is a cure for cancer, and perhaps not even a herbal formula or protocol, if underlying imbalances are not addressed. The need for pure air, water, exercise and a healthy diet - recognized by Hippocrates over 2,000 years ago - is just as important in the 21st century.

In a recent survey at least 2/3 of pharmaceutical drugs approved for cancer therapy over a 20-year period were derived from natural sources (Aithal, Kumar, Rao, Udupa & Rao, 2012). In most 'developing' countries conventional treatments for cancer are not affordable for a significant section of the population. Traditional medicine is also popular and potentially effective among patients with inoperable tumours (Vyasadeva, Dudhamal & Gupta, 2013).

In this chapter the treatment of cancer as practiced by different traditional medicine systems around the world will be explored. Although information detailing traditional anti-cancer herbs on a global scale does exist (eg. Hartwell, 1982; Kintzios & Barberaki, 2004), the majority of such information is recorded in literature specific to different cultural and geographic regions.

2. TRADITIONAL CHINESE MEDICINE (TCM)

Cancer has been recognized and treated in China for thousands of years. Zhou (2007) reviewed the history and methodologies of cancer treatment culminating in the recent development of TCM Oncology, often applied as combined therapy with western medicine. However the modernization of this approach should not sacrifice the principles and characteristics of TCM (Zhou, 2007).

In TCM, cancer is associated with abnormalities of *qi*, essence and blood, while tumours represent stagnation or stasis of *qi*. Treatment involves addressing excesses or deficiencies in these elements by the use of destagnation herbs, and by rebalancing the body-mind network (Sagar & Wong, 2001). A traditional destagnation formula has been subjected to a randomized controlled clinical trial (combined herbal and radiation treatment compared with radiation treatment only) with promising results (Sagar & Wong, 2001). In addition Chen-lian refers to a published study (in Chinese) from Shanghai Traditional Medical College that reported of 300 cases of primary cancer treated simply with herbal medicine there was a 51% success rate, although it isn't clear how success or effectiveness was measured. However the same hospital selected 60 patients with advanced squamous cancer, randomly divided them into 2 groups with one group receiving herbal medicine and the other group subjected to chemotherapy. Mean survival rates after 12 and 24 months and survival periods for the herbal group were more than double that of the chemotherapy group (Chen-lian, 1992).

In TCM the mind-body network is associated with immunity, and *Fu Zheng* herbal treatment is a specific therapy for mediating this communication network. The use of several *Fu Zheng* herbs has been shown to enhance immune modulating cytokines such as interferon and interleukin (Sanger & Wong, 2013). Table 1 is a list of the main Fu Zheng herbs. For specific details on their individual properties and applications for cancer therapy for these and other Chinese herbs, refer to 'Anticancer medicinal herbs' (Minyi, 1992) and 'Treating cancer with herbs" (Tierra, 2003).

Table 1. Fu Zheng herbs used against cancer in Traditional Chinese Medicine

Botanical name	Family	Common name	Part used
Angelica sinensis	Apiaceae	Dong quai	Root
Astragalus membranceus	Fabaceae	Huang qi	Root
Ganoderma lucidum	Polyporaceae	Ling zhi; Reishi	Fruiting body
Lycium barbarum	Solanaceae	Gogi berries	Fruit
Panax ginseng	Araliaceae	Ginseng	Root
Ligusticum wallichii	Apiaceae	Chuan wallichi	Root
Codonopsis pilulosa	Campanulaceae	Dang shen	Root
Actractyloides macrocephela	Asteraceae	Bai Zhu	Root
Poria coccus	Polyporaceae	Fu ling	Sclerotium

3. AYURVEDA

In Ayurveda, cancer is considered to be negative life energy and outside of the life force paradigm the system is built around. The lack of oxygen in tumour cells goes some way to validating this philosophy (Frawley, 1989). Treatments do not focus on herbal medicines alone, but also involve detoxifying dietary practices and adherence to a set of dietary rules (Handa, 1998; Anil, Vyas & Dwivedi, 2013). The basic ideology of Ayurveda is based on constitutional factors, especially the *Tridosha* - and all treatments are aimed at rectifying imbalances in the humors (doshas) or biotypes as they manifest in humans - *vata*, *pitta* and *kapha*. Cancer may involve all three doshas but usually starts with one that is in excess (Frawley, 1989). Failure to follow traditional dietary practices may create imbalances of one or more doshas leading to production of *Ama* (undigested food) (Anil, Vyas & Dwivedi, 2013; Vyasadeva, Dudhamal & Gupta, 2013). Excess Ama is likewise found to be a contributing factor to tumour formation, hence anti-*Ama* diets are frequently recommended with an emphasis on light and spicy foods (Frawley, 1989).

Cancer has been postulated to be a metabolic crisis whereby excess *vata* (correlated to anabolic phase) and suppressed *kapha* (correlated to the catabolic phase) interact leading to a state of cell proliferation (Balachandran, 2007). Balachandran reviews a group of Ayurvedic botanicals that have demonstrated hepatoprotective activity and which show promise for treating hepatocellular carcinomas (Table 2).

Table 2. Ayurvedic herbs evaluated for use in hepatocelluar carcinoma (Balachandran, 2007)

Botanical name	Family	Ayurvedic name
*Andrographis paniculat*a (Burm. f.) Nees.	Acanthaceae	Kalmegh
Annona x atemoya Mabb.	Annonaceae	Sitaphala
Boerhavia diffusa L.	Nyctaginaceae	Punarnava
Eclipta alba (L). Hassk.	Asteraceae	Bhringaraj
Phyllanthus niruri/amarus	Phyllanthaceae	Bhumyamalaki
Picrorhiza kurroa Royle ex Benth.	Scrophulariaceae	Katuki
Sinopodophyllum hexandrum (Royle) T.S. Ying	Berberidaceae	Giriparata
Tinospora cordifolia (Willd) Miers	Menispermaceae	Guduchi
Semecarpus anarcardium L. f	Anacardiaceae	Bhallataka

4. GRECO-ARABIC TRADITIONS

Treatments for cancer in the classic western tradition go back at least as far as Hippocrates (460-370 BC), who recommended a drink known as "elaterion" prepared from the juice of squirting cucumber (*Ecballium elaterium* (L.) A. Rich, family Curcurbitaceae) (Karpozilos & Pavlidis, 2004). The plant is described in *A Modern Herbal* (Grieve, 1931, p. 241) as a powerful hydrogogue cathartic and emetic in large doses, suggesting Hippocrates did not refrain from resorting to heroic treatments. In his famous tomb *De Materia Medica* (*ca* 65. AD), Dioscorides mentioned several plant remedies used to treat cancer though without much detail of specific actions. These include chickpea (*Cicer arietinum* L., family Fabaceae), adder-wort (*Arum dranunculus* L., family Araceae), terebinth oil and frankincense and a plaster for "hidden carcinoma" made from hedge-mustard (*Sisymbrium officinale* L. Scop., family Brassicaceae) mixed with honey (Karpozilos & Pavlidis, 2004). Chickpea is a highly nutritious legume, very popular in the Mediterranean countries, the middle-east and beyond, and recognized as a source of bioactive compounds with possible anti-cancer effects (Morrow, 2011).

Galen of Pergamon (129-199 AD) who became a prominent Roman physician and prolific author, promoted an ancient theory that cancer was caused by an inability of the spleen to clear the humor black bile, leading to congestion of bile in the veins (Karpozilos & Pavlidis, 2004; Zaid, Silbermann, Ben-Eyre & Saad, 2012). Cancer, Galenic theory suggested, was the result of a systemic malignant state, an internal overdose of black bile. "Tumours were just local outcroppings of a deep-seated bodily dysfunction, an imbalance of physiology that pervaded the entire body" (Mukherjee, 2010). Galen felt that tumours should not be cut, since the excess bile would still be present, a philosophy that influenced western medical practice until the 19th century. Specific treatments were not elaborated in his writings, although he did describe a few remedies for external growths including zinc oxide and salves made from calcining the shells of various types of shellfish (Karpozilos & Pavlidis, 2004).

Islamic medicine was strongly influenced by the Hippocratic and Galenical teachings, and while accepting the humoral origins of cancer they became more adept at identifying the various types of cancer and performing surgical removal when deemed necessary (Zaid et al., 2012). The great Persian physician Avicenna developed treatment regimes that valued gentle treatments over irritant medications. One of his most valued herbal remedies for cancer treatment was the common chicory (*Cichorium intybus* L. family Asteraceae) (Zaid et al., 2012). Historically one of the most revered spices in Islamic medicine is black cumin or black seed (*Nigella sativa* L. family Ranunculaceae), said to "cure every illness but death!" Contemporary research showed the seeds inhibit tumours in mice (Zaid et al., 2012) while the volatile oil distilled from the seeds was shown to have chemoprotective effects *in vivo* (Morrow, 2011). Thymoquinone extracted from the seeds are active against multi-drug resistant tumour lines (Kintzios & Barberaki, 2004).

While numerous other plant remedies have been used for cancer treatment in the Mediterranean and Middle-Eastern regions, popular remedies with long histories of safe use tend to be also the remedies most widely used for treating cancer or for supporting cancer patients. These include pomegranate (*Punica granatum* L. family Lythraceae), saffron (*Crocus sativus* L. family Iridaceae), stinging nettle (*Urtica dioica* L. family Urticaceae), garlic (*Allium sativum* L. family Amaryllidaceae) along with the above-mentioned *Nigella*

sativa (Zaid et al., 2012). In addition a host of Mediterranean and Middle-Eastern traditional foods - from grains and olives to beverages and spices - have been found to have protective effects against cancer. This is born out by epidemiological and experimental studies indicating that adherence to such dietary practices reduces incidence of cancer and increases survival in the modern Greek population (Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003; Benetou, Trichopoulou, Orfanos, Naska, Lagiou, Bofetta & Trichopoulos, 2008). The down side is that elements of the population may adopt diets and behaviors more in keeping with industrialized countries and subsequently the incidence of cancer increases (Moss, 2007).

As with other regions the complementary or integrative medicine movement is playing a role in the treatment of cancer in the Middle-East. In a recent survey herbal medicine was found to be the most commonly reported integrative medicine treatment in current clinical use in the region (Ben-Eyre et al., 2011). Integrative medicine represents a blend of orthodox and complementary approaches to health care, and some of the most prominent practitioners in this movement are MDs. The movement boasts numerous publications on integrative cancer treatments, including the journal *Integrative Cancer Therapies*.

5. EUROPEAN TRADITIONS

During the so-called "dark ages" ancient Greek and Roman medical texts were translated in Benedictine monasteries, and these scrolls gradually spread across Europe and to Britain (Teiten, Gaascht, Dicato & Diederich, 2013). In fact Dioscorides *De Materia Medica* is said to have provided the basis for the modern European Pharmacopoeias, well into the nineteenth century (De Voss, 2010). While influenced by Dioscorides, the 12th century German mystic and healer Hildegard von Bingen wrote her own treatises on curing cancer and other illnesses, and many of the plants she wrote about are of Northern European origin. Her work was revived in the 20th century by the Bohemian herbalist Maria Treben, whose influential book "Health from God's Pharmacy" also promoted the now world famous recipe - "Swedish Bitters" (Treben, 1980). There is a large section on treatment of malignant diseases covering many forms of cancer as well as external tumours, all supported with case studies and testimonials. The herbs that recur in almost every treatment are *Calendula officinalis* L. Asteraceae, yarrow (*Achillea millefolium* Asteraceae) and stinging nettle, while the Swedish Bitters are often used in compresses. For external tumours (including benign tumours) she used horsetail tea (from *Equisetum arvense* family Equisetaceae) and poultices in addition to the herbs mentioned above (Treben, 1980).

Numerous plants recorded from European medieval traditions are currently being clinically tested for clinical and pre-clinical cancer research. These include St. John's wort (*Hypericum perforatum* family Clusiaceae), *Arnica montana* family Asteraceae, chamomille (*Matricaria recutita* family Asteraceae), grape seed (*Vitis vinifera* L. family Vitaceae) and garlic (Teiten, Gaascht, Dicato & Diederich, 2013).

6. ANTHROSOPHICAL MEDICINE

Possibly the most mysterious European plant, and one that was worshipped by the ancients – notably but not only the Druids - is the mistletoe *Viscum album* L., family Santalaceae. Mistletoes are hemi-parasites, they grow on tree hosts from which they extract nutrients, while also having the ability to produce carbohydrates in their leaves by photosynthesis like other plants. While mistletoe has historically been widely revered as a medicine, it was really brought to prominence as a cancer treatment in the early twentieth century by Rudolf Steiner (1861-1925), the German polymath and mystic (Moss, 1998). Along with the MD Ita Wegman (1876-1943), Steiner founded Anthroposophy, a movement that still thrives today in Europe and elsewhere, and which takes inspiration from the poet and philosopher Johann Goethe (1749-1832).

Following Steiner's death, Wegman collaborated with other physicians and pharmacists to produce a range of products for cancer treatment based on mistletoe extracts, the most-well known of which is 'Iscador'. Among the numerous mistletoe products marketed today, there are five different Anthroposophical mistletoe preparations, including several versions of 'Iscador' which vary according to the host tree on which the mistletoe grows (Kielne & Bopp, ND). 'Iscador' is widely used in cancer clinics throughout Europe, most prominently in the Wegman Clinic, a general hospital run according to Anthroposophical principles. Different mistletoe preparations are taken either intravenously or orally, both as adjuncts to standard cancer therapies and as part of a natural treatment regime.

There is a large body of research literature available, and mistletoe has been investigated by the US Food and Drug Administration (FDA). Despite some concerns of toxicity it is generally well tolerated. The research findings have been reviewed by Moss (1998) and further information can be found at the Society for Anthroposophical Doctors in Germany (website at http://wissenschaft.mistel-therapie.de/?lang=1).

7. NORTH AMERICAN TRADITIONS

One of the most famous herbal cancer treatments in North America is the Essiac formula, 'discovered' by the Canadian nurse Rene Caisse, who claimed it was given to her by an Ojibway Indian, though only one of the ingredients (slippery elm) is an American species (Tierra, 2003). Caisse in turn had reputedly used it to cure breast cancer in a visiting English woman. Using this formula Caisse ran a successful cancer clinic for several decades in Canada, although her treatments were never accepted into mainstream medicine. The Essiac formula (note Caisse in reverse) was patented, while alternative and competing formulas are also in the marketplace (Moss, 1992). These formulas are still widely used.

Original Essiac Formula

Slippery elm bark (*Ulmus rubra* family Ulmaceae)
Burdock root (*Arctium lappa* family Asteraceae)

Sheep sorrel (*Rumex acetosella* family Polygonaceae)
Turkey rhubarb (*Rheum palmatum* family Polygonaceae).

Physiomedical and Eclectic Traditions

The practice of herbalism in the 19th and early 20th centuries was dominated by the Physiomedical (mainly lay practitioners) and Eclectic movements (mainly pharmacists or MDs who frequently combined botanical and non-botanical medicines), and their influence is still strong among contemporary North American herbalists and integrative medical practitioners. Harry Hoxesy was a cancer specialist with origins in Physiomedicalism. He was the son of a veterinary surgeon who adapted some of this grandfather's veterinary formulas and developed cancer treatments for humans. Despite his lack of medical education, Hoxsey was successful in establishing cancer clinics, the largest in the country during his heyday in the 1950s (Bloom, 2012). Relentless pressure from the AMA and the medical establishment in general (he was arrested more times than anyone in medical history), eventually forced Hoxsey to move his clinic from Texas to Mexico, where it still operates today (Ausubel, 2000).

Both Hoxsey and Essiac treatments are based on detoxifying herbs known as alteratives. These herbs promote elimination of metabolic wastes from the body via various channels, leading to enhanced assimilation of nutrients (Yance, 1999). Some of the ingredients (in both formulas) have notable cathartic effects due to the presence of anthraquinone glycosides. The full Hoxsey formula is listed below. Note the presence of potassium iodide, the only non-botanical ingredient – suggesting that Hoxsey was also influenced by the Eclectic system. Potassium iodide pills were issued to victims exposed to radiation at Three Mile Island and Chernobyl (Ausubel, 2000).

Hoxsey Formula

Licorice root (*Glycyrhizza glabra* family Fabaceae)
Cascara sagrada (*Rhamnus purshiana* family Rhamnaceae)
Red clover (*Trifolium pratense* family Fabaceae)
Burdock (*Arctium lappa* family Asteraceae)
Poke root (*Phytolacca americana* family Phytolaccaceae)
Queen's delight (*Stillingia sylvatica* family Euphorbiaceae)
Prickly ash bark (Zanthoxylum americanum family Rutaceae)
Buckthorn bark (*Rhamnus frangula* family Rhamnaceae)
Barberry bark (*Berberis vulgaris* family Berberidaceae)
Potassium iodide

In addition to using botanical medicines internally, Hoxsey also advocated the application of various escharіotic salves for direct application to tumours (see section below), many based on non-botanical ingredients such as antimony and arsenic (Moss, 1998). Before Hoxsey, Dr. Eli Jones, an MD and homeopath and a prominent Eclectic, gained a reputation for treating cancer, and is said to have treated over 4,000 cases of breast cancer (Naiman, 1997). His most

lasting contribution is a formula he named Scrophularia Compound (after *Scrophularia nodosa* family Scrophulariaceae) which became known as cancer syrup. Once again this compound is largely based on alterative and cathartic herbs, and may well be a precursor to Hoxsey's formula.

These are the ingredients (Tierra, 2003):

Figwort leaves and roots (*Scrophularia nodosa*)
Poke root (*Phytolacca americana*)
Yellow dock root (*Rumex crispus* family *Polygonaceae*)
False bittersweet bark and root (*Celastrus scandens* family *Celastraceae*)
Mayapple root (*Podophyllum peltatum* family *Berberidaceae*)
Juniper berries (*Juniperus communis* family *Cupressaceae*)
Prickly ash berries (*Zanthoxylum americanum*)
Guaiacum wood (*Guaiacum officinale* family Zygophylaceae)

Perhaps the most potent single herb in these formulas is poke root, and common weed in most parts of North America. Known specifically as a herb for swollen lymph glands, tumours and lumps in general, Eli Jones considered it to be the most valuable cancer remedy of all, and particularly effective for cancer of the breast, throat and uterus. The root contains antiviral proteins, increases white blood cell production and increases mitosis in lymphocytes (Yance, 1999).

It is often used as a salve for lumps and cysts of the breast. Another plant of note is the Mayapple (*Podophyllum peltatum*). This species (and *P. emodi*) has antiproliferative effects, and is the source of anticancer drugs including Etoposide, approved by the FDA for small cell lung cancer and testicular carcinoma (Kintos & Barberaki, 2004).

One European species with a tradition in cancer treatment is the sweet violet (*Viola odorata*, family Violaceae). Hildegard of Bingen made up a cancer salve containing expressed juice of violets, olive oil and goat tallow (Naiman, 1997). The prominent Physiomedical practitioner T.J. Lyle used a violet leaf infusion for reducing pain and swelling and claimed the preparation could cure cancer (Lyle, 1897). Use of violet became more widespread in the twentieth century following publication of an extensive monograph in the classic text "A Modern Herbal" (Grieve, 1931).

In the American classic "Back to Eden", the Physiomedical practitioner Jethro Kloss recommends blue violet (*V. cuculta*) for cancer and cancerous growth, in combination with red clover (*Trifolium pratense*) and vervain (*Verbena hastata*). Note that there are numerous North American species likely to have been used as a substitute for *V. odorata*. According to Naiman (1997) during the 1930s violet was used following surgery to prevent secondary tumour development.

We note that red clover was one of the alteratives in the Hoxsey formula. The founder of Physiomedicalism Samuel Thomson (1769-1843) developed a cancer plaster made from red clover blossoms, a use he may well have learned about from the Penobscot Indians (Naiman, 1997). Trifolium compound was a popular medicine during the nineteenth century, and it has been listed in the US National Formulae (Ausubel, 2000). Red clover is one of the most widely used herbs for cancer in the 20th century, including in the well-known Jason Winter's Tea.

8. Naturopathic Movements and Integrative Health

In the western nature cure movement, diet and fasting are also considered a central aspect of any cancer treatment (Wearland 1972, Bieler, 1965). These practices are now also embedded in complementary and integrative therapies practiced around the world, where they are often further integrated with traditional practices such as TCM and Ayurveda (Tillotson, 2001; Yance, 1999; Montbriand, 2007). In recent years holistic or integrative health practitioners have embraced cutting edge scientific approaches such as whole systems research based on the rationale that cancer is a dynamic and multifaceted process (Verhoef, Vanderheyden, & Fonnebo, 2006).

9. Exploring Traditional Plant Medicines for Bioactive Leads

Screening folk medicines for potential cancer drugs was initiated by the US National Cancer Institute (NCI) during the 1950s, based largely on the exhaustive literature reviews and publications of Dr. Jonathan Hartwell, one of the founders of the NCI (Moss, 1998). To this day extensive resources are being placed into screening plants for potential bioactive constituents, even though issues of intellectual property may render the process more complex (van Overwalle, 2006). Much of the screening appears to be random, however proponents of Hartwell's model argue that using traditional knowledge as a lead is a more efficient approach (Farnsworth, 1990; Kintzios & Barberaki, 2004; Miller 2011). This hypothesis was tested in a recent ethnobotanical study conducted in Vietnam by subjecting indigenous plants from a national park to typical screening biological assays, and comparing the results of a random selection with those based on ethnomedical or traditional use (Gyllenhaal et al., 2012). While the overall findings were inconclusive and failed to confirm the general hypothesis, plants used for cancer in traditional medicine did correlate with positive activity in bioassays.

10. The Role of Herbal Remedies As Adjuvants in Conventional Cancer Therapy

While traditional and complementary medicines have been and are used for primary cancer treatments in many parts of the world, the most prevalent use of these therapies in contemporary times is as supportive or adjuvant therapy for conventional treatments. This is particularly true for advanced cancer patients, where the primary treatment objectives are based more on patient comfort and quality of life than on curative treatments (Truant, Porcino, Ross, Wong & Hilario, 2013). Whilst the hope for a cure may persist into late stage cancers, a great deal of focus is on non-curative benefits such as stress reduction, boosting energy and immunity and for reduction of side effects from conventional treatment. In particular herbal medicine can be of great assistance to those suffering from nausea and vomiting which may be a result of chemotherapy, and for those undergoing radiotherapy (Tascilar, de Jong, Verweij & Mathijsson, 2008).

Management of Nausea and Vomiting

Cancer patients are very prone to digestive problems such as poor appetite, nausea and vomiting and bowel irregularities. In particular nausea and vomiting are magnified following exposure to cancer therapies, in particular chemotherapy and radiotherapy, with up to 80% of patients affected (Haniadka, Popouri, Palatty, Arora & Baliga et al., 2012). Typically these patients are given a cocktail of drugs to relieve the nausea and vomiting following aggressive cancer therapies, increasing the risk of further adverse drug reactions. Herbal medicines are a natural choice for many people, especially considering the prevalence of remedies for digestive disorders in traditional medicine across the world. Recently Haniadka et al. (2012) reviewed the use of medicinal plants for treating cancer-associated emesis. In Table 3, antiemetic herbs that have undergone scientific evaluation are listed.

Table 3. Antiemetic herbs used in cancer treatment undergoing scientific investigation (Haniadka, 2012)

Botanical name	Family	Common name	Part used
Panax ginseng	Araliaceae	Korean red Ginseng	Root
P. quinquefolius		American ginseng	Fruit
Zingiber officinalis	Zingiberaceae	Ginger	Rhizome
Cannabis sativa	Cannabinacaeae	Marijuana	Flowers, leaves
Scutellaria baicalensis	Lamaiaceae	Baical scullcap	Root
Ganoderma lucidum	Polyporaceae	Reishi	Fruiting body
Vitis vinifera	Vitaceae	Grape vine	Fruit,seeds

Radioprotection and Sensitizing with Herbs

A select group of traditional plant medicines have shown the ability to provide radioprotective effects in cancer patients undergoing radiotherapy treatment. The popular Ayurvedic formula Triphala consists of three fruits ground to a powder - *Terminalia chebula* Retz family Combretaceae, *T. belerica* (Gaertn.) Roxb.and *Phyllanthus emblica* L. family Phyllanthaceae.

Triphala was shown to provide protection against mice subjected to γ-radiation-induced sickness and mortality at doses well below the LD_{50} for the formula (Jegetia, Baliga, Malagi, & Sethukumar Kamath, 2002). While it could be argued that radioprotection hardly constitutes traditional use of the formula, the authors did indicate possible mechanisms of action, which are in keeping with traditional use. These included protection of intestinal epithelium, protection of bone marrow stem cells and associated immunomodulatory effects and free radical scavenging activity (Jegetia et al., 2002),

In addition to the benefits of radioprotection, some plant medicines are also radiosensitizing, thereby potentially enhancing the benefits of radiotherapy when used concomitantly. One traditional plant medicine - turmeric *(Curcuma longa* family Zingiberaceae) - has been shown to act as both radio-sensitizer and radio-protector *in vitro* and *in vivo* (Goel & Aggarwal, 2010).

One example is the walnut tree (*Juglans regia*), previously used as a cancer treatment in European folk medicine (Aithal et al., 2012). The naphthoquinone compound juglone, also found in various parts of the America black walnut (*Juglans nigra*), was shown to inhibit growth of melanoma cells *in vivo*, while also augmenting death of melanoma cells following exposure to radiation (Aithal et al., 2012).

11. THE USE OF TOPICAL AND ESCHARIOTIC AGENTS

Escharioticsalves have a long history of use among many cultures going back to Galen's time, and they were brought to prominence during the 19th century by the Eclectic physicians John Pattison and later by fellow Eclectic Eli Jones (see above) (Naiman, 1997). An extract of the American woodland herb bloodroot (*Sanguinaria canadensis* family Ranunculaceae) along with zinc chloride, was an ingredient in Mohs' paste used as a fixative in the chemosurgical technique for treating skin cancers, introduced by Frederick Mohs in the 1930s (Mohs, 1940). The paste may have been based on Hoxsey's escharioticcancer salve (the 'red paste'), which was applied without the accompanying surgical procedure – a practice later denounced by Dr. Mohs (McDaniel & Goldman, 2002). The paste may also have included the toxic metal antimony, which was thought to assist the infiltration of the active contents into the tumor (Ausubel, 2000).

While the Mohs technique was popular among dermatologists into the 1970s and still has its' adherents in contemporary times (Kakimoto, Tokita, Okamura, & Yoshina, 2010), Hoxsey-style pastes containing *S. canadensis* are disseminated across the Internet, often accompanied by personal testimonials (eg. http://www.blacksalveinfo.com/). A group of dermatologists have published case reports in which a basal cell- and a squamous cell-carcinoma were successfully treated with escharioticsalves purchased over the Internet (Brown, Goldstein, & Birkby, 2001).

One of the most widely used natural products for topical use on skin cancers is 'Curaderm', extracted from a species of the nightshade family known, amongst other names, as apple of Sodom – *Solanum sodomaeum* Dunal, family Solanaceae. This species, originally from the Mediterranean region, is a naturalized weed in Australia from where the product is manufactured. It has been used as a folk remedy to treat skin cancers in cattle (Moss, 1998). There are over 100 native species of the *Solanum* genus in Australia, many of which have been used for food and medicine by the Aboriginal inhabitants (Peterson, 1979). All species contain glycoalkaloids such as solasodine, with steroid-like structures that account for anti-inflammatory effects when applied to inflammatory skin lesions (Roddick, 1991). *S. sodomaeum* contains another glycoalkaloid solamargine, which when applied in the 'Curaderm' formulation can kill cancer cells (Moss, 1998). The product is widely used in Australia and elsewhere.

CONCLUSION

Cancer therapy in the modern world can still learn from traditional medicine. One of the challenges is to better integrate complimentary and traditional medicines in public health care systems, and blueprints do exist (Hussain & Malik, 2013). There always has been and probably always will be a level of scepticism regarding the use of complementary and traditional medicines for cancer therapy among conventional physicians (Tascilar et al., 2008). *However* a vast body of empirical and scientific evidence now exists to support the use of traditional herbal medicine both as adjuvants or alternatives to conventional cancer therapies, and around the world the public have demonstrated a willingness to embrace this approach.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 4

BIOACTIVE PLANTS AND HEALTH BENEFITS

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ABSTRACT

Plants have a long history of use in the treatment of a wide range of diseases. Medicinal plant research has led to novel developments in drug discovery. Research and development opportunities exist to discover innovative and useful biological activities. This review focuses on the noteworthy bioactive compounds, current findings regarding health benefits and important medicinal plants. A brief overview is given of the synthesis of plant compounds, as well as the classification of the different groups of bioactive plant compounds in line with their clinical significance. Particular areas of health concern where natural products play a vital role are highlighted, including non-transmissible diseases such as cancer, cardiovascular disease and diabetes, and infectious diseases including malaria, HIV and tuberculosis.

1. INTRODUCTION

Plants produce a diverse range of organic compounds, the majority of which do not appear to contribute directly to growth and development (Croteau et al., 2000). Such substances, known as secondary metabolites, are often differentially distributed among restricted taxonomic groups within the plant kingdom (Croteau et al., 2000; Hartmann 2007). Initially considered waste products, research has revealed that organisms have evolved to produce these multifarious, complex and frequently toxic chemicals for communication, defense and predation (Cragg et al., 2009). Due to their large array of biological activities, plant secondary metabolites have been used in traditional medicine since time immemorial

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(Bourgaud et al., 2001). The first records of medicinal plant use from Mesopotamia, dating back from about 2600 BC, include plants which are still in use today namely licorice (*Glycyrrhiza glabra*) and myrrh (*Commiphora* species) (Gurib-Fakim 2006). The isolation of morphine ('principium somniferum') by Friedrich Wilhelm Sertürner in 1806 is commonly accepted as the beginning of plant secondary product research (phytochemistry) (Hartmann 2007). Decades later, the first synthesis of a secondary product, indigo, by von Baeyer in 1886, provided a milestone in synthetic organic chemistry (Hartmann 2007). Natural products and their derivatives have been the single most productive source of leads for the development of drugs and represent over half of all the drugs in clinical use worldwide (Gurib-Fakim 2006; Harvey 2008). Presently a quarter of all prescribed pharmaceuticals in developed countries contain compounds that are directly or indirectly, by means of semi-synthesis, derived from plants (Oksman-Caldentey et al., 2004).

Natural product molecules characteristically have more chiral centers, fewer hetero atoms, less heavy atoms, and more diverse ring systems when compared to synthetic organic compounds (Pan et al., 2012). Furthermore, synthetic pharmaceuticals are based upon single chemicals, whilst phytomedicines commonly wield their beneficial effects through the synergistic action of several phytochemical compounds acting at single or multiple target sites linked with a physiological process (Briskin 2000). In the twentieth century, natural extracts were largely replaced by synthetic molecules in a synthetic-chemistry-dominated pharmaceutical industry (Raskin et al., 2002). Today, despite the past successes, many pharmaceutical companies have reduced the use of natural products in drug discovery screening. One of the key motives underlying this decline is that the pharmaceutical industry has principally changed its tactic of drug discovery to the rapid high-throughput screening of molecular target-based pure compound chemical libraries, which have been generated mostly using combinatorial chemistry (Pan et al., 2012). Furthermore issues in natural products research including difficulties in access and supply, complexities of natural product chemistry as well as concerns about intellectual property rights have also played a role (Harvey 2008). Nonetheless with the enormous diversity in chemical structures, natural compounds continue to offer promising drug sources and natural products have provided improvements in both activity and pharmacokinetic properties of existing drugs during pharmacomodulation (Gordaliza 2007). The large number of natural product-derived compounds in different stages of clinical development indicates that the use of natural products is still a feasible source of new drug candidates (Mishra et al., 2011) and the efficacy of natural products as sources of novel structures, but not necessarily the final drug entity, is still very active (Newman et al., 2007). This review focuses on the significant bioactive compounds, current findings regarding health benefits and the importance of medicinal plants in drug discovery.

2. Common Bioactive Compounds in Medicinal Plants

2.1. Biosynthesis of Plant Compounds

The crucial process of photosynthesis transforms carbon dioxide and water into carbohydrates, which is the carbon source for the synthesis of organic compounds in plants. All plant constituents are synthesized via a few general pathways (Samuelsson 1999) and

these are summarised briefly in Figure 1. Carbohydrates are degraded to pyruvic acid, which is then oxidised to acetate that can condense to produce fatty acids and polyketides. Another biosynthetic pathway leads from acetate via mevalonic acid to terpenes and steroids. A third route generates amino acids, which can also be formed directly from pyruvic acid. The shikimic acid pathway is another route which leads from carbohydrates to amino acids, and shikimic acid is also the precursor for the biosynthesis of tannins. An important pathway leads from amino acids to proteins, purines and alkaloids.

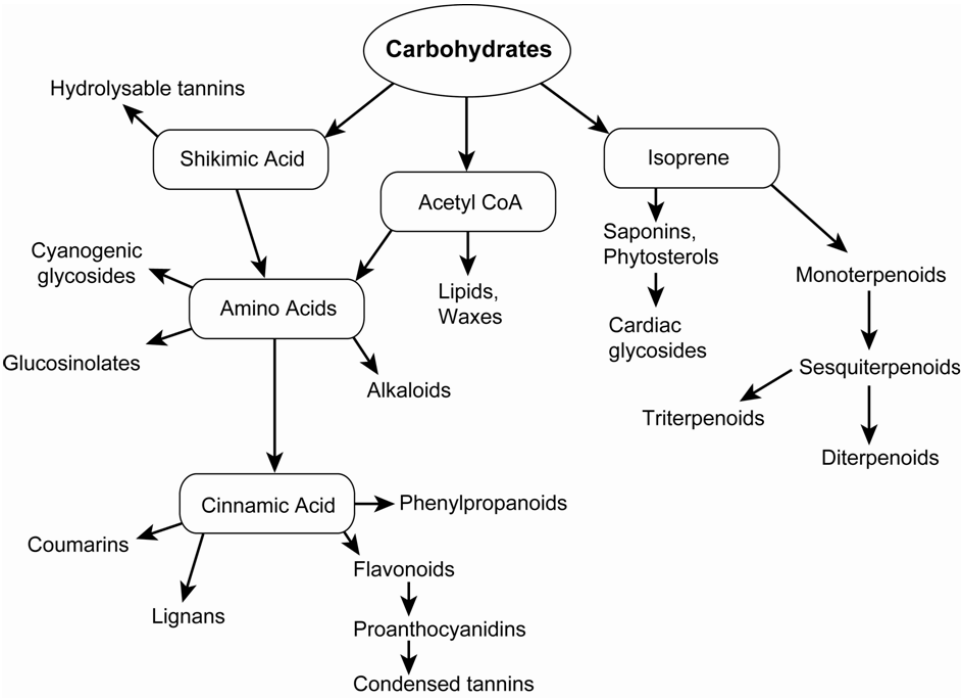

Figure 1. Synthesis of bioactive compounds in plants. All pathways begin with carbohydrates formed during photosynthesis. Rectangles represent key intermediate compounds. Modified from Yarnell (2007).

2.2. Classification of Bioactive Plant Compounds

Primary metabolites of plants are integral to the functioning of the plant and include carbohydrates, lipids, amino acids and proteins. Such constituents may have therapeutic relevance, for example complex polysaccharides administered orally have often been shown to have immunostimulating effects (Yarnell 2007). For example, the immune-stimulating effects of leaf and flower polysaccharides of *Echinacea purpurea* may be due to changes in various interleukin levels (Parnham 1999).

The majority of medicinally active compounds from plants comprise secondary metabolites. Secondary metabolites are low molecular weight compounds with no role in primary plant metabolism, and several tens of thousands of these compounds have been isolated and their structures characterized (Van Wyk et al., 2004). The three major groups of secondary metabolites include nitrogen-containing substances, terpenes and phenolics.

Nitrogen-containing compounds, of which more than 14 000 have been described, consist mainly of alkaloids, amines, non-protein amino acids, cyanogenic glycosides and glucosinolates (Van Wyk et al., 2004).

The classification of secondary metabolites can be accomplished in various ways, for example on the basis of their clinical function. This is complicated by the lack of an exclusive connection of clinical outcome to closely related substances, as chemically dissimilar molecules may produce similar clinical effects (Bernhoft 2010). Alternatively they can be classified botanically based on the families or genera of plants producing the secondary compounds, as closely related plant species may produce chemically similar active compounds (Bernhoft 2010). For chemists and pharmacognosists, it is useful to classify secondary metabolites by their biosynthetic pathways and structure (Yarnell 2007). Representations of the structural types of secondary metabolites are provided in Figure 2. In this section, some of the major classes of bioactive plant compounds will be discussed briefly in terms of their biosynthetic groupings, but with an emphasis on clinical relevance.

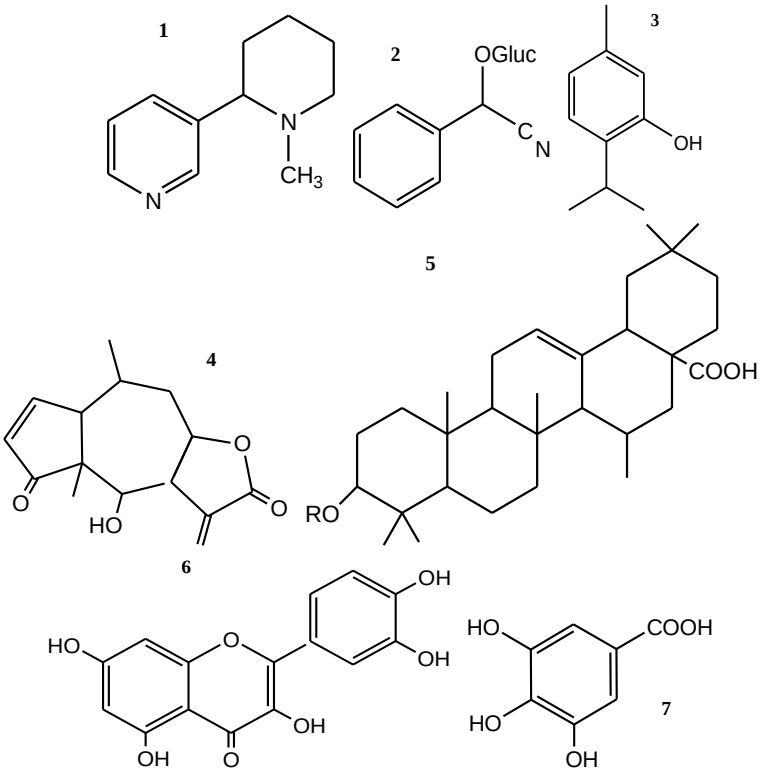

Figure 2. Representative structures of some secondary metabolites (1 = alkaloid, 2 = cyanogenic glycoside, 3 = monoterpene, 4 = sesquiterpene, 5 = triterpene, 6 = flavonoid, 7 = gallic acid, a phenolic acid).

2.2.1. Alkaloids

Alkaloids are heterocyclic, nitrogen-containing compounds which usually have potent activity and a bitter taste (Bernhoft 2010). They have been detected in about 15% of plants, occurring also in bacteria, fungi and animals (Van Wyk et al., 2004). There are various groups of alkaloids, including tropane alkaloids which occur in the Solanaceae (nightshade)

family, e.g. *Atropa belladonna* (deadly nightshade), *Datura* species (thorn apples) and *Hyoscyamus niger* (henbane). These compounds have anticholinergic activity and are used to reduce smooth muscle spasms, hypersecretion and pain (Bernhoft, 2010). Pyrrolizidine alkaloids are produced in the Asteraceae (daisy) family, particularly *Senecio* species, and alkylate DNA following metabolic activation in the liver (Van Wyk et al., 2004). There are varied additional classes of alkaloids, many with distinctive physiological effects. Important medicinal alkaloids include colchicine, vinblastine and taxol which inhibit the assembly or disassembly of microtubules (Van Wyk et al., 2004). Quinine from *Cinchona* species (Rubiaceae) inhibits the metabolism of the malaria parasite, and galanthamine from *Galanthus* species (Amaryllidaceae) inhibits acetylcholinesterase and is used in treating Alzheimer's disease (Van Wyk et al., 2004).

2.2.2. Terpenoids

The terpenoids represent a large class of secondary metabolites, and are synthesized via the five-carbon building block isoprene (Bernhoft 2010). Main groups are monoterpenes (with 10 carbon atoms), sesquiterpenes (15 carbons), diterpenes (20 carbons), triterpenes (30 carbons), steroids (27 or fewer carbons), tetraterpenes (40 carbons) and polyterpenes. Terpenes may have complex structures because of various chemical groups and secondary ring formations. Mono- and sesquiterpenes are often volatile and can be distilled as essential oils (Van Wyk et al., 2004), for example menthol, thymol and thujone. They occur in several families including the Asteraceae, Lamiaceae, Lauraceae, Rutaceae and Zingiberaceae (Van Wyk et al., 2004). Terpenes often show antimicrobial and cytotoxic activities against a range of organisms and may be used to treat bacterial and parasitic infections as well as respiratory disorders (Van Wyk et al., 2004).

2.2.3. Phenolic Compounds: Phenylpropanoids, Coumarins, Flavonoids and Tannins

Phenylpropanoids are derived from phenylalanine and tyrosine via deamination and are building blocks for many phenolic compounds (Van Wyk et al., 2004). An example is salicylic acid and its derivatives (from *Salix* species) which inhibit cyclooxygenase, a key enzyme in prostaglandin biosynthesis. Phenylpropanoids act as building blocks for coumarins, which are common in the Apiaceae, some members of the Fabaceae, and the Rubiaceae families (Van Wyk et al., 2004). Phenylpropanoids can also form complex dimers known as lignans, for example podophyllotoxin which is a potent inhibitor of microtubule formation, thus preventing cell division (Van Wyk et al., 2004)

Phenylpropanoids can condense with a polyketide moiety to form flavonoids, chalcones, catechins and anthocyanins (Van Wyk et al., 2004). Flavonoids have a central three-ring structure and proanthocyanidins are oligomers of flavonoids. Both these groups of compounds can occur as glycosides and they often have a general antioxidant effect, with several structures reducing inflammation or carcinogenicity (Bernhoft 2010).

Tannins are widely distributed in the plant world. There are two types of tannins, namely condensed tannins which are large polymers of flavonoids, and hydrolysable tannins consisting of polymers with a monosaccharide core and several catechin derivatives attached. Although the two tannin types share many properties, hydrolysable tannins are less stable and are more likely to be toxic (Bernhoft 2010). Tannins bind indiscriminately to proteins and the larger tannins are used as astringents to treat diarrhoea and bleeding (Bernhoft 2010).

2.2.4. Glycosides

Glycosides consist of secondary metabolites that are bound to a monosaccharide, an oligosaccharide or to uronic acid (Yarnell 2007). The saccharide or uronic acid component is the glycone and the molecule to which the glycone is attached via a glycosidic linkage is the aglycone (Yarnell 2007). The main groups of glycosides are cardiac glycosides, cyanogenic glycosides, glucosinolates, saponins and anthraquinone glycosides, and flavonoids also often occur as glycosides (Bernhoft 2010).

The aglycones of cardiac glycosides are steroidal and they function to inhibit the Na+/K+-ATPase pumps in the cell membrane (Bernhoft 2010). These pumps are concentrated in cardiac cells, being critical for their functioning, and the compounds have serious effects on heart tissue. Cardiac glycosides are present for example in species of the Scrophulariaceae, particularly *Digitalis purpurea*. Over 60 different cyanogenic glycosides are widely distributed among plant species, especially the Rosaceae, Fabaceae, Gramineae and Araceae (Van Wyk et al., 2004). They have aglycones derived from amino acids and can release hydrogen cyanide which is lethal at high dosages (Bernhoft 2010).

Most saponins occur as glycosides and have aglycones consisting of either pentacyclic triterpenoids or tetracyclic steroids (Bernhoft 2010). The saponin glycosides are large molecules with a hydrophilic glycone and a hydrophobic aglycone, producing emulsifying properties and they can therefore function as detergents. The attached sugar molecules render the saponins water soluble, and the presence of saponins in an aqueous extract can be detected from the foam which forms following vigorous shaking of the solution (Van Wyk 2009). Some saponins have demonstrated immune modulating and antineoplastic effects (Bernhoft 2010). Glucosinolates have sulphur-containing, pungent amino acid-derived aglycones (Bernhoft 2010) and are associated with the Brassicaceae (brassica) family.

3. BIOACTIVE PLANTS AND HEALTH BENEFITS

3.1. Cancer

The worldwide burden of cancer continues to intensify largely due to the aging and growth of the global population and a cumulative adoption of cancer-causing behaviors (particularly smoking) within economically developing countries (Jemal et al., 2011). In many economically developing countries, female breast, lung, and colorectal cancers are occurring in high frequencies (Jemal et al., 2011) with cervical cancer often the most common cancer among women (IARC 2014). The first plant-derived agents to advance into clinical use were isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. At the time, the plant was being investigated as a source of a potential oral hypoglycemic agent as per its traditional use in diabetes treatment, but the opportune observation of the reduction of white blood cell counts and bone marrow depression in rats led to the isolation of vinblastine and vincristine (Cragg et al., 2009). Amongst the anticancer drugs developed from around 1940 to date, of the 175 small molecules, 85 (48.6%) are either natural products or directly derived therefrom (Newman et al., 2012). Interaction with the cellular protein tubulin is one of the key mechanisms of action of natural products (Kingston 2009). Two renowned anticancer agents derived from plants are taxol and camptothecin. Taxol, a microtubule agent, was

isolated from *Taxus brevifolia* guided by bioassay on various extracts and chromatographic fractions. Taxol binds to a protein, tubulin, thus inhibiting cell division (Wall et al., 1995). Camptothecin, an alkaloid found in the wood bark of *Camptotheca acuminata*, a tree native to China, was isolated in 1966 (Wall et al., 1966) and has shown excellent antitumour activity (Du 2003; Huang et al., 2013). Currently there are numerous compounds of natural origin in different stages of clinical trials for treatment of various cancers (Cragg et al., 2011; Shah et al., 2013).

Chemoprevention, a mode of cancer control by which the occurrence of the disease can be wholly prevented, slowed down, or reversed by use of nontoxic natural or synthetic products, is a promising and pragmatic approach to reducing the risk of cancer (Pratheeshkumar et al., 2012). Substantial interest has developed on the role of plant products and spices to lessen tumour development. Numerous studies have shown that phytochemicals, including curcumin, resveratrol, epigallocatechin gallate, apigenin, quercetin, genistein, lycopene, ursolic acid, isothyocynates and perillyl alcohol exert anticancer effects via multiple signal transduction pathways thus consumption of natural products rich in these compounds could be beneficial for cancer prevention (Pratheeshkumar et al., 2012). Turmeric is a spice prepared from the root of *Curcuma longa*, of which curcumin is one of the constituents. Curcumin has been investigated as both a chemotherapeutic and chemopreventive agent in many different animal models of carcinogenesis (Epstein et al., 2010). Its non-toxicity and good tolerability in humans, in conjunction with encouraging results from *in vitro* and *in vivo* studies as well as early human clinical studies, support the continuing research and development of curcumin as both a preventive and disease-modifying agent (Epstein et al., 2010).

3.2. Cardiovascular Diseases

In 2008, an estimated 17 million people died from cardiovascular diseases (CVDs), representing 30% of all deaths globally (WHO 2011). Developing counties are unduly affected with over 80% of CVD deaths taking place in low- and middle-income countries (WHO 2011). Risk factors for CVD include elevated levels of plasma total cholesterol, LDL cholesterol (LDL-C), triglycerides (TGs) and glucose, and the amount of oxidised LDL also contributes to atherosclerosis (Tappia et al., 2013). Several lipid-lowering drugs, such as statins, are used as first-line therapy in hypercholesterolemia (Bellosta et al., 2012) but problems associated with the long term use of statins include interactions with other drugs (Taylor et al., 2013). Extensive evidence demonstrates that plant-based diets including whole grains as the main form of carbohydrate, unsaturated fats as the predominant form of dietary fat, plenty of fruit and vegetables, and sufficient n-3 fatty acids can play a vital role in preventing CVD (Hu 2003).

Herbal treatments have been used to treat patients with a range of CVD-related issues, including congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency and arrhythmia (Mashour et al., 1998). Many of these still require rigorous scientific assessment, investigation of toxic effects and drug-drug interactions (Mashour et al., 1998). Most plant-based medicines commonly have multiple cardiovascular effects that often overlap, and it is possible that the dilution of active components in herbal medicines results in fewer adverse effects than allopathic medicines

(Mashour et al., 1998). A number of bioactive compounds in plants, including isoflavones, diosgenin, resveratrol, quercetin, catechin, sulforaphane, tocotrienols and carotenoids, reduce the risk of cardiovascular diseases and aid in cardioprotection (Vasanthi et al., 2012). The cardioprotective effects of the various phytochemicals may be a result of their antioxidative, antihypercholesterolemic, antiangiogenic, anti-ischemic, anti-platelet aggregation and anti-inflammatory activities that reduce the risk of cardiovascular disorders (Vasanthi et al., 2012).

Several medicinal plants contain cardiac glycosides which have positive inotropic actions on the heart. Congestive heart failure has been treated for many decades by the drugs digitoxin, derived from either *Digitalis purpurea* or *D. lanata*, and digoxin, from *D. lanata* (Mashour et al., 1998). Cardiac glycosides are potent molecules with a low therapeutic index (Mashour et al., 1998). Other sources of cardiac glycosides include *Apocynum cannabinum* (black Indian hemp), *Carissa spectabilis* (wintersweet), *Cheiranthus cheiri* (wallflower), *Helleborus niger* (black hellebore), *Nerium oleander* (oleander), *Thevetia peruviana* (yellow oleander) and *Urginea maritima* (squill).

The beneficial effects of willow bark (*Salix alba*) have been known for centuries (Fuster et al., 1993). The active compound is the bitter glycoside, salicin, from which the anti-inflammatory and analgesic derivative acetylsalicylic acid, or aspirin, was prepared (Flower et al., 1985). The anti-platelet effect of this compound was only discovered in the late 1960s (Weiss et al., 1967) and its use in the therapy and prevention of CVD conditions has since become widespread, owing to the critical role of platelets, platelet products and thrombosis in CVD (Fuster et al., 1993). In the United States, an estimated 36% of the adult population, equating to more than 50 million people, take aspirin regularly for CVD prevention (Ajani et al., 2006). Among individuals with known CVD, this percentage increases to over 80%, translating into 10 to 20 billion aspirin tablets consumed annually in the US alone for CVD prevention (Campbell et al., 2007). Aspirin therapy has proven value in the treatment of acute myocardial infarction and also in the long-term use in patients with a wide range of prior manifestations of CVD (Hennekens et al., 1997). However, aspirin therapy should be an adjunct and not an alternative to the management of other risk factors (Hennekens et al., 1989). Reserpine, an alkaloid derived from the roots of *Rauwolfia serpentina*, was one of the first drugs used on a large scale to treat systemic hypertension (Mashour et al., 1998). The compound reduces blood pressure by decreasing cardiac output, peripheral vascular resistance, heart rate and renin secretion, but with the advent of other antihypertensive drugs with fewer adverse effects on the central nervous system, its use has decreased (Mashour et al., 1998). Garlic (*Allium sativum* L.) possesses many beneficial effects on the cardiovascular system, including lowering blood pressure, inhibiting platelet aggregation, enhancing fibrinolytic activity, reducing serum cholesterol and triglyceride levels, and protecting the elastic properties of the aorta (Kleijnen et al., 1989).

As well as lowering blood levels of total cholesterol and LDL-C, some natural medicine products have been demonstrated to lower circulating TG and glucose levels, and inhibit lipid oxidation (Tappia et al., 2013). Reductions in the levels of total cholesterol, LDL-C or triglycerides have been reported for red yeast rice (*Monascus purpureus* Went.), Goji berry (*Lycium barbarum* L.), Indian gooseberry (*Emblica officinalis* Gaertn.), artichoke (*Cynara cardunculus* var. *scolymus* L.) and rhubarb (*Rheum rhaponticum* L.). Garlic, fenugreek (*Trigonella foenum-graecum* L.), curcumin (*Curcuma longa* L.) and ginger (*Zingiber officinale* L.) exhibit cholesterol-lowering and hypoglycemic effects. Improved lipid profiles and hypoglycemic effects have been observed with licorice roots (*Glycyrrhiza glabra* L.),

seabuckthorn (*Hippophae rhamnoides* L.), milk thistle (*Silybum marianum* L.*)* and the tree bark of *Terminalia arjuna* L. (Tappia et al., 2013). Larger clinical trials need to be undertaken to verify the effects on lipids and glycemia. The lipid-lowering effect of plant sterols (phytosterols) such as sitosterol and campesterol is mediated by competitive inhibition of cholesterol absorption and by transcriptional induction of genes implicated in cholesterol metabolism in both enterocytes and hepatocytes (Genser et al., 2012). Some approaches may be beneficial as adjuncts to conventional management of cardiovascular disease, but no evidence exists to support the role of bioactive plant compounds as primary treatment (Miller et al., 2004).

3.3. Diabetes

Diabetes is a cluster of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association 2013). Diabetes generally falls into two broad etiopathogenetic categories; namely types-1 and -2. In type-1 diabetes, the cause is an absolute deficiency of insulin secretion whereas in type-2 diabetes, the cause is an amalgamation of resistance to insulin action and an inadequate compensatory insulin secretory response (American Diabetes Association 2013). Type-2 diabetes is the major form of diabetes, accounting for approximately 90–95% of all diabetic cases (Hui et al., 2009). Control over hyperglycemia can theoretically be achieved by different mechanisms namely an increase in insulin secretion; a decrease in nutrient ingestion; an increase in peripheral glucose uptake; or a decrease in hepatic glucose production (Bedekar et al., 2010). The current understanding of antihyperglycemic activity of plants has led to numerous dietary supplements however only one clinical drug has gone from plant to pharmacy (Gunn et al., 2012). Metformin (dimethylbiguanide) is the most widely prescribed drug in the treatment of diabetes, the origin of which can be traced back to *Galega officinalis* (goat's rue or French lilac) (Bailey et al., 2004). After the discovery of its anti-diabetic effects, guanide was chemically modified as a biguanide to improve efficacy and reduce toxicity (Chan et al., 2013). Despite being introduced clinically in the 1950s, the exact mechanism of action of metformin has not been fully elucidated (Viollet et al., 2012). However, clinical trials suggest that metformin may also have therapeutic potential in other health conditions, including CVDs and the prevention or treatment of cancer (DeCensi et al., 2010; Viollet et al., 2012).

Ginseng (*Panax* sp.) is currently the most studied medicinal plant species for hypoglycemic activities (Prabhakar et al., 2011). Allegedly *Panax ginseng* (Chinese, Korean or red ginseng) has premier therapeutic potency, followed by *Panax quinquefolius* (American ginseng) with medium potency, while *Panax japonicus* (Japanese ginseng) is considered low potency grade ginseng (Hui et al., 2009). However a systemic review to evaluate the current evidence of *P. ginseng* in patients with type-2 diabetes revealed that the results are not convincing and that more rigorous studies are needed (Kim et al., 2011). Bitter melon (*Momordica charantia* L.) is utilised for the treatment and management of diabetes in parts of Asia and Africa (Harinantenaina et al., 2006). Despite the abundant data from biochemical and animal studies, available clinical data on *M. charantia* are often unsound due to small sample size, lack of control and poor study designs (Gunn et al., 2012). A systematic review and meta-analysis to evaluate the effect of herbal supplements on glycemic control in type-2

diabetes identified nine randomized, placebo-controlled trials (n = 487 patients) (Suksomboon et al., 2011). Results showed that supplementation with *Ipomoea batatas* (sweet potato), *Silybum marianum* (milk thistle), and *Trigonella foenum-graecum* (fenugreek) may improve glycemic control in type-2 diabetes. Such effect was not observed with *Cinnamomum cassia* (cinnamon). However, considering the limitations of the existing studies and high heterogeneity of the results for milk thistle and fenugreek, further large controlled trials using homogeneous preparations are necessary to better clarify the effects of these herbs on glycemic control in type-2 diabetes patients.

3.4. HIV/AIDS

Worldwide, approximately 35 million people are infected with human immunodeficiency virus (HIV), with the majority of them living in Africa and Asia (UNAIDS 2013). Antiretroviral therapy (ART) has emerged as a potent force for saving lives. Yet notwithstanding the unprecedented growth in numbers of people on HIV treatment in the last few years, nearly half of all who are eligible do not have access (UNAIDS 2012). The life cycle of HIV, the causative agent of acquired immune deficiency syndrome (AIDS), offers various points for chemotherapeutic attack. Many anti-HIV agents inhibit the actions of the viral enzymes, including reverse transcriptase (nucleoside and non-nucleoside reverse transcriptase inhibitors; NRTIs and NNRTIs), protease (protease inhibitors), and integrase (integrase inhibitors) (Lee 2010). To date, there are 24 drugs for HIV treatment approved by the Food and Drug Administration of the U.S.A. (Hupfeld et al., 2009). A number of secondary metabolites such as terpenoids, coumarins, alkaloids, tannins and flavonoids have shown promising anti-HIV activity (Hupfeld et al., 2009). However, in many countries, the inclusion of anti-HIV traditional medicines and other natural products in official HIV/AIDS policy is an extremely sensitive and contentious issue (Chinsembu 2009). This is because firstly, traditional medicines and other natural products can easily become a culprit for abjuration and inertia to roll-out ART (Chinsembu 2009) and secondly certain medicinal plants may interfere with antiretroviral (ARV) treatment (Mills et al., 2005). Two common African herbal medicines used for HIV/AIDS treatment in sub-Saharan Africa include *Hypoxis hemerocallidea* (African potato), and *Sutherlandia* however low-level evidence of harm recognizes the potential for drug interactions with ARVs (Mills et al., 2005). Andrographolide, a diterpenoid lactone isolated from *Andrographis paniculata*, was investigated to assess effects on plasma virion HIV-1 RNA levels and $CD4^+$ lymphocyte levels (Calabrese et al., 2000). The trial was interrupted at 6 weeks attributable to adverse events including an anaphylactic reaction in one patient. Furthermore, short term results indicated that there were no statistically significant changes in mean plasma HIV-1 RNA levels. A series of polycyclic coumarins with anti-HIV activity originally isolated from several tropical trees of the genus *Calophyllum* in Malaysia were identified as NNRTIs that exhibited anti–HIV-1 activity and other unique properties (David et al., 2002). The most potent of these isolated compounds, calanolide A, is the only anti-HIV natural product undergoing clinical trials (David et al., 2002; Mehellou et al., 2010). A Cochrane review in which nine randomized placebo-controlled trials involving 499 individuals with HIV infection and AIDS testing eight different herbal medicines concluded that there is inadequate evidence to support the use of plant-based medicines in HIV-infected individuals and AIDS

patients (Liu et al., 2005). Nonetheless, the shortcomings of conventional ART such as resistance, toxicity, limited availability, high cost and lack of any curative effect (Margolis et al., 2013) continue to add optimism in the use of ethnomedicinal plants and other natural products for the management of HIV/AIDS (Chinsembu et al., 2010).

3.5. Malaria

Malaria is a major worldwide public-health issue. Approximately half the world's population is at risk of malaria, and *Plasmodium falciparum* malaria—the deadliest form of the disease—is the cause of about one million deaths annually (Hay et al., 2010). The parasite is now resistant to a number of antimalarial drugs thus plants offer an attractive potential for new metabolites with an original mode of action which can be active on resistant strains (Bero et al., 2011). In traditional medicine, plants are used by traditional health practitioners to treat malaria and its symptoms (Willcox et al., 2004). In Mali, for example, certain communities use herbal remedies as the first line treatment for more than 80% of malaria episodes (Diallo et al., 2006). *Argemone mexicana*, a medicinal plant used for uncomplicated malaria, seemingly prevents severe malaria without completely clearing parasites in most patients. A study, in a high transmission area of South Mali, explored whether residual parasitaemia at day 28 was associated with subsequent malaria episodes and/or anaemia. After three months of follow-up in a high transmission region there was no difference in incidence of uncomplicated or severe malaria or anaemia between patients treated first-line with the locally produced *A. mexicana* decoction and patients treated with artesunate/amodiaquine, or between patients with and without total parasite clearance at day 28 (Willcox et al., 2011).

Xu et al. (2013) reviewed recent developments in antimalarial natural products and reported 171 structures isolated from medicinal plants between January 2010 and April 2012. Such structures comprised of alkaloids, terpenoids, phenolics and other metabolites. From the World Health Organization (WHO) listed antimalarials, 15 of the 21 are derived from nature, either as natural extracts, semisynthetics or natural product analogs (Duffy et al., 2012). Quinine, the original natural product used in antimalarial chemotherapy was identified from cinchona tree bark, and purified in 1820 (Wells 2011). Cinchona tree, belonging to the family Rubiaceae, is native to tropical South America. Peruvian Indians were the first to discover the medicinal properties of the bark of the tree. Cinchona trees remained the only source of quinine until 1944 (Kalotka-Kreglewska 2011). Quinine and related cinchona alkaloids including quinidine, cinchonine and cinchonidine are all effective against malaria (Achan et al., 2011). Artemisinin was first isolated from the leaves of the sweet wormwood (*Artemisia annua*) in 1971 (Wells 2011). Artemisinin-based combination therapies are now recommended by the WHO as first-line treatment of uncomplicated falciparum malaria in all areas in which malaria is endemic (WHO 2006). Atovaquone, artemisinin and its semi-synthetic derivatives as well as clindamycin, erythromycin, azithromycin, chlortetracyline, tetracycline, oxytetracycline and doxycycline, are significant examples of the contribution of natural products for the development of antimalarial drugs, particularly in light of chloroquine-resistant parasites (Ginsburg et al., 2011).

3.6. Tuberculosis

Tuberculosis (TB), a contagious disease caused by infection with *Mycobacterium tuberculosis*, has become an increasingly serious global health concern. About one-third of the world's population is infected with TB, although most never develop the active TB disease (WHO 2008). Globally, there were an estimated 9.2 million new cases and 1.7 million deaths in 2006 (WHO 2008).

In sub-Saharan Africa, the persistent increase of TB may potentially be attributed to the AIDS pandemic, combined with inadequate healthcare systems (Zager et al., 2008). Of the estimated 1.7 million people who died of TB in 2006, 14% were co-infected with HIV (WHO 2008). Other species of *Mycobacterium*, such as *M. bovis* which primarily infects cattle, may also infect humans, particularly immunocompromised individuals. Much progress has been made against TB in many countries (Zager et al., 2008), but the emergence of *M. tuberculosis* strains resistant to currently used anti-TB drugs is a major threat. This drug resistance, coupled with the long duration of treatment and the toxic effects of currently used anti-TB drugs, necessitates the search for new classes of anti-TB drugs.

A large number of recent reviews have emphasised the potential of plant species and natural products as active antimycobacterial extracts and chemicals (Cantrell et al., 2001; Newton et al., 2002; Okunade et al., 2004; Gibbons 2005; Nayyar et al., 2005; Pauli et al., 2005; Negi et al., 2010; Salomon et al., 2012; García et al., 2012; Liu et al., 2012). Plant-derived antimycobacterial compounds belong to an extraordinary array of classes, among them alkaloids, terpenoids, coumarins, peptides and phenolics. During the last two decades, there has been much progress made in developing new techniques to evaluate the antimycobacterial potential of a large number of compounds (García et al., 2012). With the improvement in time-saving assays, many naturally occurring compounds have been discovered with promising inhibitory activity against *M. tuberculosis* and, in some cases, the mechanism of action has also been determined (Tripathi et al., 2005). Natural products and their derivatives have been reported to have significant growth inhibitory activity against *M. tuberculosis*. A review covering literature published from 2006 to 2011 on natural products with *in vitro* growth inhibitory activity against sensitive and resistant *M. tuberculosis* strains reported 278 natural products and some derivatives isolated from plants, algae, fungi, cyanobacteria, and sponges (García et al., 2012). Molecules with anti-TB selectivity indexes (SIs) higher than 10 were considered to be promising leads for development of new antitubercular drugs (García et al., 2012). For example, aegicerin, ergosterol peroxide, parguesterol A, and fischambiguiene B were described as remarkable scaffolds for drug development, because of their potent antimycobacterial activity, their low or negligible toxicity against Vero cells and their selectivity indexes higher than 10.

3.7. Womens Health

The idea of using hormones for contraception was first proposed in the 1920s when the ovarian hormones, oestrogen and progesterone, and their role in reproduction were discovered (Quarini 2005). At that time, the single source of hormones was animal tissue; and 5 kg of pig ovaries were required to produce 30 mg of oestrogen (Quarini 2005). In the early 1940's, the organic chemist, Marker, discovered that some plants contained hormone-like substances and

succeeded in developing a five-step, high-yield conversion of diosgenin into progesterone (Djerassi 2011). The source of the diosgenin, was extracted from *Dioscorea mexicana* roots, a plant which grows in Mexican jungles (Dhont 2010).

It is known that a large portion of women use plant-based remedies during pregnancy, many of them without informing their doctor or midwife (Holst et al., 2009; Warriner et al., 2013). Ginger has long been used as a traditional remedy for treating sickness, including nausea and vomiting associated with early pregnancy (NVP) (Tiran 2012). Ginger use during pregnancy is well studied (Dante et al., 2013) and evidence suggests that it is a safe and effective treatment for NVP (Ding et al., 2013; Heitmann et al., 2013). In a cohort of women at moderate risk of pre-eclampsia, the administration of garlic during the third trimester was effective in reducing hypertension however did not prevent pre-eclampsia, which was the primary study outcome (Ziaei et al., 2001).

Vasomotor symptoms such as hot flushes and night sweats, related to insomnia and cognitive dysfunction, frequently occur in women after menopause and are due to lowering of oestrogen levels in the hypothalamus attributable to ovarian failure (Powles 2004; Pachman et al., 2010). Such symptoms are the chief motive for women taking oestrogen (occasionally with a progestin) as hormone replacement therapy (Powles 2004). For almost two decades there has been extensive controversy about the use of hormone therapy (HT) among postmenopausal women (Genazzani et al., 2006). Although many women view HT favorably for climacteric symptom relief (Tao et al., 2011), the European Menopause Survey (2005) revealed that many women either opt not to use any treatment or use a herbal/natural remedy driven by fear of breast cancer (Genazzani et al., 2006). Phytoestrogens are polyphenolic non-steroidal compounds of oestrogenic activity derived from plant origin (Gencel et al., 2012). Hundreds of foods contain phytoestrogens with most belonging to one of three classes: isoflavones, lignans or coumestans (Bedell et al., 2014).

Isoflavones are found in beans with soybeans and soy products being the major dietary source. Lignans are found in high fiber foods such as unprocessed grains, cereal brans and beans, with flaxseed containing the largest content. Foods comprising the highest amount of coumestans include alfalfa and clover sprouts (Bedell et al., 2014). A recent prospective randomized, double-blind, placebo controlled trial evaluated the effect of red clover isoflavone supplementation over vasomotor and overall menopausal symptoms in postmenopausal women (Lipovac et al., 2011). The isoflavone supplement showed more efficacy than the placebo in reducing vasomotor frequency and overall menopausal intensity. Many postmenopausal women report memory loss and cognitive decline as common features of midlife transition. A systematic review to evaluate the evidence regarding the efficacy of herbal and dietary supplements on cognition in menopause revealed that available evidence from 12 randomized clinical trials failed to demonstrate conclusively that any herbal or dietary supplement improved cognition in menopause (Clement et al., 2011). Black cohosh (*Cimicifuga racemosa*) has been used as a natural remedy by women for over a century and is currently advocated as an alternative therapy for menopausal symptoms (Mahady et al., 2002). However unresolved issues regarding the quality, safety, and efficacy of black cohosh during menopause remain (Soni et al., 2011).

3.8. Other Health Benefits

Medicinal plants have an almost limitless capacity for producing interesting chemicals with potential for the treatment of ailments affecting humans as well as animals, including livestock and companion animals. Ethnoveterinary medicine incorporates the use of plant extracts in the treatment of animal diseases. The efficacy of a large number of plant species tested against animal disease-causing microbes and parasites, as well as ticks which carry many diseases, were reviewed by Eloff et al. (2009). An advantage of testing plant-based products for use in animal health is that the necessity for costly and time-consuming human clinical trials is avoided.

A comprehensive overview of the use of plants for treating infectious diseases would fill several volumes. The number of articles published on antimicrobial activity of medicinal plants has expanded impressively over the past few years (Rios et al., 2005), indicating increasing levels of interest in this field. Multidrug resistance is encountered in an increasing number of disease-causing microbes, and the rapid progression of such resistance is driving the search for new strategies and alternative therapies for treating infections. Methicillin resistant *Staphylococcus aureus* (MRSA) is no longer confined to hospital wards but has spread to communities (Witte et al., 2004) and much effort has been devoted to discovering new anti-MRSA entities. A well-known example of a plant product with excellent activity against MRSA (as well as anti-inflammatory activity), is *Melaleuca alternifolia*, or tea tree oil (Carson et al., 2006). Tea tree oil has anti-inflammatory activity as well as antimicrobial efficacy against bacteria, fungi, viruses and protozoa, and it has been postulated that the multicomponent nature of the oil may lower the chances of resistance occurring spontaneously, since multiple simultaneous mutations may be required to overcome all of the antimicrobial actions of each of the components (Carson et al., 2006). Two clinical trials conducted on the anti-MRSA activity of tea tree oil have been reviewed by Slover et al., (2009), and the oil was held to be effective and safe in decolonisation of MRSA although further trials are needed to determine optimal concentrations to be used in topical products.

Resistance to current drugs has also emerged as a significant problem in parasites such as nematodes and protozoa, including malaria parasites. Antiparasitic activity of plant extracts and purified compounds derived from active plants is a rewarding sphere of research. Several antiviral plant compounds have shown competitive *in vitro* and *in vivo* antiviral activities with those of synthetic antiviral drugs, contrary to antibacterial and antifungal plant substances (Vlietinck et al., 1991). Natural products also interfere with a range of viral targets, from adsorption of the virus to the host cell to release from the cell, indicating complementary mechanisms of action to those of current antiviral drugs (Vlietinck et al., 1991).

Immune modulators have received much attention in recent years. In one study Borsuk et al. (2011), reported that burdock (*Arctium lappa*) and bur marigold (*Bidens tripartite*) extracts stimulated the humoral immune response in mice, and nettle (*Urtica dioica*) and licorice (*Glycyrrhiza glabra*) extracts stimulated cellular response and nonspecific resistance, with their effects superior to those of *Echinacea purpurea* tincture, a well-known immune booster. The immune stimulating properties of *Echinacea* extracts, including results of several human clinical trials, have been reviewed (Aridoğan 2009), with the conclusion that *Echinacea* is indeed effective in reducing the duration of severity of symptoms of illness although the exact mechanism of its immunostimulatory effect is still unknown.

Bioactive plants have several more interesting and useful biological activities, including analgesic, anti-inflammatory, anti-pyretic and antidepressant efficacy. It is clear that although many studies have been conducted using *in vitro* test systems, more clinical studies need to be conducted to verify the efficacy and safety of biologically active plant extracts and purified compounds before they can be used to their full potential.

CONCLUSION

Despite important examples of traditionally used, plant-based medicines that have inspired modern drugs, the customary approach of bioassay-guided fractionation can be arduous, and it is also dependent on the availability and accessibility of a suitable assay (Harvey 2008). Nonetheless, plants remain an abundant and economical renewable resource distinctively adapted to intricate biochemical synthesis (Raskin et al., 2002). Considering the accumulative cost of energy and chemical raw materials, coupled with the environmental concerns associated with conventional pharmaceutical manufacturing, plants continue to be a very lucrative option for the future (Raskin et al., 2002). In the search for new natural products, certain compounds may be deemed useful scaffolds for structure-activity relationship studies and advancement of new drugs (García et al., 2012). Although active phytochemicals have been identified from numerous plants, countless pathways for the biosynthesis of specific medicinal compounds and the biotic and abiotic factors regulating their production remain vague (Briskin 2000). A challenge for phytochemical-based therapeutics is to incorporate the ability to detect and genetically manipulate multifaceted biosynthetic pathways in plants with better characterization of genetic targets for the prevention and treatment of multifaceted diseases (Raskin et al., 2002). One of the key problems with evaluating health benefits of natural products is the lack of clinical trial data. Numerous trials have significant flaws in study design and reporting, including uncertainty regarding the maximum safe dosage, appropriate period of treatment, consequences of over-dosage, and possible herb-drug interactions, with a general lack of scientific robustness in terms of data validity and reliability (Ding et al., 2013; Pallivalappila et al., 2013). Nonetheless, the wealth of hitherto unexplored plant biodiversity provides limitless possibilities for discovery of novel therapeutic molecules with diverse and useful mechanisms of action to combat the array of infectious and non-infectious diseases affecting humans and animals. Focus areas for future studies include designing and conducting acceptable clinical trials with promising natural products or potentiated extracts to validate the use of these preparations in standard healthcare practices.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 5

BIOACTIVE COMPOSITION OF PLANTS AND PLANT FOODS

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ABSTRACT

The recent changes in demographic trends of society as well as the emergence of new research amalgamations of nutritional and pharmaceutical sciences have provided great opportunities for the utilisation of plant food products. There is currently significant interest in the nutraceutical industry, and the search for new health-exhibiting and somewhat toxicity-inducing naturally occurring plant compounds is on the rise. Bioactive components in plants are essentially secondary metabolites that can display pharmacological or toxicological response in humans and animals alike. These metabolites are produced concomitantly with the primary biosynthetic compounds that are responsible for plant growth, development, signalling and protection, and once ingested can produce beneficial health effects on many levels. Furthermore, the beneficial health properties of bioactive compounds are primarily due to their possession of exceptional antioxidant properties when applied in relatively small amounts. This unique feature extends to the majority of their conjugate metabolites to a similar extent as observed in their pure forms. Most of the plant species are capable of producing these compounds from relatively small levels to exceptionally high concentrations however, the variety of externally controlled factors are also influential for the levels found in plants themselves. The aim of this chapter is to provide an introduction to key plant bioactive compounds classification, description of some most important plant bioactive compounds as well as putative mechanisms of action that these bioactive compounds possess and their significance in the use in today's nutraceutical industry.

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1. INTRODUCTION

Presently, the pharmaceutical and food industry domains share very similar interests in characterisation, production and methods of identification of many purified compounds from natural plant sources. The search for the *natural compounds* in the form of a plant extract or as a single component that are able to provide medicinal and/or health benefits, including the prevention and treatment of disease is one of the fundamental topics in many laboratories around the world. This amalgamation of the pharmaceutical and nutrition industries has provided a sharp rise in the investigation of nutraceuticals and is further fuelled by increased consumer interest in naturally occurring compounds. Even some of the therapeutic areas of oncology have established their foundations from the abundant diversities that natural products possess and their ability to interact with many target specific receptors within the cell. Hence natural ingredients have been a major source of inspiration for some of the most fundamental and important drug discoveries approved by the Foods and Drugs Administration (Mishra and Tiwari, 2011) as well as being the single and most productive lead source for the development of new drugs, particularly anti-cancer and anti-infective products (Harvey, 2008).

In support of the current population demographic trends of our aging society and the formation of a booming nutraceuticals industry, there is an overwhelming accumulation of evidence suggesting that diet itself contains cancer preventive agents, implying that many cancers may be prevented by dietary modifications (Stan et al., 2008). Further, it is becoming increasingly evident that many components within foods may aid in the prevention of multiple mechanisms of carcinogenesis through the detoxification of carcinogenic intermediates (Chen et al., 2011), inhibition of proliferation of cancer cells (Kavandi et al., 2013), selective promotion of apoptosis in cancer cells (Deng et al., 2013, Gao et al., 2013, Kavandi et al., 2013, Bak et al., 2013) and via the antioxidant activity of various plant bioactive compounds (Fattahi et al., 2013, Kogiannou et al., 2013, Lee et al., 2013, Nam and Kim, 2013, Sakulnarmrat et al., 2013, Tsuji et al., 2013, Yu et al., 2013).

Therefore, the aim of this chapter is to provide an introduction to the classification of plant bioactive compounds, a description of some of the most important plant bioactive compounds as well as putative mechanisms of action that these bioactive compounds possess, and finally their significance within the nutraceutical industry.

2. OXIDATION, FREE RADICALS AND ANTIOXIDANTS

The oxidation process in biological systems focuses on oxygen as an important acceptor of electrons, which leads to the formation of active oxygen and free radical species. A free radical can be defined as any molecular species, containing one or more unpaired electrons, which is capable of independent existence (Halliwell and Gutteridge, 1989). There is a whole variety of free radicals that can be generated in biological systems and their role is dependent on the nature of the radical itself and on its molecular composition.

Table 1. A selection of biologically important free radicals (Devasagayam et al., 2004)

Free Radical	Symbol	Half-life (s)	Implications
Reactive Oxygen Species (ROS)			
Superoxide	$O_2^{\bullet-}$	0.05 (Fridovich, 1983, Gutowski and Kowalczyk, 2013)	Biologically toxic and produced by NADPH oxidase (Kroller-Schon et al., 2014)
Hydroxyl radical	$^{\bullet}OH$	10^{-9} (Gutowski and Kowalczyk, 2013)	Implication with neurological autoimmune diseases (Allan et al., 1987, Kundu et al., 2012, Orciani et al., 2013)
Hydrogen Peroxide[1]	H_2O_2	Enzymatic (Sies et al., 1992, Gutowski and Kowalczyk, 2013)	Pathogenesis of ischemia, inflammation and atherosclerosis (Gunaydin and Demiryurek, 2001, Osato et al., 1995)
Peroxyl radical	$ROO^{\bullet}$	7 (Sies et al., 1992, Gutowski and Kowalczyk, 2013)	Inductors of neurodegenerative and inflammatory diseases (Spiteller, 2006)
Organic Hydroperoxide	ROOH	540 (Liddell et al., 2006)	Generated during bacterial host interactions (da Silva Neto et al., 2012)
Singlet Oxygen	O_2	10^{-6} (Sies et al., 1992, Gutowski and Kowalczyk, 2013)	Major ROS involved with provoking cellular damage and rapidly oxidising cellular components (Fenoglio et al., 2013)
Reactive Nitrogen Species (RNS)			
Nitric Oxide	$NO^{\bullet}$	1-10 (Gutowski and Kowalczyk, 2013, Sies et al., 1992)	Important function as EDRF as antioxidant but in presence of $O_2^{\bullet-}$ forms peroxynitrite (Halliwell and Gutteridge, 1989, Stocker and Keaney, 2004)
Peroxynitirite	$ONOO^-$	0.05-1 (Gutowski and Kowalczyk, 2013, Sies et al., 1992)	Potent inducer of lipid peroxidation (Violi et al., 1999)
Peroxynitrous acid	ONOOH	<1 (Lesshem, 2000)	Modifies human endothelial cell matrix, gene expression and decreases cell adhesion (Chuang et al., 2014)
Nitrogen Dioxide	NO_2	120 (Vanloon and Duffy, 2011)	Oxidises membrane and lipid proteins (O'Donnell et al., 1999)

[1] Note: Although commonly placed on a free-radical list, Hydrogen peroxide is not a free radical but rather a molecule that is toxic to most cells in range between 10 - 100µM. Several enzymes can generate H_2O_2 in vivo such as L-amino acid oxidases, xanthine and urate (Gutowski and Kowalczyk, 2013).

In functional contrast, some free radicals (Table 1) are involved in a range of biologically important reactions such as aetiology of different diseases as well as processes of aging (Nazarewicz et al., 2013, Panis et al., 2012, Al-Saeedi, 2014, Kalwa et al., 2014, Tsai et al., 2014). In general these can be divided in two categories, reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their production is exceptionally well regulated for the maintenance of healthy tissue as well as functioning as signalling molecules (Devasagayam et al., 2004). For example, nitric oxide ($NO^{\bullet}$), formed from intracellular L-arginine metabolism, has an important function as an endothelial-derived relaxation factor (EDRF), which causes smooth muscle cells to relax (Halliwell and Gutteridge, 1989, Stocker and Keaney, 2004). Due to $NO^{\bullet}$ having an unpaired electron in the highest orbital, it has the ability to reduce other molecules and can behave as a potential antioxidant. However, in the presence of another free radical, superoxide anion ($O_2^{\bullet -}$), $NO^{\bullet}$ is rapidly inactivated and forms peroxynitrite ($ONOO^-$), a potent inducer of lipid peroxidation (Violi et al., 1999).

Antioxidants, on the other hand, are defined as substances which, when present in much smaller quantities than substances that they protect, are effective at protecting against oxidative damage (Halliwell and Gutteridge, 1989). There is a whole array of substances that may prevent or delay the oxidation of other substances. Some antioxidants are able to neutralise free radicals by acting as scavengers (eg. Mannitol, superoxide dismutase, glutathione), as chain breaking antioxidants neutralising intermediate peroxyl radicals (eg. α-tocopherol, ascorbic acid) and as preventative antioxidants able to bind metal cations, preventing metal ion production of free radicals (eg. Haem, albumin) (Halliwell and Gutteridge, 1989, Stocker and Keaney, 2004).

Antioxidants can also be distinguished by their lipid solubility (Figure 1). Some are lipophillic and are soluble in the core of the lipoprotein particles (α- and β-tocopherols and β-carotene) (Bjornson et al., 1976, Drevon, 1991, Hacquebard and Carpentier, 2005) while others are hydrophilic and can be transported around the body in plasma as their free forms such as catechins (Fung et al., 2013), ascorbic acid (Wilson, 2005), and although a metabolic end product of purine metabolism, uric acid (So and Thorens, 2010). Furthermore, some of these antioxidants can be metabolised into their methylated forms for example, in quantities that can still pose a significant importance in beneficial effects associated with its parent forms (Renouf et al., 2011). The phytochemicals referred to as flavonoids have also been shown to decrease the oxidation induced by the free radical $ONOO^-$ and to reduce the modification of amino acids in the LDL protein, $apoB_{100}$ for example (Oldreive et al., 1998, Grace et al., 1998, Kono et al., 1997, Pannala et al., 1997).

Apart from classifying the antioxidants based on their mechanism of action and solubility, antioxidants can also be separated into the high and low molecular weight compounds. One of the most interesting groups that fit both high (3000Da+) and low molecular weight (500-3000Da) are the compounds collectively termed polyphenols (Hattenschwiler and Vitousek, 2000). The recent advances in identifying, separating, extracting and purifying varieties of plant polyphenols have provided readily available products to be used in their pure form *in vitro* as well as *in vivo* experimentation. These compounds have been used as pure non-altered compounds in their parent forms as well as incorporated within the food product providing it as a functional food product. Some of the most studied flavonoids are found in green tea (Kuo et al., 2005, Lin and Lin-Shiau, 2006, Rietveld and Wiseman, 2003, Stangl et al., 2007, Yamamoto et al., 1997) however a variety of other plants were also investigated for their polyphenolic content and antioxidant activities

(Kellogg and Lila, 2013, Mercado-Mercado et al., 2013, Tutel'ian and Lashneva, 2013, Miraballes et al., 2013, Rubio-Moraga et al., 2013, Nawrocki et al., 2013, Tommonaro et al., 2013, Rosenblat et al., 2013, Ciesla et al., 2013). However, it is important to note that there are several different factors that are influencing the bioavailability of the plant-based antioxidants such as food matrix (including the combination of different antioxidants within the same food matrix), absorption (including rate, place and competitive prospects of absorption) and metabolism (how is the compound metabolised within the system).

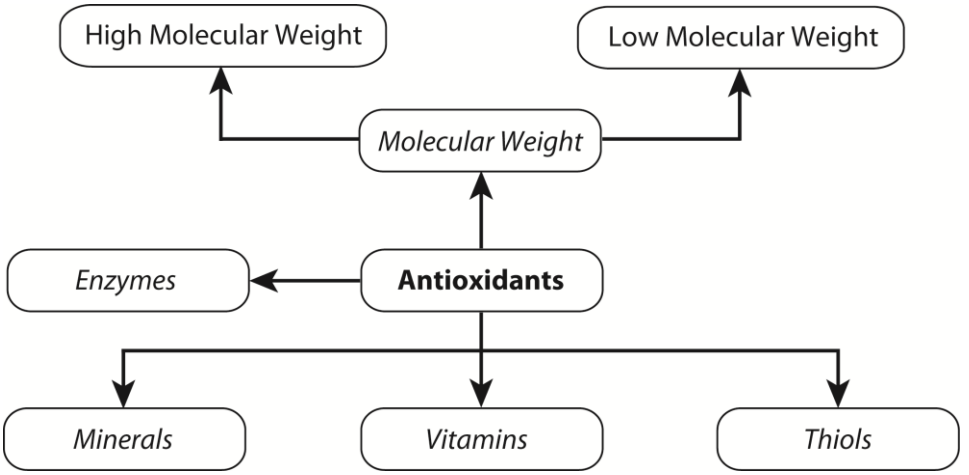

Figure 1. Classification of plant-derived antioxidants.

3. Antioxidant Composition of Plants

Nowadays it is widely accepted that plants are one of the major sources of antioxidant compounds (Abe et al., 2000a, Anandh Babu et al., 2006, Carlsen et al., 2010, Coelho et al., 2013, Cragg and Newman, 2005, Cragg and Newman, 2013), and the levels of total antioxidants significantly varies between the variety of plants and plant food categories. According to analysis by Carlsen and colleagues (Carlsen et al., 2010), who examined the total content of more than 3100 foods used worldwide, suggested that two points need to be taken into account; firstly, the plant food categories can be divided into eight main categories (Table 2); berries (including berry products), fruit and fruit juices, grains and grain products, herbal and traditional plant medicines, legumes, nuts and seeds, spices and herbs and overall vegetables. Secondly, the total antioxidant composition is significantly varied between the two different types of analysis methods used for the determination of total antioxidant content of the same or similar plant product.

Although there is a number of plant (food) groups that contain a significant amounts of total antioxidants according to Halvorsen and colleagues (Halvorsen et al., 2006), plant products with the highest amounts of total antioxidants are ground cloves (125.55 mmol/100g) and dried oregano leaves (40.29 mmol/100g). It is important to note that values for these two herbs are determined in dried or processed final food products rather than in their natural state. This can indicate that the total content of antioxidants after drying and/or

processing, these plant-derived antioxidants have remained active within the plant structure and quite possibly remained unchanged.

The antioxidant action is essentially the fundamental mode of operation for majority of bioactive compounds. The plant based foods such as fruits, vegetables and herbs in particular are the main sources of bioactive phytochemicals and as such may provide beneficial health effects beyond the basic nutrition such as reduction of risk of some of the chronic diseases.

Table 2. Total antioxidant content of some plant food products

Plant food category	Range for total antioxidant content (mmol/100g)	Source
Berries	0.06 – 261.53	(Carlsen et al., 2010)
	0.98 – 4.06	(Halvorsen et al., 2006)
	1.02 – 39.46	(Halvorsen et al., 2002)
Fruit and fruit juices	0.03 – 55.52	(Carlsen et al., 2010)
	0.05 – 2.52	(Halvorsen et al., 2006)
	0.04 – 11.33	(Halvorsen et al., 2002)
Grains and grain products	0.00 – 3.31	(Carlsen et al., 2010)
	0.01 – 0.99	(Halvorsen et al., 2006)
	0.04 – 1.09	(Halvorsen et al., 2002)
Traditional Plant medicines	0.28 – 2897.1	(Carlsen et al., 2010)
	Not included in analysis	(Halvorsen et al., 2006)
	Not included in analysis	(Halvorsen et al., 2002)
Legumes	0.00 – 1.97	(Carlsen et al., 2010)
	0.01 – 1.18	(Halvorsen et al., 2006)
	0.12 – 1.89*	(Halvorsen et al., 2002)
Nuts and seeds	0.03 – 33.29	(Carlsen et al., 2010)
	0.03 – 13.13	(Halvorsen et al., 2006)
	0.23 – 20.97	(Halvorsen et al., 2002)
Spices and Herbs	0.08 – 465.32	(Carlsen et al., 2010)
	0.80 – 125.55	(Halvorsen et al., 2006)
	Not included in analysis	(Halvorsen et al., 2002)
Vegetables	0.00 – 48.07	(Carlsen et al., 2010)
	0.02 – 4.69	(Halvorsen et al., 2006)
	0.02 – 2.46	(Halvorsen et al., 2002)

*This value represents the total antioxidant content of the pulses rather than legumes.

4. CATEGORISATION OF PLANT BIOACTIVE COMPOUNDS

The majority of the non-nutritive compounds present in plant foods have shown to exhibit a strong potential to improve human health. In general these compounds were collectively termed *bioactive compounds*, as these are capable of producing pharmacological or toxicological effects in humans and animals (Bernhoft, 2010, Denny and Buttriss, 2007). Although the minerals and vitamins are also active components of plants, and these can in fact induce beneficial and toxicological effects when ingested in large quantities as well, the

main focus of describing the plant bioactive compounds is orientated to the compounds that are produced as secondary metabolites. Therefore, the most appropriate definition of plant metabolites would be secondary metabolites that can induce pharmacological or toxicological effect in humans and animals (Bernhoft, 2010).

The classification of plant bioactive compounds can be divided using several different criteria based on; clinical function related to their toxicological or pharmacological effect; biological/ botanical approach considering their respective families and genera; and chemical classification that is predominately orientated on the plant bioactive compounds interaction to biochemical pathways (Bernhoft, 2010, Carlsen et al., 2010, Cragg and Newman, 2005, Cragg and Newman, 2013). However, some of the major classes of plant bioactives can be essentially divided into categories such as polyphenols, phenolic compounds, carotenoids and phytosterols.

Polyphenols

Polyphenols are ubiquitous compounds that are classified as wide and complex group of plant secondary metabolites. To date there are over 8000 compounds identified and the structures of these compounds range from very simple molecules (phenolic acids) to exceptionally complex and highly polymerised structures such as proanthocyanidins (Borriello et al., 2010, Dai and Mumper, 2010, Franz et al., 2011, Recio et al., 2012, Schaffer et al., 2012, Lorrain et al., 2013). Their importance and regulation of various metabolic activities in plant physiology are exceptional, playing an important function in structure and growth (Lewandowska et al., 2013, Cho et al., 2013, Orabi et al., 2013, Mildner-Szkudlarz et al., 2013, Vossen et al., 2012, Arung et al., 2011), pigmentation (Arung et al., 2011, Vossen et al., 2012, Mildner-Szkudlarz et al., 2013) as well as a resistance to various strains and types of pathogens (Ahmad et al., 2013, Betts et al., 2011, Mihai et al., 2012). In addition to the plant protective and structural aspects, plant polyphenols play significant importance in the provision of slight astringent and bitter tastes to the products, such as green and black tea (Balentine et al., 1997). However, this flavour characteristic in the tea is predominately ascribed to the catechins that are described in detail later in this chapter.

The occurrence of polyphenols in nature, from a structural perspective, is chiefly in the form of conjugated complexes via their hydroxyl groups and various glucose residues. This conjugation is necessary in order for polyphenols to be more adequately stored in plant vacuoles (Lu et al., 2013, Mullen et al., 2013, Liu et al., 2013, Del Rio et al., 2013). Furthermore, once polyphenol conjugates are assembled in plant vacuoles, they appear to be moderately resistant to external stressors such as heat and oxygen levels (Fischer et al., 2013, Li et al., 2013, Czibulya et al., 2012, Muhamad et al., 2012). Interestingly, once the polyphenols are isolated and purified, their stability in suspensions and even incorporation in food matrices is very limited (Li et al., 2011, Pacheco-Palencia et al., 2008, Wang et al., 2008, Wang and Zhou, 2004) unless it is manipulated via the use of various reducing agents such as ascorbate, Tris(2-carboxyethyl)phosphine and/or their combinations (Dube et al., 2010).

The polyphenols are most commonly found in fruits like grapes, apples and pears as well as cherries, and various types of berries. These fruits contain exceptionally high amounts of polypohenols in a range between 200-300 mg of polyphenols/100 g of fresh fruit (Sutherland

et al., 2006, Leppert et al., 2006, Kaliora et al., 2006, Chan et al., 2005, Zern and Fernandez, 2005, Urquiaga and Leighton, 2005, Peregrin, 2005, Manach et al., 2005a, Graziani et al., 2005, Moskaug et al., 2005, Manach et al., 2005b, Scalbert et al., 2005). However, the most significant interest from the nutraceutical industry is that even the products that can be manufactured from these plants can contain quite substantial polyphenol levels. It is obvious that the polyphenolic content is going to be to a lesser quantitative extent found in the extracts when compared to the original plant source, however there are still ample levels of purified/extract found in these products.

The polyphenolic compounds can chemically be defined by the presence of at least one aromatic ring bearing one or more hydroxyl substituents (phenols) that include their functional derivatives such as esters or glycosides. The polyphenols are generally divided into two groups based on their molecular weight, low (500-3000Da) and high molecular weight (3000Da+) (Hattenschwiler and Vitousek, 2000). Furthermore, polyphenols can also be classified into different groups based on the number of phenolic rings that they contain and on the basis of structural elements that these rings are connected to one another such as phenolic acids, flavonoids, stilbenes and lignans.

Flavonoids

The most predominant plant secondary metabolites with over 4500 different naturally occurring compounds are referred to as flavonoids. These compounds have been identified to inhibit the production and scavenging properties of most common free radicals such as superoxide anion, hydrogen peroxide and hydroxyl radical.

Anthocyanins

Anthocyanins are groups of compounds that are responsible for the naturally occurring colours in numerous fruits, vegetables, flowers and grains. The colours are predominately associated with blue and red colourings and these molecules occur naturally in plants as glycosides bound to sugar groups and sugars (glucose, galactose, rhmanose, xylose or arabionose) are bound to aglycon (Pojer et al., 2013). From the chemical perspective (Figure 2), anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of flavium salts possessing characteristics of typical polyphenolic structure (Wallace, 2011). Although there are over 635 anthocyanins already identified (Vuong et al., 2014, Wallace, 2011), only six anthocyanidins namely, cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petuindin account for over 90% of the currently identified anthocyanins in nature. This is mainly due to their very poor stability and predomination of glycosylated forms with or without aromatic aliphatic acid conjugations (Wallace, 2011).

The anthocyanidins are well represented in the human diet with the predominant occurrence in various types of grapes, berries and other types of red, blue and purple fruit (Table 3) (Pojer et al., 2013). Furthermore, depending on the type of diet, the daily intake of anthocyanidins in human is estimated to be 12.53 mg/day (Wu et al., 2006).

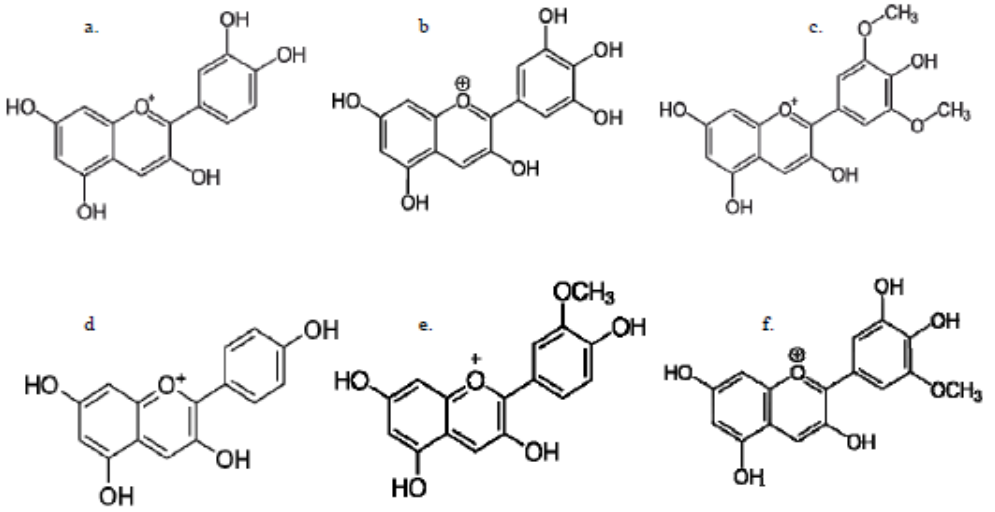

Figure 2. Structures of six most predominant anthocyanidins found in nature: Cyanidin (a); Delphinidin (b); Malvidin (c); Pelargonidin (d); Peonidin (e); and Petunidin (f).

Table 3. Dietary sources of anthocyanins and some of the glycosylated conjugates that are commonly distributed in plants (Neveu et al., 2010)

Anthocyanidin	Type of plant	Scientific name	Average Content (mg/100g)
Cyanidin	Red Raspberry (Raw)	*Rubus idaeus* L.	0.53
	Strawberry (Raw)	*Fragaria* L.	0.50
	Common black bean (Whole Raw)	*Phaseolus vulgaris* L.	1.63
Delphinidin (Delphinidin 3-O-glucoside)	Aestival Grape (Black)	*Vitis aestivalis* Michx.	16.61
	Red Raspberry (Raw)	*Rubus idaeus* L.	0.21
	Black Current (Raw)	*Ribes nigrum* L.	86.68
	Lowbush Blueberry (Raw)	*Vaccinium augustifolium* Aiton	15.17
	Grape Black	*Vitis vinifera* L.	2.63
Malvidin (Malvidin 3-O-glucoside)	Grape Black	*Vitis vinifera* L.	39.23
	Lowbush Blueberry (Raw)	*Vaccinium augustifolium* Aiton	26.06
	Highbush blueberry (Raw)	*Vaccinium corymbosum* L.	11.18
Pelargonidin	Strawberry (Raw)	*Fragaria* L.	4.31
	Common black bean (Whole Raw)	*Phaseolus vulgaris* L.	0.95
Peonidin	Common black bean (Whole Raw)	*Phaseolus vulgaris* L.	1.36
Petuindin	Lowbush Blueberry (Raw)	*Vaccinium augustifolium* Aiton	11.20
	Aestival Grape (Black)	*Vitis aestivalis* Michx.	6.20
	Highbush blueberry (Raw)	*Vaccinium corymbosum* L.	6.09

Historically, anthocyanidins have been studied primarily because of their involvement as phytoprotective agents. Hwever in more recent times, the interest in these compounds is primarily seen from their antioxidant capacity particularly in the prevention of cardiovascular disease (Wallace, 2011, Cassidy et al., 2013, Das et al., 2011, Flamini et al., 2013, Toufektsian et al., 2008), while several other beneficial health outcomes of higher intake were also reported; such as improvement in vision, anti- diabetic/obesity/inflammatory effects as well as chemoprevention and cancer protection (Pojer et al., 2013).

Chalcones

Chalcones (Figure 3) are aromatic ketones and enones that act as precursors of other flavonoids such as Quercetin, and are well known for their anti-cancer properties (Sharma et al., 2013). They are composed of three-carbon α, β-unsaturated carbonyl system and there are several anti-inflammatory, antitumour and antiviral effects of these compounds in the recent literature. Furthermore, naturally occurring chalcones are mostly found in hydroxylated forms (Wang et al., 2005, De Vincenzo et al., 2000) and due to their relatively simple chemical structure and significant pharmacological activities, there are several entities that have been identified and proposed as lead compounds for anti-cancer drug development (Bukhari et al., 2013a, Bukhari et al., 2013b, Kamal et al., 2013, Sharma et al., 2013).

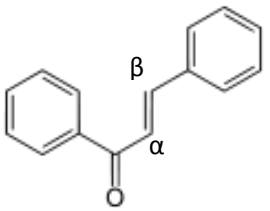

Figure 3. Chemical structure of a Chalcone.

The chalcones are constituents of important groups of natural compounds and their presence is found in fruits (citrus, apples) as well as vegetables (tomatoes, bean sprouts) and various plants and spices (Orlikova et al., 2011). Although there are several different naturally occurring chalcone derivatives, narigenin chalcone that is the most abundant in tomato skin has shown to dose-dependently inhibit the production of TNF-α as well as NO in LPS stimulated macrophages (Hirai et al., 2007), as well as suppression of various inflammatory conditions. As such, they have gained significant interest for the potential treatment of various types of cancer (Yadav et al., 2011).

Flavanols

Flavanols are polyphenolic compounds that have been found in various fruits, vegetables, teas and variety of different pods, beans, herbs and spices (Table 4). Their presence is probably the most ubiquitous of all polyphenols found in plants and studies using these compounds (whether pure or as a part of the plant extract) as well as their derivatives for

various different chronic diseases, tumours and cancers are on the increase (Vuong et al., 2014, Nagao et al., 2007, Abe et al., 2000b, Yang and Xiao, 2013). One of the most representative compounds of this group are the catechins, and these have shown to possess very high antioxidant activity. Furthermore, there is increasing evidence from epidemiological, clinical and experimental studies which suggest that the tea polyphenols have various biological activities, such as anti-fungal (Hirasawa and Takada, 2004), anti-inflammatory (Katiyar and Mukhtar, 2001, Kawai et al., 2004) and antioxidative (Rietveld and Wiseman, 2003) properties. They have also been linked with lower incidence of CVD (Sano et al., 2004), reduced mortality due to cardiovascular disease (Kuriyama et al., 2006) and recent reviews have linked these compounds with prevention and treatment of various types of cancer (Vuong et al., 2014).

Catechins (Figure 4) have been shown to be the most prominent and biologically active compounds in green tea, almost all of which can be extracted with hot water during the tea brewing process (Vuong et al., 2010, Vuong et al., 2011). This group of polyphenols consists of eight catechins and their structures are characterised with multiple hydroxyl groups on two or three benzene rings depending on the compound.

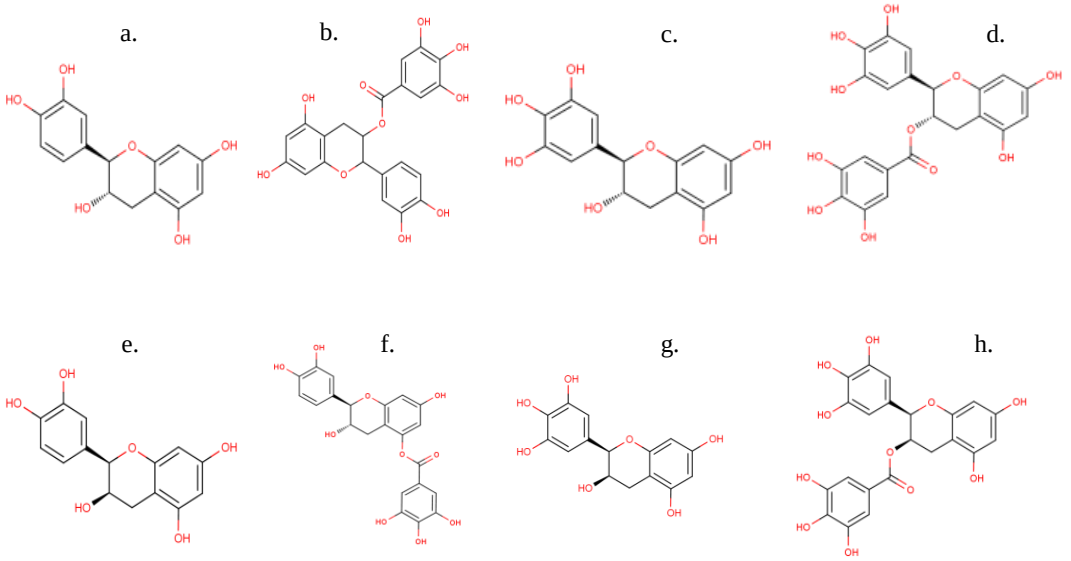

Figure 4. Chemical structure of some of the most predominant catechins found in plants Catechin (a); Catechin 3-O-gallate (b); Gallocatechin (c); Gallocatechin 3-O-gallate (d); Epicatechin (e); Epicatechin 3-O-gallate (f); Epigallocatechin (g); Epigallocatechin 3-O-gallate (h).

Flavanones

Flavanones are a very unique class of compounds whose presence, in contrast to other polyphenols such as favanols that are found in vast ranges of different foods is reserved predominately for citrus fruits (Manach et al., 2003), and some herbs such as Mexican oregano (*Lippia graveolens* Kinth) (Neveu et al., 2010). Orange juice in particular has been reported to predominately contain hesperidin (hesperidin-7-rutinoside) and narirutin

(narinegenin-7-rutinoside) (Figure 5). The hesperidin is represented in about 90% of the total flavanone content of the orange juice with the reminder of flavanones being assigned to narirutin (Coelho et al., 2013). Also, the hesperidin concentrations in orange juice were reported to be anywhere between 20-59mg/100ml while narirutin between 1.6-8.4mg/100ml (Manach et al., 2003). Furthermore, the hesperidin was also identified in dried peppermint (*Mentha x piperita* L. (pro sp.)) with levels around 480mg/100g while narirutin levels were around 127mg/100g (Neveu et al., 2010).

Table 4. Plant sources of catechins (Neveu et al., 2010)

Flavanol	**Source**	**Scientific name**	**Average Content (mg/100g)**
Catechin	Cocoa (powder)	*Theobroma cacao* L.	107.75
	Plum, prune, juice	*Prunus domestica* L.	24.70[a]
	Strawberry (Raw)	*Fragaria* L.	6.36
	Grape Black	*Vitis vinifera L.*	5.46
Catechin 3-O-gallate	Tea, Black, Infusion	*Camellia sinensis* (L.) O. Kuntze	4.74 [a]
	Peppermint tea	*Mentha x piperita L.* (pro sp.)	0.45 [a]
Gallocatechin	Tea, Black, Infusion	*Camellia sinensis* (L.) O. Kuntze	14.01 [a]
	Tea, Green, Infusion	*Camellia sinensis* (L.) O. Kuntze	2.26 [a]
	Broad bean pod, raw	*Vicia faba* L.	9.68
Gallocatechin 3-O-gallate	Tea, Black, Infusion	*Camellia sinensis* (L.) O. Kuntze	4.74 [a]
	Peppermint tea	*Mentha x piperita* L. (pro sp.)	0.45 [a]
Epicatechin	Cocoa (powder)	*Theobroma cacao* L.	158.30
	Blackberry, raw	*Rubus* L.	11.48
	Tea, Green, Infusion	*Camellia sinensis* (L.) O. Kuntze	7.93 [a]
Epicatechin 3-O-gallate	Tea, Green, Infusion	*Camellia sinensis* (L.) O. Kuntze	7.50 [a]
	Peppermint tea	*Mentha x piperita* L. (pro sp.)	9.24 [a]
Epigallocatechin	Tea, Green, Infusion	*Camellia sinensis* (L.) O. Kuntze	19.68 [a]
Epigallocatechin 3-O-gallate	Tea, Green, Infusion	Camellia sinensis (L.) O. Kuntze	27.16 [a]

[a] Represents value in mg/100ml.

One of the relatively recent flavanones identified is Alpinetin (Figure 6) found in Zingibercaceae (*Alpinia katsumada* Hayata) a seeds commonly used in Korean traditional medicine (Hu et al., 2013, Suo et al., 2014) as well as found in plants of the ginger family, such as turmeric and cardamom (Suo et al., 2014). Although previous studies have identified that extracts of this plant contains variety of active ingredients, alpinetin has recently been identified as an inducer of apoptosis of pancreatic cancer cells (Vuong et al., 2014).

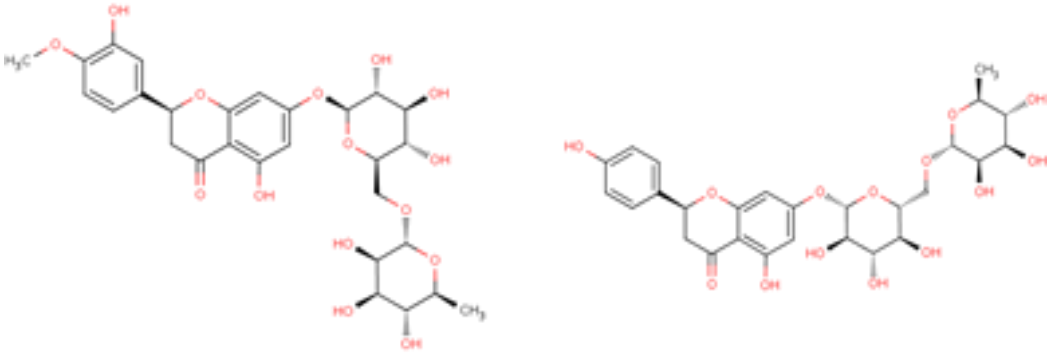

Figure 5. Chemical structures of hesperidin (a) and narirutin (b).

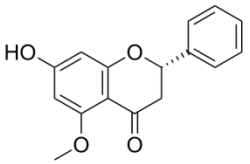

Figure 6. Chemical structure of alpinetin.

Flavones

Flavones are polyphenolic compounds that are commonly found in the skins of the fruits (Vuong et al., 2014) and in some of the common culinary herbs as well as in various vegetables (Neveu et al., 2010). Structurally, flavones are very similar to other polyphenols consisting of 2-phenylchromen-4-one backbone with the number of hydroxyl groups attached to various positions. Two of the most common flavones found in plants are apigenin (Lefort and Blay, 2013) and luteolin (Lopez-Lazaro, 2009) (Figure 7).

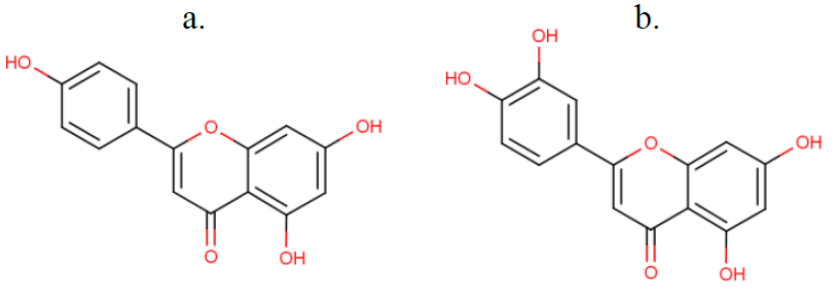

Figure 7. Chemical structures of apigenin (a) and luteolin (b).

Apigenin is one of the most commonly occurring flavones present in abundance in some common fruits and vegetables such as celery, tarragon, fresh sage, marjoram chamomile and wheat sprouts (Neveu et al., 2010, Patel et al., 2007). This flavone has recently gained interest for its beneficial health promoting effects as it has a low intrinsic toxicity and has been

reported that it can exhibit differential effects in normal versus cancer cells when compared to other structurally related flavonoids (Babcook and Gupta, 2012, Shukla and Gupta, 2010, Tong and Pelling, 2013). To date there is a very limited evidence that pure apigenin can promote adverse effects in vivo predominately due to the complexity of the compound itself as it is not very stable in its pure form and it cannot be dissolved in organic or aqueous solutions (Patel et al., 2007). However, the natural sources are commonly found to contain glycosylated apigenin that is more stable and provides better bioavailability. Despite this, apigenin has been used for centuries as traditional or alternative medicine such as use of passion flower in treatment of asthma and Parkinson's disease, as well as being a one of the main constituents of chamomile tea flower (Patel et al., 2007). The concentration ranges for different plant sources of this flavone vary from 50µg/100g (fresh onion) to 4.40 mg/100g (dried marjoram) (Neveu et al., 2010).

Luteolin is another flavone common to fruit and vegetables, most abundant in celery, broccoli, carrots, cabbages, apple skins, sage, oregano, and green and black olives, among others. The concentration ranges for this flavone in some commonly occurring foods ranges from 0.1mg/100g (Pistachios) to 56.33 mg/100g (Oregano) (Neveu et al., 2010). Similar to other flavones and flavonoids, luteolin is commonly found in plants in its glycosylated forms and once consumed, glycoside is hydrolysed to free luteolin during absorption (Lamy et al., 2008, Lin et al., 2008, Seelinger et al., 2008).

Flavonols

Similar to flavanols, flavonols are also one of the most widely distributed polyphenol groups found in nature. Flavonols are predominately present as diverse glycosides with a sugar moiety bound to the C-3 position. They can be found in a variety of different fruits and vegetables such as onions, apples, cider, grapes, wines and tea (Table 5). The most abundant flavonol (and some claim the most studied one) is quercetin while the other two most predominant plant food flavonols include myricetin and kaempferol (Figure 8) (Hollman et al., 1999, Perez-Vizcaino and Duarte, 2010). Flavonols are characterised by 2 benzene rings that are connected by an oxygen containing pyrene ring. These three rings are planar and flavonol molecules are relatively polarised in this structural appearance (Chen et al., 2010).

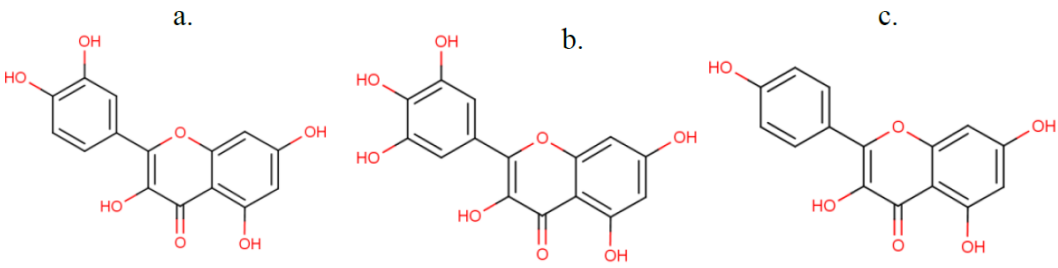

Figure 8. Chemical structures of the three main flavonols found in plant foods: quercetin (a), myricetin (b) and kaempferol (c).

Quercetin-type flavonols (primarily found as quercetin glycosides) are one of the most abundant flavonoid molecules. These compounds are found in variety of different foods such as apples, berries, brassica vegetables, capers, grapes, tomatoes and a variety of different seeds. Quercetin is also found in some of the botanical medicines such as Ginko biloba, *Hypericum perforatum* (St John's wort) and *Sambucus canadensis* (elder) (Kelly, 2011). Due to its presence in a variety of different plant foods commonly eaten by humans, quercetin is the major bioflavonoid that is present in the human diet with levels of up to 25mg per day (Lamson and Brignall, 2000).

Myricetin is a naturally occurring flavonol with the hydroxyl substitutions on 3, 5, 7, 3', 4' and 5' positions (Figure 8). It is most commonly found in plants of berries, fruits and vegetables and it is relatively hard to be found in its "pure" form as like most of the flavonols it is present predominately as glycoside. Interestingly, the myricetin content of berries is proportionally increased as the fruit ripens (Ong and Khoo, 1997). In addition, most medicinal applications of myricetin are predominately due to its antioxidant role.

Kaempferol is yellow in colour and it is most commonly found in many edible plants such as apples, grapes and tomatoes as well as kale and some green tea samples (Kim and Choi, 2013). Furthermore, it is readily present in the plant products commonly used in the traditional medicines such as Ginko biloba, *Tilla spp*. and propolis (Calderon-Montano et al., 2011).

Table 5. Concentrations of three major flavonols (quercetin, myricetin and kaempferol) in selected plant foods (mg/100g) (Bhagwat et al., 2013)

Plant food	Quercetin	Myricetin	Kaempferol
Apples	4.27	0.02	0.00
Apricots	2.08	0.00	0.00
Cranberries	15.09	0.09	6.78
Kale	7.71	26.74	0.00
Onions	21.42	0.62	0.02
Tea	2.74	0.88	0.89

Isoflavones

In contrast to flavonoids, the prevalence of isoflavones in food is narrowly distributed. Isoflavones are diphenolic compounds that are present in plants such as soybean and red clover (Birt et al., 2001). These compounds have structures similar to mammalian estrogens and once consumed can display both estrogenic and non-estrogenic effects (Vitale et al., 2013, Mahmoud et al., 2014). Soybeans contain compounds such as genistein and daidzein (Figure 9) with concentrations reported up to 3mg/g. Although these two isoflanoes are predominately found in soybean, it was also reported that Daidzein is a component in peanuts (0.48mg/100g) and genistein was found in common black beans (0.6 mg/100g) (Neveu et al., 2010). Furthermore, each of these compounds can be found in different forms such as unconjugated, sugar-conjugates (isoflavone glucoside) acetylglucosides and malonylglucosides (Vitale et al., 2013).

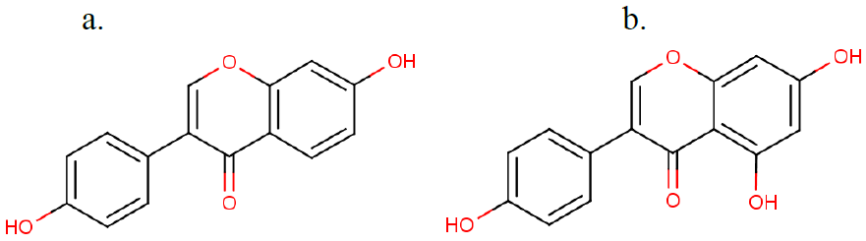

Figure 9. Chemical structures of Daidzein (a) and Genistein (b).

Phenolic Acids

Phenolic acids are polyphenolic compounds that are not of flavonoid origin and can be further divided into two main types; benzoic and cinnamic acid derivatives (Table 6; Figure 10). The fruits and vegetables mainly contain free phenolic acids with the conjugates of these polyphenolic compounds predominately found in grains and the seeds (Tsao, 2010).

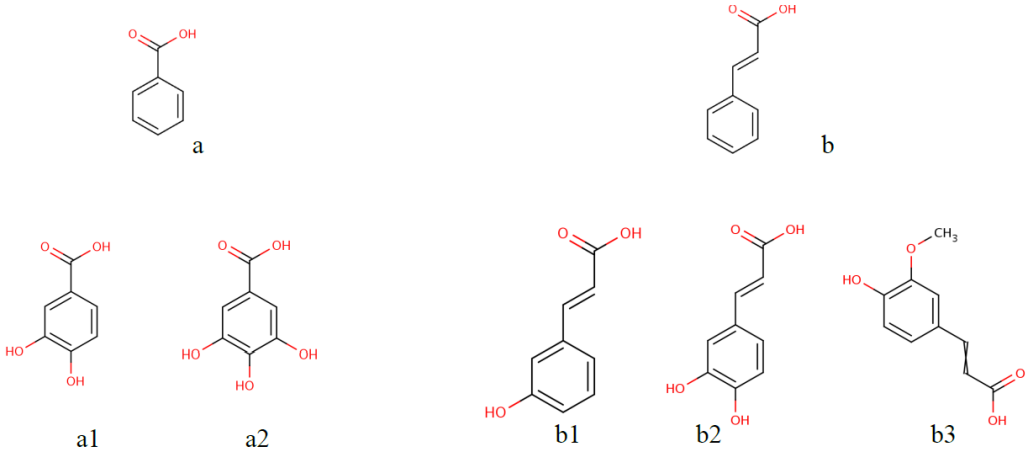

Figure 10. Chemical structures of the two most commonly found polyphenolic acids: benzoic (a) with its derivatives Protocafechuric acid (a1) and Gallic acid (a2); as well as Cinnamic (b) acid with its derivatives Coumeric acid (b1); Caffeic acid (b2) and Ferulic acid (b3).

The hydroxyl derivatives of benzoic acid are found in relatively low quantities in plants that are consumed in the human diet, except in foods such as onions and certain red fruits that have concentrations reported to be of few tens of mg/kg. However, tea is the most important source of Gallic acid where concentrations were reported of up to 4.5g/kg.

The hydroxyl derivatives of Cinnamic acid are reported to be more common than derivatives of benzoic acid consisting of predominately Coumaric, Caffeic and Ferullic acid (Norskov et al., 2013, Ono et al., 2004, Phan et al., 2003, Woodward et al., 2011). These acids are predominately found in the bound glycosylated forms rather than in free forms and caffeic acid in general, is the most abundant phenolic acid that represents between 75 and 95% of the hydroxylcinnamic acids in fruit. Ferullic acid was reported to be the most predominant in grains such as wheat grain (200mg/100g fresh), however this phenolic acid was also found in aubergines (60mg/100g) (Manach et al., 2004).

Table 6. Benzoic and Cinnamic acid (and their derivatives) composition in some of the plant foods (Manach et al., 2004)

	Source	**By weight (mg/100g)**
Hydroxybenzoic acid	*Blackberry*	8 – 27
Protocatechuic acid	*Raspberry*	6 – 10
Gallic acid	*Black Currant*	4 – 13
Hydroxycinnamic Acid	*Bluberry*	200 – 220
Caffeic Acid	*Kiwi*	60 – 100
Chlorogenic Acid	*Cherry*	18 – 115
Coumaric Acid	*Plum*	14 – 115
Ferulic Acid	*Aubergine*	60 - 66

Stilbenes

Stilbenes are relatively small molecular weight polyphenolic compounds (200-300g/mol) sharing structures very similar to estrogen and that naturally occur in a wide range of plant food sources (Roupe et al., 2006). Although these are well distributed in plant food sources, they are found in only small quantities in the human diet (Manach et al., 2004). Despite these relatively small quantities, one of the most predominant and the most widely published stilbenes is resveratrol with over 5000 references in the MEDLINE database.

Resveratrol

Resveratrol (Figure 11) is a natural phytolaexin 3,4',5-trihydroxystilbene that is abundantly present in a small number of plant species (Stewart et al., 2003). It was firstly isolated and identified in 1940 from the dried root of white hellebore plant but there was little interest in this compound until the early 1990's (Raederstorff et al., 2013, Renaud and de Lorgeril, 1992) (Langcake and Pryce, 1977).

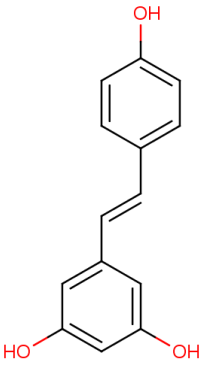

Figure 11. Chemical structure of resveratrol.

Resveratrol is found in the seeds and skin of grapes and due to this site specific plant occurrence, it is commonly found in greater quantities in red wines. Although the resveratrol is found in white (0.01mg/100ml) and rose (0.04mg/100ml) wines, the levels are significantly smaller than when compared to red (0.19 mg/100ml) wines (Guerrero et al., 2009, Pedroza et al., 2013, Fernandez-Mar et al., 2012). Furthermore, the composition of resveratrol in wine varies based on a variety of different factors, such as geographical region, grape variety and climatic factors that the grapes are exposed to (Gambuti et al., 2007).

Apart from being found in wine and grapes, resveratrol was also detected in raw strawberries (0.35mg/100g), in redcurrants (1.57 mg/100g) (Ehala et al., 2005) as well as peanuts (0.014µg/g) (Sanders et al., 2000).

Carotenoids

The term *carotenoids* refers to the class of natural, fat-soluble pigments with colours ranging from light yellow to dark red (Hernandez-Marin et al., 2013, Abdel-Aal el et al., 2013, Waramboi et al., 2013, Pugliese et al., 2013, Gallon et al., 2013, Pan et al., 2013, Manthorpe and Lockley, 2013, Kilcrease et al., 2013, Iqbal et al., 2013, Alavizadeh and Hosseinzadeh, 2013). The carotenoids are synthesized by nearly all photosynthetic bacteria (Wang et al., 2012, Takaichi et al., 2012, Mlalazi et al., 2012, Banares-Espana et al., 2013), cyanobacteria (Leema et al., 2010, Wu et al., 2012, Chen et al., 2012, Domonkos et al., 2013), algae (Domonkos et al., 2013, Cui et al., 2013) and higher plants including fruits and vegetables (Teixeira et al., 2013, Takemura et al., 2013, Pons et al., 2013, Fernandez-Orozco et al., 2013). To date more than 600 different types of carotenoids have been identified in nature and although the number of carotenoids is relatively large, only about 10% of the identified carotenoids are metabolised and utilised by humans (Hammond and Renzi, 2013). Essentially, humans and animals are not able to synthesize carotenoids *de novo* and the source of carotenoids is strictly related to dietary intake (Lugtenburg and Dawadi, 2012).

Carotenoids can be classified based on their structure, cyclization, structural alterations and biological function (Figure 12) (Namitha and Negi, 2010). Structurally, carotenoids are a class of hydrocarbons consisting of eight isoprenoid units (tetraterpenoids) with the central carbon chain as a unique structural feature consisting of alternating single and double bonds. This 40-carbon polyene chain can be bound to the cyclic or linear (non-cyclic) end groups. Symmetry is maintained by the two central methyl groups in a 1,6-positional relationship as well as non-terminal methyl groups that begin the 1,5 positional relationship (Namitha and Negi, 2010). Furthermore, structural division of carotenoids can be assigned as containing carbon and hydrogen (α-carotene, β-carotene, β-cryptoxanthin) and oxygenated derivatives of these compounds, commonly referred to as xanthophylls.

One of the most important and widely used classification of carotenoids in health sciences is based on their biological function, which is essentially dependant on their structural function (Namitha and Negi, 2010). The most commonly referred benefits of the carotenoids in biological systems are their ability to act as very strong antioxidants (Sindhu et al., 2013, Meinke et al., 2013, George et al., 2013, Xue et al., 2013, Otsuka et al., 2013, Holzapfel et al., 2013). This property can be ascribed to the alternating pattern of the single and double bonds in the main backbone and to the nature of the end groups of the polyene chain (Namitha and Negi, 2010).

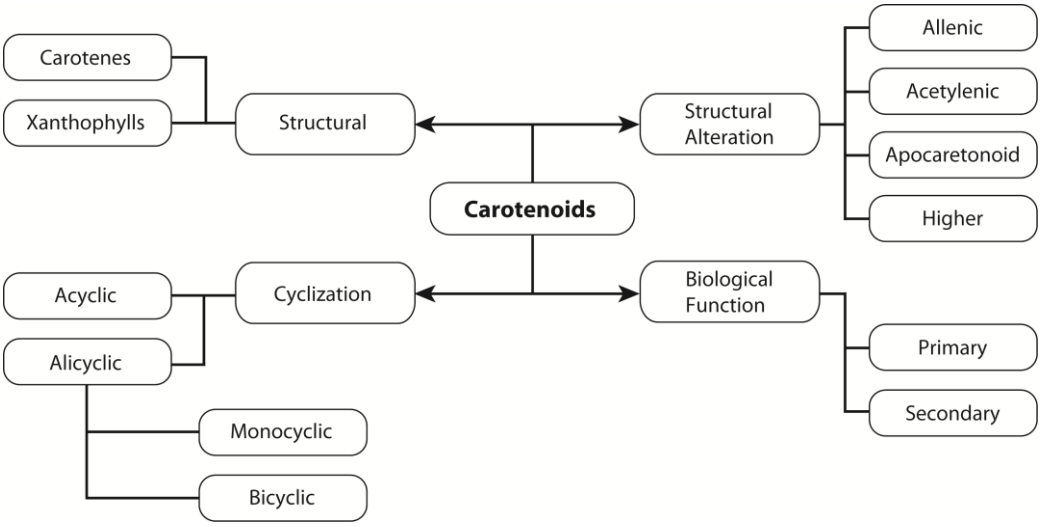

Figure 12. Classification of carotenoids based on their structure, cyclization, structural alteration and biological function (Skibsted, 2012, Kaiser et al., 2012, Namitha and Negi, 2010).

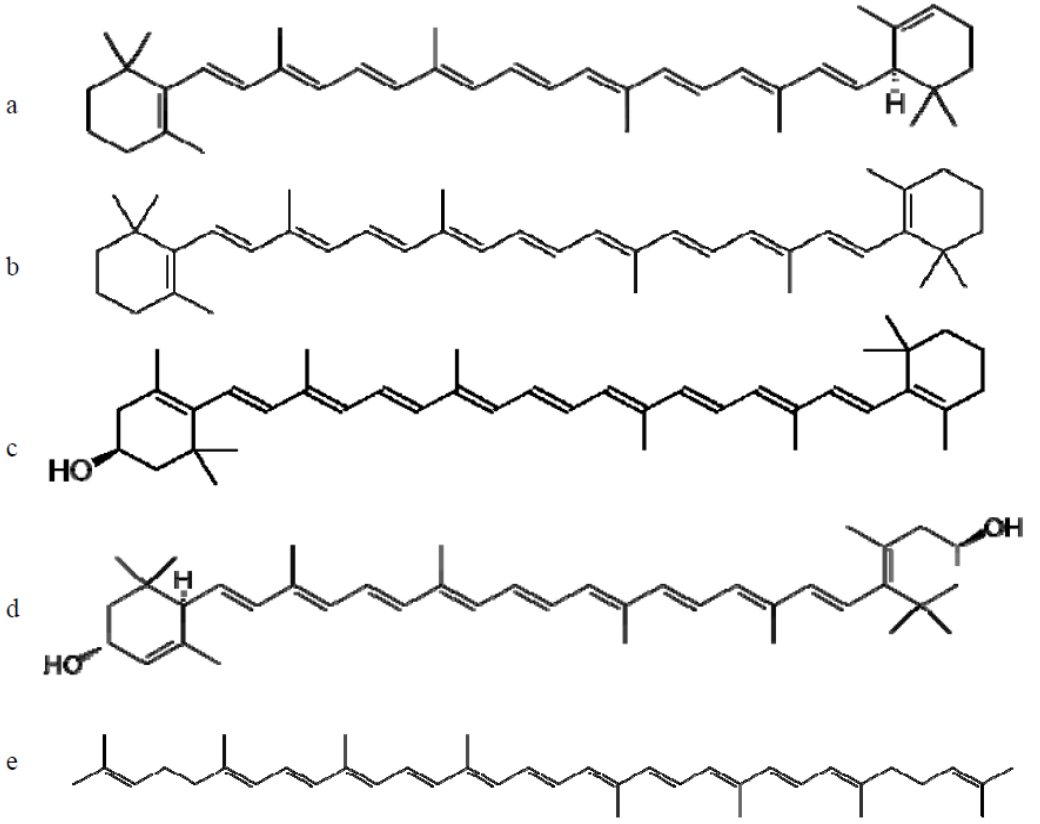

Figure 13. Chemical structures of commonly found plant carotenoids a. α-carotene; b. β-carotene; c. β-cryptoxhantin, d. Lutein, e. Lycopene.

The five most distributed carotenoids in nature are α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene (Figure 13) and their appearance in a variety of different fruits, vegetables and germ crops and with foods that are deeply pigmented, presents one of the main plant bioactive constituents. The most studies carotenoid is β-carotene and it is also one of the major carotenoids in the human diet (Hammond and Renzi, 2013, Sies et al., 1992, Wang and Wang, 2011).

In some food databases the concentrations of lutein and zeaxanthin are commonly added as a sum of both values, due to two reasons; firstly, the concentrations of these two bioactives are very low in foods and secondly, depending on the method of extraction and analysis it is relatively hard to determine the identity of these two carotenoids (United et al., 2014).

Table 7. Carotenoid content of some of the plant foods expressed as mg/100g

	α-Carotene	**β-Carotene**	**β-Cryptoxanthin**	**Lycopene**	**Lutein or Zeaxanthin**
Carrots	3.477	8.285	-	0.001	0.256
Kale	0.054	5.927	0.081	-	8.198
Onions	-	0.001	-	-	0.004
Beetroot (Beets)	-	0.002	-	-	-
Mango	0.009	0.64	0.01	0.003	0.023
Brussels Sprouts	0.006	0.45	-	-	1.59
Grapefruit	0.003	0.686	0.006	1.419	0.005
Papayas	0.002	0.274	0.589	1.828	0.089
Grapes	0.001	0.039	-	-	0.064
Pears	0.001	0.039	-	-	0.064
Tangerines	0.101	0.155	0.407	-	0.138
Peas	0.021	0.449	-	-	2.477
Rocket lettuce (Arugula)	-	1.424	-	-	3.555
Spinach	-	5.626	-	-	12.198
Kiwifruit	-	0.052	-	-	0.122

Phytosterols

Phytosterols is the term commonly used for the description of bioactive non-nutrient substances with structurally similar configurations to cholesterol. These compounds are commonly divided in two forms; (i) unsaturated, that are present in many plants, and (ii) saturated, also referred to as stanols that are found only in small amounts in cereals and some fruits and vegetables (Orzechowski et al., 2002). There are over 250 different phytosterols identified in various plant and marine materials and most frequently occurring phytosterols belong to the 4-desmethyl sterols, namely β-sitosterol (the most abundant), campesterol and stigmasterol (Brufau et al., 2008). From the structural (Figure 14) and biosynthetic

perspective, although these compounds appear similar to cholesterol, and belong to the family of triterpenes with tetracyclic ring and a side chain linked to carbon 17, β-sitosterol and campesterol have ethyl and methyl groups at C-24 respectively and stigmasterol is identical to β-sitosterol however it has an extra double bond at C-22 (Brufau et al., 2008, Marangoni and Poli, 2010, Ostlund, 2002).

The main phytosterol plant food sources include vegetable oils, nuts, grains, as well as sprouts, cabbages, cauliflowers and green and black olives (Marangoni and Poli, 2010). Although vegetable foods contain significant amounts of individual phytosterols, the current food databases do not have comprehensive estimates of phytosterol content (Ostlund, 2002).

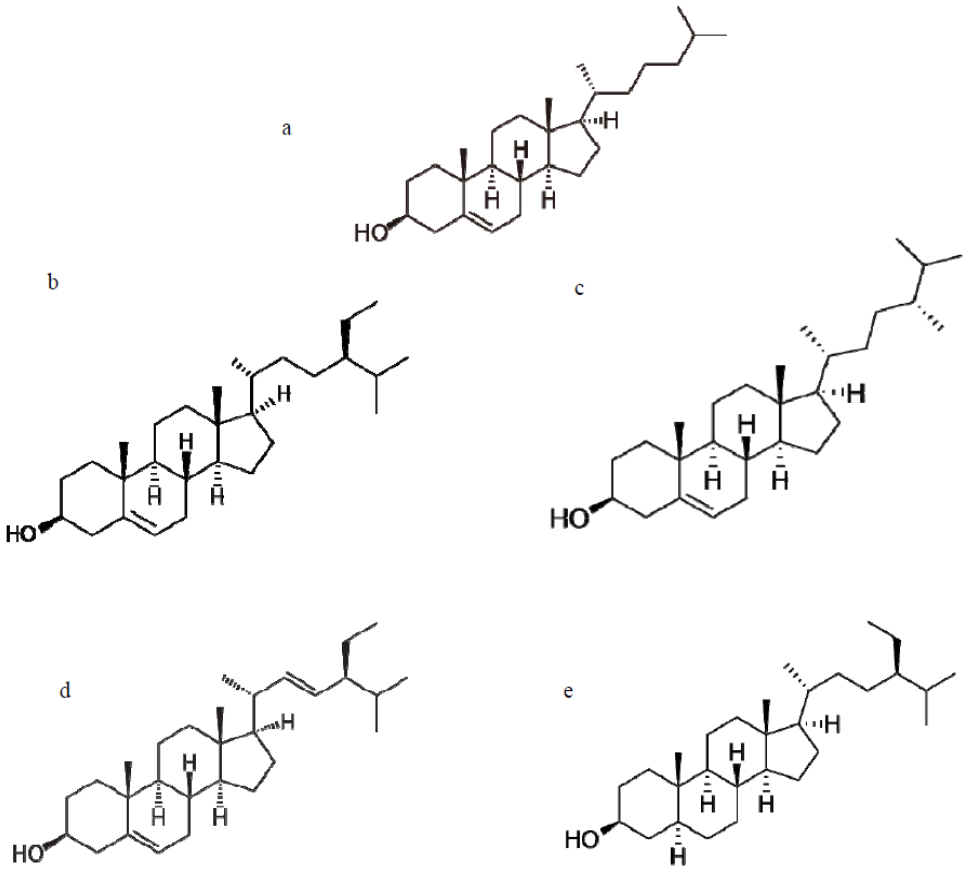

Figure 14. Chemical structures of cholesterol (a) and some of the commonly found plant foods phytosterols, β-sitosterol (b), campesterol (c), stigmasterol (d) and sitostanol (e).

CONCLUSION

There are numerous examples from epidemiological studies, as well as from randomised clinical trials that indicate the beneficial health effects of diets rich in plant foods. Although these effects were initially ascribed to the micronutrient component of the plants, the emergence of plant bioactives and their health benefits is on a continuous rise. In the last

decade, there was an absolute explosion of research in identifying new bioactive compounds as well as their metabolites and conjugates. This is also fuelled with the development of new and more sensitive laboratory techniques and improvements in the laboratory instrumentation resulting in new food groups being identified to contain some of the bioactive components that have not been previously determined. All of the plant bioactive components have displayed various protective effects against some chronic diseases, as well as providing a path for the development of new pharmaceuticals that can assist and treat illnesses, including a variety of different cancer types. On numerous occasions these bioactive compounds have also provided a "backbone" structure for the synthesis of new pharmaceuticals and the amalgamation of nutrition and pharmaceutical science is evident not only in drug development but also in the development of functional food products that contain one or more of plant bioactives.

Table 8. Phytosterol content of some plant foods and plant food products expressed as mg/100g (United et al., 2014)

	Phytosterols	**β-sitosterol**	**Stigmasterol**	**Campesterol**
Rice bran oil	1190	-	-	-
Seasame oil	865	-	-	-
What Germ oil	553	-	-	-
Safflower oil	444	-	-	-
Poppyseed oil	276	-	-	-
Apricot Kernel oil	266	-	-	-
Almond oil	266	-	-	-
Olive oil	221	-	-	-
Peanut oil	207	-	-	-
Grapeseed oil	180	-	-	-
Walnut oil	176	-	-	-
Pistachio nuts	214	198	5	10
Pecan Nuts	85	78	2	4
Lentils	57	47	4	6
Fava Beans	22	18	1	3
Grape Leaves	21	20	2	-

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 6

EXTRACTION AND ISOLATION OF PLANT BIOACTIVES

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ABSTRACT

Natural bioactive compounds derived from plant materials play an important role in human health. It is desirable to find the most suitable methods for extracting and isolating those compounds from plant matrices. Techniques for extraction and isolation of bioactive compounds have been developed over the years. This chapter presents a general overview of the techniques involved in extraction and isolation of bioactive compounds from plants. Particularly, traditional extraction methods (steam distillation and solid-liquid extraction), novel extraction techniques (microwave assisted extraction, ultrasound assisted extraction, supercritical fluid extraction, and pressurised fluid extraction) and isolation techniques (ion-exchange and high performance liquid chromatography) are reviewed. Comparisons of different extraction techniques, various applications and recommendations with specific examples for each technique are also discussed. Furthermore, the different evaluation techniques *in vitro* and *in vivo* for bioactivities and antioxidant activities of crude extracts or isolated/purified compounds are also presented.

1. INTRODUCTION

Plant bioactive compounds linked with the treatment and prevention of human diseases are currently under investigation in many laboratories and industries. Valuable natural compound extracts from plants are widely used as drugs, functional food ingredients or

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nutraceuticals due to consumer growing interest in health food products. Accordingly, the extraction and isolation of the extracts are an area of importance to academia and industry.

The conventional extraction methods by steam distillation and solid-liquid extraction are considered as either time consuming or polluting, due to potential environmental and health concerns. They have recently been challenged by novel extraction techniques, namely microwave assisted extraction, ultrasound assisted extraction, supercritical fluid extraction, and pressurised liquid extraction. This chapter provides theoretical background on the conventional and novel extraction techniques. Some applications for the extraction of bioactive compounds from plant matrices and their comparisons are also discussed. Furthermore, isolation techniques such as ion-exchange and high performance liquid chromatography are also presented.

2. EXTRACTION TECHNIQUES OF PLANT BIOACTIVE COMPOUNDS

2.1. Sample Pre-Treatment before Extraction

Figure 1 shows the general steps for extracting and isolating bioactive compounds from plant material. Pre-treatment of the plant material is desirable to ensure optimal contact between the solvent and the plant matrix during the extraction process. The plant characteristics of composition, structure, moisture content, particle size and solvent-to-solute ratio are considered important parameters. Depending on the extraction method chosen and characteristics of plant material, the pre-treatment may differ.

Generally, the pre-treatment comprises three main steps being drying, grinding and sieving. Water from raw material can be removed using different drying methods, such as air, vacuum, freeze or microwave drying. Depending on the desirable quality of the extract, a suitable drying method can be chosen. When the conventional and cheap air drying method is applied, the bioactive compounds, which are sensitive to high temperature and oxygen in the air, can easily degrade. Alternatively, the vacuum, freeze and microwave drying methods can be used. Vacuum drying lessens the effect of heat and oxygen on the product. Freeze drying can be used for the samples which are very sensitive to heat and oxygen. However, it takes a lot of time to freeze dry and it is expensive due to high capital and energy costs related to the operation of the refrigeration and vacuum systems. Therefore, microwave drying can be applied as an alternative due to the decrease in the drying time of the sample without, or with insignificant nutrient loss (Wojdyło et al., 2014). Furthermore, microwave drying under vacuum, called the vacuum-microwave drying technique, has been successfully applied for many natural plant materials (Figiel, 2009). As an example, Popovich et al. (2005) reported that freeze drying and vacuum-microwave drying techniques improved both extraction efficiency and actual retention of individual ginsenosides in North American ginseng (*Panax quinquefolius*). Drying pre-treatments can have significant effects on yield and quality of bioactives in subsequent extraction (Kha et al., 2014a). In all cases, other than the quality of the pre-treated material, the availability of equipment should be also considered.

The grinding step is conducted to reduce the material size, increasing the surface area for easier extraction, while the sieving step ensures the particles of the material are similar in size and therefore improves homogeneity of the particles before extraction. Depending on the

extraction methods applied, the particle size plays an important role, which will be discussed in the next section.

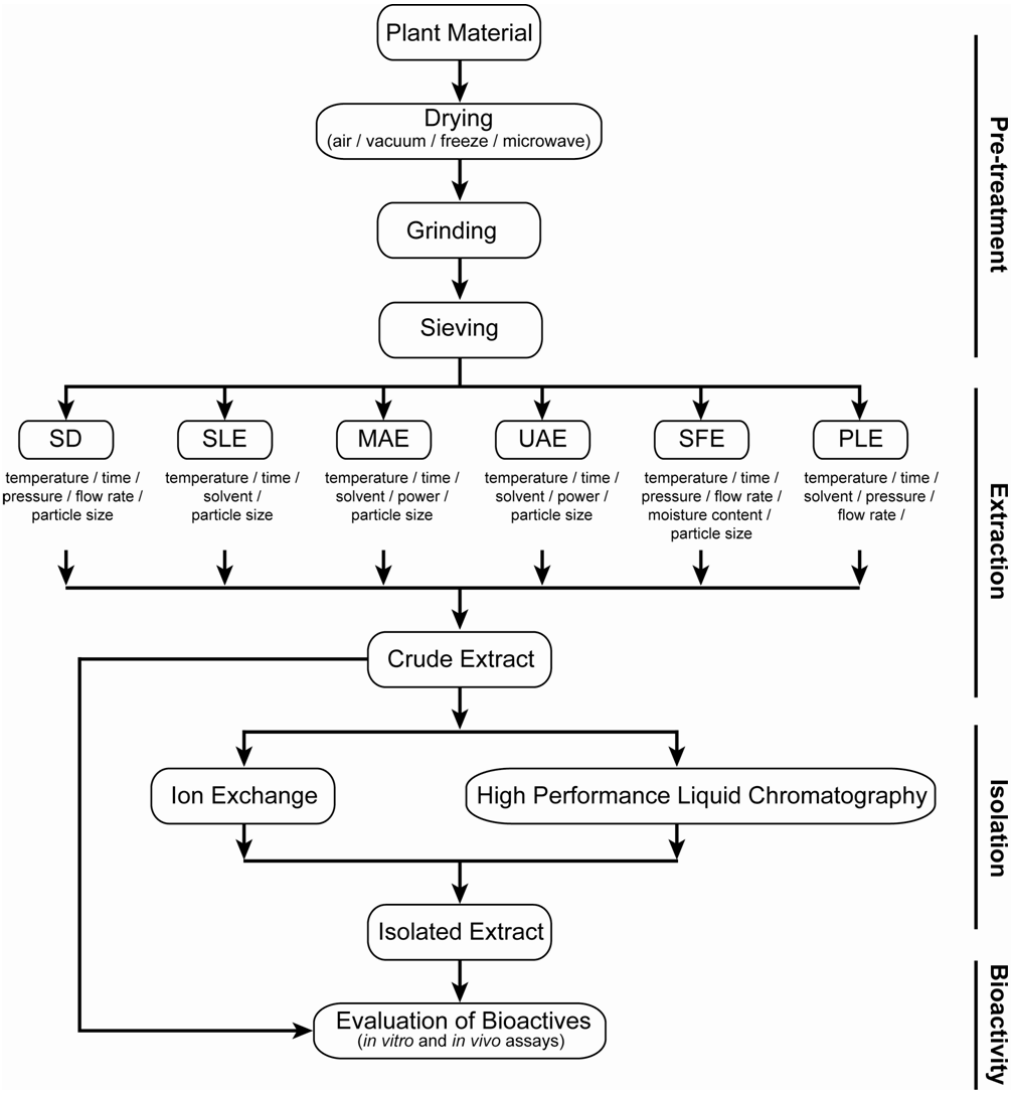

Figure 1. General steps for extracting, isolating and evaluating bioactive compounds from plant material (SD: steam distillation; SLE: solid-liquid extraction; MAE: microwave assisted extraction; UAE: ultrasound assisted extraction; SFE: supercritical fluid extraction; PLE: pressurised liquid extraction).

2.2. Steam Distillation (SD)

2.2.1. Extraction Mechanisms

Depending on the type of steam and raw material, the steam distillation process can be divided into three categories; direct steam distillation (steam distillation), water distillation (hydro-distillation) and dry steam distillation.

In direct steam distillation, the raw material is placed onto a screen or perforated grid attached above the bottom of a still. The material is not in direct contact with water. The water is boiled inside or outside the still. The saturated steam having low pressure flows up through the material and the bioactive components evaporated in the steam are condensed and collected. In contrast, water distillation is a process in which the material comes in direct contact with the boiling water. The material is completely immersed or is floating on the boiling water. It is necessary to stir or mix during the process to prevent agglutination of the material, which can form large compact lumps. Dry steam distillation is similar to the direct steam distillation, in that the raw material is inserted into the still and steam flows through it. However, the steam is generated outside the still in the superheated (dry) state at moderate pressure.

In general, the mechanism of the steam distillation process takes place in three main subsequent steps: (1) an increase in temperature is promoted by the boiler, the volatile compounds are released from inside the material matrix to its outer surface; (2) the compounds are vaporised and diffused from the material surface into the steam (mass transfer); (3) the vapour compounds, being carried along by the steam, are condensed and collected.

2.2.2. Applications of SD for Plant Extraction

Steam distillation has been widely applied to extract essential oils (consisting of volatile and hydrophobic compounds) from aromatic, condimentary and medicinal plants. The essential oils are usually distilled from the whole plant or the individual parts such as flower, seeds, root and peel. Generally, individual parts of the plant material are chosen for the steam distillation in the laboratory, whereas the whole plant material is used in industry. Compared with an industrial scale, results for the chemical composition of the essential oils obtained under laboratory conditions are more reproducible and exhaustive. In industry, the steam distillation process is carried out only until the desired chemical composition of the essential oil is reached, as it may not be economic to continue processing for the small amount of residual oils.

Prior to steam distillation, pre-treatment of the plant materials (seeds and fruits) by grinding is necessary. This step allows the breaking down of the cells of the plants in which the essential oils are located. As a result, the steam easily contacts the oil, resulting in an increase in the vaporisation rate. Other parts of the plant materials such as roots and stems should be cut in small pieces to expose a greater number of oil containing cells. However, flowers and leaves may be directly distilled without grinding if their structure is sufficiently permeable.

Typical studies reported that distillation conditions including operating pressure, distillation time, steam flow rate and particle size significantly affect the distillation yield and quality of essential oils from different plant materials (Rouatbi et al., 2007, Manzan et al., 2003, Hanci et al., 2003). Results from those studies indicated that different operating conditions resulted in different yields and quality of the oil extract. This is because different plant materials and different distillation systems were used. Therefore, it is important to note that optimisation of those conditions could enhance the yield and quality during the distillation process of a specific plant material.

2.3. Solid-Liquid Extraction (SLE)

2.3.1. Extraction Mechanisms

Depending on the purpose of the process, solid-liquid extraction has different names, such as lixiviation, washing, percolation, leaching or solvent extraction. The main mechanism of the solid-liquid extraction is the liquid phase diffusion inside the solid (mass transfer mechanism) during a separation process. In foods, this process involves the mass transfer of more than one chemical component, which is known as the solute (also called the extract) separated from a solid to a liquid phase (solvent). Solvents such as water, ethanol and hexane are commonly used in the extraction of food components, but the use of water as a natural solvent or a chemical free solvent is becoming preferable in the food extraction industry.

During extraction, the movement of a soluble substance through the solid matrix using a specific solvent occurs. The solvent is transferred onto the surface of the solid matrix and penetrates inside by diffusion. After that, the solute is dissolved into the solvent until a constant concentration is reached. The movement of the soluble substance is due to the different concentration gradient at the solid-liquid interface. The substance will be then separated by different means such as filtration, evaporation and centrifugation.

In order to obtain the highest content of the target compounds and lowest content of unwanted compounds, it is important to note that there are many factors affecting the extraction rate. They include the choice of solvent, food material property and its preparation, and extraction temperature.

- The choice of the extraction solvent is generally based on the physical properties of the solvent, cost and toxicity. The important characteristics in the proper selection of the solvent are high selectivity, capability of only dissolving the wanted compounds, high stability, low viscosity, low toxicity and inflammatory properties and low cost.
- Food material properties and their preparation play an important role in the solid-liquid extraction. In most cases, the bioactive compounds are located inside intracellular space, capillaries or cell structures. Therefore, it is desirable to grind the raw material before the extraction in order to increase the contact area between the solid matrix and the solvent due to a breakdown of the cell structures (Figure 1). It is also important to note that the preservation of the bioactive compounds during the preparation steps needs to be considered.
- In general, higher extraction temperature enhances higher diffusion rate and higher solubility of the bioactive compound. However, thermolabile compounds can be degraded at elevated temperatures.

2.3.2. Applications of SLE for Plant Extractions

In the food industry, the solid-liquid extraction process can be performed in batch, semi-batch or continuous batch modes. The type of the equipment chosen will depend on the material, the bioactive compounds required and the cost. One or multiple stages applied in batch operations, with a new solvent in each stage, can be carried out in the extraction process. A stage refers to an operation in which the solid and liquid phases are in contact for a specific time until the equilibrium is reached. After that a mechanical separation is applied to separate the two phases. However, it is difficult to reach the equilibrium in one stage.

Therefore, it is necessary to determine the actual stages by calculating the extraction efficiency during the solid-liquid extraction.

This extraction has been extensively used in the food industry to extract many important food substances such as lipids, sucrose, proteins, and functional compounds, among others. For instance, vegetable oils can be extracted from different plant materials such as olives, peanuts, sunflower, palm, rape, canola, sesame, and soy, using the solid-liquid extraction. The type of solvent used in the extraction is an important factor affecting the extraction efficiency. The most used solvents are hexane, heptane, cyclohexane and acetone, of which commercial hexane is the most common solvent used in the solvent extraction for vegetable oil. Although concern of organic solvent safety is not discussed in this chapter, management and control of solvent emission during the extraction is important to protect workers and the environment. It should be also noted that many solvents, which are toxic for humans and dangerous for the environment must be separated from the extract, particularly when it is applied into foods (Joana Gil-Chávez et al., 2013). Because of consumer concern about potential residual solvent in the extract, hydraulic pressing (expression) for top quality virgin oils is increasing in demand and SLE is usually used only on the leftover from that process.

Water-soluble compounds are also extracted using this extraction process. For example, sucrose extracted from beets is extracted using hot water as the solvent. Beets are cut into thin slices, placed in an extractor bed and then immersed in the water under pressure. After an appropriate time, the extract is filtrated and then concentrated in an evaporator into a syrup to be crystallised. The sugar crystals are separated by centrifugation. Another example is the use of hot water to extract coffee and tea. This extraction is an important stage in the production of instant coffee and tea products. Temperature is the most important factor that influences the quality of the extract. For coffee, high temperature of 100^{o}C is preferred, because insoluble carbohydrates become more soluble, resulting in an increase in the soluble solid content in the final extract. However, undesirable aromas and flavours may be also extracted at higher temperature due to hydrolysis of substances. For tea, the extraction process can be performed in fixed-bed extractors. The water temperature of 70^{o}C in the first stage and 90^{o}C in the last stage are preferred. Furthermore, to facilitate the extraction process, vacuum is often applied to the extractor.

2.4. Microwave Assisted Extraction (MAE)

2.4.1. Extraction Mechanisms

Microwaves are non-ionising electromagnetic waves within the frequency band of 300 MHz to 300 GHz in the electromagnetic spectrum, corresponding to wavelengths between 1 mm to 1 m. Generally, microwave applications are performed at 915 MHz (United States), 896 MHz (United Kingdom) and 2450 MHz (worldwide) for all practical purposes. According to Singh and Heldman (2001), as the velocity of light is 3×10^{8} m/s, the microwave wavelengths at 915 MHz and 2450 MHz are calculated as 0.328 and 0.122 m, respectively. Therefore, the frequency of 915 MHz is considered as most useful for industrial applications with its greater penetration depth, whereas domestic microwave ovens use the frequency of 2450 MHz.

When microwaves penetrate deeply into a material, the absorption of microwaves by the dielectric component of this material results in release of their thermal energy to it. The two

main mechanisms of heating using microwaves are ionic polarisation and dipole rotation (Kaufmann and Christen, 2002). Food materials contain water molecules, which are the most common, and generally have a random orientation. When an electric field is applied, the molecules orient themselves according to the polarity of this field. As the electric field is alternated rapidly, the polar molecules rotate to follow the applied field. This leads to friction with the surrounding medium, resulting in heat being generated. For ionic polarisation, this happens when ions in food solutions move due to their inherent charges as the electric field is applied. Kinetic energy of the moving ions is converted into heat by the resulting collisions between ions; there is a rapid increase in temperature as a consequence (Kadam et al., 2013, Zhang et al., 2011).

Recently, the development of microwave-assisted extraction has been applied to heating the moisture inside a plant cell using microwave radiation, thus evaporation and high pressure on the cell wall are generated. The physical properties of the biological tissues (cell membrane and organelles) are modified by the generated pressure inside the material. The cell membrane is ruptured, the bioactive compounds from the ruptured cells are released into the medium. The penetration of extraction solvent through the porous biological matrix is facilitated. Therefore, the extraction yield of desired compounds could be increased. In addition, the use of this extraction also offers several benefits such as less organic solvent use, reduced processing time and uniform heating (Azmir et al., 2013, Uquiche et al., 2008). These advantages have recently led to microwave-assisted extraction to be considered as one of the most novel techniques for the extraction of bioactive substances from different plant materials.

2.4.2. Applications of MAE for Plant Extraction

As presented above, the microwave-assisted extraction of bioactive compounds from plant materials shows more advantages and is considered as a potential alternative to conventional solid-liquid extraction. For example, MAE could significantly shorten the extraction time of saponins from ginseng, from 12 h using conventional solvent extraction methods to a few seconds (Kwon et al., 2003). Importantly, the extraction yield, the quality and the nature of ginsenosides (tri-terpene saponins) extracted were significantly improved. Similarly, the extraction yield of ginsenosides Rg1 (0.28%) and Rb1 (1.31%), and the extraction time (15 min) from ginseng using MAE was better than that from the conventional solvent extraction (0.22%, 0.87% and 10 h, respectively) (Shu et al., 2003). Furthermore, shorter time, higher yield and better chemical composition of essential oils from different plant materials such as *Eucalyptus citriodora* (Gupta et al., 2013), Walnut (*Juglans regia* L.) leaves (Boukhari et al., 2013) and Dwarfed *Cinnamomum camphora* var. *Linaolifera fujita* twigs (Wei et al., 2013) were also obtained using microwave assisted hydrodistillation, compared to the conventional hydrodistillation. MAE was also employed to extract other bioactive compounds such as polyphenols and caffeine from green tea leaves, and carotenoid rich oil from Gac fruit aril. Results showed that the higher extraction yields for a shorter time was obtained compared to the conventional extraction methods (Nkhili et al., 2009, Pan et al., 2003, Kha et al., 2013). Therefore, it can be concluded that the shorter extraction time, the higher extraction yield and the better quality of the bioactive compounds from plant materials can be achieved using MAE.

Many studies have confirmed that the extraction yield and quality of bioactive compounds are affected by MAE conditions including microwave power, extraction

temperature, extraction time, type of solvent, type of material and particle size (Zhang et al., 2011, Azmir et al., 2013, Routray and Orsat, 2012) (Figure 1). It is strongly recommended that preliminary experiments for selecting the most suitable extraction conditions need to be carried out to obtain higher extraction efficiencies. Further investigation on the chosen operating conditions should be also performed to optimise the MAE process.

2.5. Ultrasound Assisted Extraction (UAE)

2.5.1. Extraction Mechanisms

Ultrasound technology utilises mechanical waves at frequencies above human hearing (16 Hz to 16-20 kHz). The applications of ultrasound in the food industry can be divided basically into two different categories; low-intensity and high-intensity ultrasound. The power levels used in low-intensity ultrasound are very small, typically less than 1 W cm^{-2}. This ultrasonic wave is most commonly applied in the food analysis due to no physical and chemical alterations in the physicochemical properties of the materials, such as firmness, sugar content and acidity (McClements, 1995, Soria and Villamiel, 2010). In contrast, the high-intensity ultrasound uses higher power levels, typically from 10 to 1000 W cm^{-2}. The ultrasound power can alter the properties of food physically and chemically. During the past years the high-intensity ultrasound, which is applied at higher frequencies up to 2.5 MHz, has attracted attention due to strong physical disruption of tissues for extraction purposes.

The main principle of the ultrasound technique used in the extraction is the propagation of ultrasound pressure waves, resulting in cavitation. In liquid systems, longitudinal waves are formed via a series of compression and rarefaction waves of elastic materials (Soria and Villamiel, 2010, Knorr et al., 2011). A sufficiently high intensity results in local pressure waves below the vapour pressure of the liquid, generating a constant growth of gas bubbles being distributed throughout the liquid. These bubbles will reach a critical size, then become unstable and violently collapse (cavitation). High temperature of 5000 ^{o}K and high pressure up to 100 atm are momentarily generated when the bubbles collapse, thus producing very high shear energy waves and turbulence in the cavitation zone (Patist and Bates, 2008). Therefore, it can be seen that the combination of heat, pressure and turbulence are responsible for a variety of effects of high-intensity ultrasound in the extraction. For instance, the release of extractable compounds is facilitated due to the fast changes in pressure and temperature (cavitation phenomena), which cause shear disruption of cell membranes of the plant matrix. They also enhance the mass transfer by disrupting the plant cell walls (Chemat et al., 2011) and hence extraction rate.

In addition to the extraction mechanism, it is also desirable to understand the process parameters, which affect extraction rate, to achieve the maximum yields. There are a number of parameters influencing the extent of cavitation phenomena in ultrasound-assisted extraction, including temperature, pressure, frequency and the medium viscosity. For example, more rapid formation of cavitation bubbles occurs at higher temperatures due to increasing vapour pressure and reducing tensile strength (Earnshaw, 1998). At lower frequencies (e.g. 20 kHz), in addition, larger bubbles are formed when higher energies accumulated are produced due to the implosion of cavitation bubbles. However, at higher frequencies (above 2.5 MHz), bubbles are not formed and cavitation does not occur. The medium viscosity is another important parameter determining the extent of cavitation.

Cavitation bubbles form less easily in a highly viscous environment and decrease the effectiveness (Earnshaw, 1998, Patist and Bates, 2008). This is overcome by increasing temperature, which results in reducing viscosity, a more violent collapse occurs. Moreover, surface tension, nature and concentration of dissolved gas and presence of solid particles also affect the effectiveness of cavitation (Soria and Villamiel, 2010).

2.5.2. Applications of UAE for Plant Extraction

UAE is also known as a novel technique for extraction of bioactive compounds from plants. Reducing extraction time, increasing yield and producing high quality of the plant extracts are the main benefits of this method. In order to achieve an efficient and effective ultrasound-assisted extraction, it is of interest to understand the influence of the extraction conditions and the plant characteristics.

For the conditions of ultrasound-assisted extraction (Figure 1), proper choice of the solvent is the key to successful extraction. The important criterion is the extent of ultrasound cavitation in the solvent. For instance, the physical properties such as surface tension, viscosity and vapour pressure of the solvent affect the intensity of cavitation in a liquid medium. In addition, the solubility of the analytes of interest and the interactions between the solvent and plant matrix should also be considered. Other important factors including ultrasound power, temperature and extraction time also need to be taken into account. It is necessary to control the extraction temperature during UAE because ultrasound generates heat. As a result, it is also important to minimise the sonication time to avoid degradation of bioactive compounds.

Various target bioactive compounds from plant materials including carotenoids (Vilkhu et al., 2008, Xu and Pan, 2013, Ye et al., 2011, Kha et al., 2014b), polyphenols (Ghafoor et al., 2009, Ilbay et al., 2013), anthocyanins (Galván D'Alessandro et al., 2013, Oancea et al., 2013) and saponins (Wu et al., 2001), have been successfully extracted using UAE technique. UAE conditions such as ultrasound power, temperature and extraction time, type of solvent, ratio of solvent to solid significantly affected the extraction yield of the bioactive compounds. Reduction in extraction time and improvement in quality of bioactive components are also the two main advantages of this technique. Thus, it is interesting to note that implementation of UAE may improve throughput in commercial bioactive compound production process.

Similar to MAE, UAE can also be coupled to a variety of the conventional extraction methods such as solvent extraction. For example, an ultrasound device being placed in an appropriate position of a Soxhlet extraction unit, known as Sono-Soxhlet extraction, could improve extraction efficiency of bioactive compounds. Djenni et al. (2013) reported that the Sono-Soxhlet extraction for olive oil provided significant improvement in time (30 min) without degradation of fatty acid composition, compared with the conventional Soxhlet extraction methods (8 h).

2.6. Supercritical Fluid Extraction (SFE)

2.6.1. Extraction Mechanisms

The fluid used in this extraction is in its supercritical state, which has its pressure and temperature above their critical values. In this state, unique properties of the supercritical

fluid such as density, viscosity and diffusivity are intermediate between those of a gas and a liquid. In particular, the lower viscosity and higher diffusion coefficient are evident, as compared to that of a liquid. The density of the fluid is similar to that of a liquid. Those properties depend on the pressure, temperature and composition of the fluid (Camel, 2001). Importantly, the density, the dissolving power of the fluid, can be adjusted by changing both temperature and pressure of the fluid. Therefore, these properties offer a number of advantages including shorter extraction times, higher extraction yields and better retention of nutritional and valuable bioactive compounds (Herrero et al., 2006).

It is important to select the most suitable supercritical fluid in this extraction technique. In general, many solvents can be used as supercritical fluids such as ethylene, methane, nitrogen, xenon and fluorocarbons. Among those, carbon dioxide (CO_2) has been a preferred solvent for SFE, known as supercritical carbon dioxide (SC-CO_2) extraction. The main reasons are the low critical temperature of CO_2 (31^oC) and the low critical pressure (74 bar), which enables the extraction process at low temperature and moderate pressures. Due to its low polarity, CO_2 is good for extraction of low or non-polar compounds, but not suitable for polar compounds. To overcome this drawback, the use of a small amount of chemical modifier or co-solvent can significantly enhance the solubility of the polar compounds in SC-CO_2. The co-solvents include hexane, methanol, ethanol, isopropanol, acetonitrile, dichloromethane among others. Of those, ethanol is the most suitable co-solvent because of its lower toxicity and miscibility in CO_2 (Joana Gil-Chávez et al., 2013). In all cases, criteria for selection of the best co-solvent are the properties of sample, desired compounds of interest and results of preliminary experiments (Azmir et al., 2013).

In order to obtain a successful SFE of bioactives from plant materials, many factors (Figure 1) need to be considered, including temperature, pressure, extraction time, particle size, moisture content of feed material, flow rate of fluid, solvent and co-solvent choices and solvent-to-feed ratio (Camel, 2001, Azmir et al., 2013). These factors will be discussed in the following section.

2.6.2. Applications of SFE for Plant Extraction

For vegetable oils, hydraulic pressing and/or traditional solvent extraction have been commonly used. High extraction efficiency is usually achieved using the organic solvent extraction, however, solvent elimination after the extraction is an inconvenient step. Furthermore, the main drawbacks of the traditional solvent extraction are thermal degradation of the bioactive compounds and the incomplete solvent elimination (refer to section 2.3). Since CO_2 is the main solvent used in the SC-CO_2 extraction, it is most effective when the desirable compounds are nonpolar. Importantly, separation of the solute from CO_2 solvent can be easily obtained by depressurising the SC-CO_2 (Martínez and de Aguiar, 2014). Therefore, the SC-CO_2 extraction can be employed as an alternative to traditional extraction methods with hazardous solvents. In recent years, vegetable oils from plant materials have been extracted using SC-CO_2 extraction technique (Santos et al., 2013, Tomita et al., 2013, Kha et al., 2014c). According to those studies, material characteristics (particle size and moisture content) and SC-CO_2 extraction conditions (pressure, temperature, time and flow rate) significantly influenced the extraction yield of the vegetable oils. In fact, a faster rate of the CO_2 diffusion is achieved when using a smaller particle size because of increasing surface area to volume ratio of material and rupturing cell membranes (Del Valle and Uquiche, 2002). As a result, grinding the sample to an appropriate particle size is recommended. This is

because there can be a problem with channelling inside the extraction bed if very fine particles are used. Furthermore, it should be noted that when filling the vessel, it is necessary to ensure a homogeneous bed of material to avoid channelling. Moisture content of the material is also an important parameter in the SC-CO_2 extraction. High moisture content can cause mechanical problems such as restrictor clogging due to ice formation. To overcome this, addition of anhydrous Na_2SO_4 and silica gel to the wet plant sample to capture the moisture can be used (Lang and Wai, 2001). However, the preferred pre-treatment (refer to section 2.1) is to dry the plant materials to appropriate moisture content before the extraction. It has been reported that the moisture content of the plant materials should not be higher than 12% because water can cause unwanted difficulties such as ice formation in pipelines (Fornari et al., 2012).

As presented in section 2.6.1, there are numerous extraction factors such as pressure, temperature, time and flow rate influencing the extraction efficiencies. It is generally agreed that applying higher pressure and temperature increases mass transfer and release of bioactive compounds from the plant matrix. However, high pressure and temperature also produces more undesirable compounds in the extract. Generally pressure and temperature can be controlled to optimise the extraction yield of carotenoids. Many studies reported that the extraction pressures between 30 and 40 MPa resulted in the maximal extraction efficiencies of β-carotene and lycopene from difference plant matrices (such as tomato and carrot). For extraction temperature, the low temperature of 60^{o}C gave the highest extraction efficiencies of β-carotene and lycopene, which were reported in many studies (Şanal et al., 2004, Nobre et al., 2009). Furthermore, the high extraction temperature of 80^{o}C also favours lycopene extraction from plant materials (Sabio et al., 2003, Rozzi et al., 2002). However, as isomerisation into its *cis* form at temperature higher than 80^{o}C is promoted, the lower temperature is recommended. Therefore, it is necessary to investigate the most important factors affecting the efficiencies and then optimise the conditions.

The use of co-solvents to enhance the extraction efficiencies has been limited due to safety and environmental concerns. An alternative solvent such as vegetable oil, which is relatively cheap and safe, is of growing interest for extracting high molecular weight compounds such as carotenoids. For example, α- and β-carotene and lutein were extracted using SC-CO_2 extraction at pressure of 27.6 - 41.4 MPa and temperature of 40 - 70^{o}C with canola oil as a co-solvent (Sun and Temelli, 2006). Results indicated that significant improvement in the carotenoid yields was achieved compared with the SC-CO_2 extraction without modifier. Likewise, extraction yield of lycopene from tomato was also significantly increased using hazelnut oil as a modifier (Vasapollo et al., 2004, Ciurlia et al., 2009).

2.7. Pressurised Liquid Extraction (PLE)

2.7.1. Extraction Mechanisms

Pressurised liquid extraction is also known as pressurised fluid extraction, accelerated solvent extraction, high-pressure solvent extraction and enhanced solvent extraction. The extraction conditions used in PLE are in the ranges of 50 - 100^{o}C and 3.5 - 20 MPa, respectively. Organic solvents are employed at elevated pressures and temperatures to enhance the extraction process. The pressure causes the solvents to increase above their atmospheric boiling point temperature. The increased temperature accelerates the extraction

kinetics by increasing solubility and mass transfer properties. The viscosity and surface tension of solvents are decreased by the increased temperature, allowing a better penetration of the material matrix and weakened interactions of bioactive compounds and the matrix. Diffusivity of the solvent is also enhanced at elevated temperature, resulting in faster extraction. Moreover, elevated pressure keeps the solvents in the liquid state and forces the solvent to move rapidly into the matrix, thus facilitating the extraction process (Kaufmann and Christen, 2002, Kadam et al., 2013).

In addition to the use of the organic solvents, water can be employed as a solvent in the pressurised liquid extraction for extracting desired bioactive substances. This technique is known as pressurised hot water extraction or subcritical water extraction. In order to achieve the highest extraction recoveries, there are a number of extraction conditions, which need to be optimised, including the extraction solvent, the temperature, the pressure, the time, and solvent flow rate. More detail of each parameter used in the pressured liquid extraction process has been intensively reviewed by Nieto et al. (2010).

2.7.2. Applications of PLE for Plant Extraction

Bioactive compounds, such as polyphenols and anthocyanins, from plant materials have been successfully extracted using PLE technique. The extraction efficiencies of those bioactive compounds depend on a variety of factors, including characteristic and type of solvent, temperature, time and flow rate. An aqueous mixture of ethanol and water is the most common solvent used in PLE, and their characteristics can be controlled by temperature (Wijngaard et al., 2012). For example, polyphenols from apple pomace (Wijngaard and Brunton, 2009) and anthocyanins from dried red grape pomace (Monrad et al., 2010) were extracted by PLE using the mixture of ethanol and water as the solvent. The use of ethanol-water mixture was more effective than water solvent only in extracting flavonoids from spinach using PLE (Howard and Pandjaitan, 2008). This is because one solvent (ethanol) can improve the solubility of the compounds, and desorption of the compounds from the plant matrix can be promoted by water (Mustafa and Turner, 2011). However, due to the presence of organic ethanol solvent used in PLE, there is still a need to consider the process cost of the ethanol as well as its recovery (Wijngaard et al., 2012).

As indicated earlier, an increase in temperature can improve the extraction efficiency of the desirable compounds due to an increase in mass transfer rate. For instance, the extraction efficiencies of polyphenols increased with increasing the temperatures (García-Marino et al., 2006). However, a degradation of the phenolic acids and anthocyanins at higher temperature was observed (Singh and Saldaña, 2011, Monrad et al., 2010). In addition, Maillard reaction products, which are undesirable, may be also produced at high temperature during PLE (Wijngaard and Brunton, 2009). Therefore, it is important to choose an appropriate temperature for extracting specific bioactive compounds in PLE.

Solvent flow rate during PLE is also an important factor influencing the extraction efficiency. According to Srinivas et al. (2010), the flow rate significantly affected the solubility of quercetin compound in water at different temperatures. Since there are not many published reports about the effect of flow rate in PLE, in addition to other PLE conditions, it is important to carry out more research into the effective dissolution of target compounds for enhancing the extraction efficiency.

2.8. Comparisons of Different Extraction Techniques

The different extraction techniques including SD, SLE, MAE, UAE, SFE and PLE have been presented in the previous sections. Depending on the availability of equipment, the target bioactive compounds and the processing cost, a proper choice of the extraction technique or a combination of different extraction methods can be made. A comparison of six extraction methods is presented in Table 1 in terms of extraction time, sample size, solvent use, solvent type, required investment, advantages and drawbacks.

It is well known that the conventional extraction methods such as steam distillation and solid-liquid extraction take a very long time to complete the extraction process of desired bioactive compounds. Another drawback of the conventional methods is the likely degradation of thermolabile compounds due to the high temperature applied. In addition, the large amounts of organic solvent used and wasted in the solid-liquid extraction incur costs for solvent disposal and environmental control measures, and contribute to the criticism of the conventional extraction methods (Luque de Castro and García-Ayuso, 1998).

To overcome these limitations of the conventional methods, the development of innovative technologies for extracting bioactive compounds from plant materials addresses specific human requirements for health and safety. The main advantages of the novel extraction methods including MAE, UAE, SFE and PLE are shorter extraction time, higher extraction yield and better retention of valuable bioactive compounds. The use of SFE in the extraction of the bioactive compounds, which can be used as nutraceuticals and pharmaceuticals, has been reported. The extracts containing high bioactive compounds can be used to treat or prevent disease (Henry and Yonker, 2006). Similar to PLE, SFE uses nil or a small amount of organic solvent (as a co-solvent) in the extraction, so is considered as more environmentally friendly than the conventional extraction methods. However, the main drawbacks of SFE technique are economics and onerous operating conditions, thus its use so far is limited to areas such as essential oil extraction and coffee decaffeination (Wang and Weller, 2006).

There have been many studies reporting the benefits of MAE and UAE methods for extracting bioactive compounds. Similar to SFE, the MAE and UAE methods can operate at low temperature, allowing the extraction of thermolabile compounds from various plants. Between them, UAE device is cheaper and its operating process is easier compared with MAE. Like Soxhlet extraction, UAE can be used with any solvent, in contrast, the extraction solvent used in the MAE must absorb microwave energy. Overall, MAE and UAE techniques are comparable to other innovative extraction techniques such as SFE and PLE and are considered as strong novel extraction methods in terms of process simplicity, low investment cost and practicality (Wang and Weller, 2006).

Although these methods are very promising as alternatives to the conventional extraction methods, they have been performed only at laboratory or bench scale, except for several industrial applications of SC-CO_2 extraction (Wang and Weller, 2006). It is important to conduct more research to up-scale these novel extraction methods. It is also important to be aware of new developments of other novel techniques such as pulsed-electric field assisted extraction (Delsart et al., 2012, López et al., 2009).

Potentially, from understanding the advantages and drawbacks of different extraction methods (Table 1), there are opportunities to combine different extraction methods to overcome the limitations and retain the advantages.

Table 1. A comparison of different extraction techniques for bioactive compounds from plant materials

Name	Steam Distillation (SD)	Solid-Liquid Extraction (SLE)	Microwave Assisted Extraction (MAE)	Ultrasound Assisted Extraction (UAE)	Supercritical Fluid Extraction (SFE)	Pressurised Liquid Extraction (PFE)
Description	Sample is inserted into the still and steam flows through.	Sample is contacted with solvent and stirred or flowed.	Sample is immersed in solvent and subjected to microwave.	Sample is immersed in solvent and subjected to ultrasound.	Sample is placed in a vessel and crossed to the supercritical fluid.	Sample is placed in a vessel and subjected to pressurised liquid.
Extraction time	10 - 300 min	2 - 8 h	3 - 30 min	10 - 60 min	10 - 60 min	5 - 20 min
Sample size	1- 50 g	1 - 5 g	1 - 10 g	1 - 30 g	1 - 5 g	1 - 3 g
Solvent use	30 - 500 mL	50 - 300 mL	10 - 40 mL	50 - 200 mL	2 - 5 mL (solid trap) or 30 - 60 mL (liquid trap)	15 - 60 mL
Solvent type	Water	Organic solvent	Water and/or organic solvent	Water and/or organic solvent	CO_2 and/or organic solvent as a co-solvent	Water and/or organic solvent (ethanol)
Investment	Moderate	Low	Moderate	Low	High	High
Advantages	High distillation efficiency; environmental friendly	High extraction efficiency; use as a standard method; easy to use	Rapid; easy to handle; moderate solvent consumption; environmental friendly	Easy to use	Rapid; low solvent consumption; no filtration required	Rapid; low solvent consumption; no filtration required
Drawbacks	Time-consuming, large energy consumption; possible degradation of thermolabile solutes	Time-consuming; large amount of solvent use; possible degradation of thermolabile solutes; further filtration step required; unsafe.	Extraction solvent must absorb microwave energy; further filtration step required.	Large amount of solvent consumption; further filtration step required.	Many parameters to optimise.	Possible degradation of thermolabile solutes.

Adapted from Chemat et al. (2011).

Several combined extraction techniques, including ultrasound with microwaves, ultrasound with microwaves or with solid phase, ultrasound or microwaves with pressurised liquid, and pressurised liquid or supercritical fluid with solid phase, were successfully applied to extract bioactive compounds from plant materials (Rostagno et al., 2010, Mustafa and Turner, 2011). For example, Lianfu and Zelong (2008) successfully extracted lycopene from tomato using the combination of ultrasound and microwaves. The results confirmed that the shorter extraction time, higher yield of lycopene and less amount of solvent used were obtained in this combined extraction method compared with the ultrasound-assisted extraction on its own.

3. ISOLATION TECHNIQUES OF PLANT BIOACTIVE COMPOUNDS

After the extraction of bioactive compounds from plant materials by different extraction techniques as discussed in section 2, the crude extract obtained usually contains other unwanted compounds, which may interfere with a bioassay. Therefore, isolation is essential in order to achieve concentrated target bioactive compounds. In this section, two isolation techniques of bioactive compounds, including ion-exchange and high performance liquid chromatography, are presented.

3.1. Ion-Exchange

3.1.1. Isolation Mechanism

Ion-exchange is applied for isolation and purification of crude extract containing undesirable compounds. This method has been preferred due to being a low cost isolation method, and it is more important if the separation of the bioactive compounds from waste products will balance the waste disposal costs. Importantly, this method can be used in conjunction with other process such as membrane filtration to obtain higher purity of bioactive compound.

The ion-exchange process is a mass transfer in which the extract containing a solute (liquid phase), also known as the adsorbate, is transferred to the solid phase due to retention on the solid's surface or to reaction with the solid (or the adsorbent). In other words, the ion-exchange is a replacing process of ions of a liquid phase with others contained in the solid phase, which is called ion-exchange resin. This is a chemical adsorption (or chemisorption), where ion-exchange process occurs at the specific points of the resin with an opposite charge, implying in chemical bonds (Ibarz and Barbosa-Cánovas, 2003).

In the ion-exchange method, resin is the most widely used as ion exchanger, which is a synthetic agent. Ion-exchange resins can be anion exchange resin or cation exchange resin, depending on the type of ions in the extract (Calvo and Cocero, 2009).

- Anion exchange resin can be strong base type of $-N-(CH_3)_3X$ or $-N-(CH_3)_2(C_2H_4OH)X$, or weak base type of $-NR_2$, $-NHR$, $-NH_2$.
- Cation exchange resin can be type of strong acid ($-SO_3H$) or weak acid (-COOH and $-PO_3H$).

Ion-exchange can be also a physical adsorption where different stages of mass transfer occurs in physical adsorption processes. First, external mass of ion A from the liquid phase transfers to the resin's surface, then ion A diffuses through the pores of the resin until the exchange points are reached. After that, ion-exchange process occurs, in which the ion A is physically exchanged by ion B so that ion A is bound to the resin, whereas ion B passes to the liquid phase. Ion B diffuses through the pores of the resin until reaching the surface of resin. Finally, the external mass of ion B from the resin surface transfers to the liquid phase (Ibarz and Barbosa-Cánovas, 2003).

Compared to chemisorption, the forces between adsorbate-adsorbent interactions are usually weak, such as van der Waals or hydrogen bonds. Desorption of physically adsorbed molecules can be carried out easily by increasing the temperature or by reducing the concentration. This is because the physical adsorption often implies the formation of a multilayer of adsorbate, whereas the chemisorption is restricted to a monolayer of molecules. As a result, the physical adsorption requires less heat (<80 kJ/mol) compared to the chemisorption (80 - 400 kJ/mol). For those reasons, in industrial separation, ion-exchange based on the physical adsorption is preferred (Calvo and Cocero, 2009).

3.1.2. Application of Ion Exchange for Isolation of Bioactive Compounds

Resin has been widely used as adsorbent, a solid material having capacity to adsorb another substance (solute). The most important properties of adsorbent are a large contact surface and porosity. Depending on the type of bioactive compounds isolated, different adsorbents can be used. There are a variety of adsorbents commonly used in the ion-exchange methods. For example, silica has been investigated for the isolation of vitamin E from Palm fatty acid distillate (Chu et al., 2004). Vitamin E from solutions with different polar and nonpolar solvents was also isolated using mesoporous carbons CMK-1 and CMK-3 (Hartmann et al., 2005). Other adsorbents such as macroporous copolymer MA-DVB beads (Xu et al., 2000) and Fe_3O_4 magnetic nanoparticles (Li et al., 2014) have been used to isolate flavonoid compounds from crude extract of *Ginkgo biloba* leaves. Interestingly, waste product such as rice hulls can be also used as adsorbent to isolate β-carotene (Chen et al., 2003) and lutein (Palaniappan and Proctor, 1990) from soybean oil.

In addition to the selection of the best adsorbent, operating conditions, such as temperature, time, pH, agitation, polarity of the extract, adsorbent mass, initial concentration of bioactive compounds, ratio of adsorbent to solute (Silva et al., 2007, Barboza et al., 2003, Ma and Lin, 2004) and other characteristics including particle size, porosity, specific surface area and pore volume distribution (Scordino et al., 2004), affect the adsorption kinetics and equilibrium. As a result, it affects the adsorption capacity of an adsorbent or the isolation efficiency. The impact of those parameters on the isolation efficiency can be calculated from a sorption isotherm or a sorption equilibrium. The sorption isotherm can be defined as the balance between adsorbent and adsorbate in the given conditions. The mathematical models of Freundlich and Langmuir are usually chosen to fit experimental data obtained in batch experiments. The sorption isotherms can be then used to predict the behaviour of the adsorbents in dynamic systems such as fixed-bed chromatographic processes (Silva et al., 2007, Barboza et al., 2003).

One of the main limitations of ion-exchange method for isolation of bioactive compounds is the recovery of the adsorbate from the adsorbent. As a result, there is a need of a further step for selection of the most suitable eluent to wash and collect the adsorbate. The most

common eluents used for recovery of bioactive compounds are hydro-alcoholic mixtures and organic solvents.

3.2. High Performance Liquid Chromatography (HPLC)

3.2.1. Basic Principle

HPLC has been widely used for analysing and isolating the bioactive compounds in food research laboratory and industry. The principle of the HPLC technique is the mass transfer of analytes between the stationary (immobile packing within the column) and mobile (organic solvent) phases. The bioactive compounds must be dissolved in the mobile phase and then forced to flow via the stationary column. They can be isolated based on the different equilibrium distribution coefficient of different materials in the stationary phase and the mobile phase. The quantities of different bioactive compounds are not the same in the two phases due to differences in their physical and chemical properties.

Fractionating bioactive compounds from plant materials into different groups of compounds prior to HPLC/MS (mass spectrometry) analysis is a necessary step because the complex matrix of bioactive compounds in plant materials can result in a decrease in the sensitivity of determination. Preparative HPLC is one of the automated and easy-to-use techniques for purifying compounds of interest in mixtures. Generally, some of the concepts of preparative HPLC are similar to the analytical HPLC but also have some differences (Vera and Michael, 2009). The differences between analytical and preparative HPLC are shown in Table 2.

Purity of the product, yield and throughput are the three key criteria used for judging the result of a preparative run. In many cases, if a high purity of compound is needed to be isolated for the activity testing, and hence, the yield and throughput are then less important. Therefore, it is dependent on the application where a compromise of these three parameters is often necessary for optimising a purification run. Choosing the appropriate stationary (column) and mobile phases are also important parameters in preparative chromatography. The choice of column (such as adsorption, reverse-phase, size exclusion and ion exchange chromatography) is critical to provide the best selectivity for the compounds of interest and reproducible preparative HPLC method. Parameters including solvent viscosity, sample solubility in the mobile phase, pH, volatility of solvents/buffers, solvent cost and safety hazards should also be taken into consideration when choosing an optimal mobile phase (Vera and Michael, 2009).

3.2.2. Application of HPLC for Isolation of Bioactive Compounds

Two alkaloids were successfully separated and purified from *Huperzia serrata*, a traditional medicine possessing positive effects on learning and memory, by using a preparative HPLC (Zhang et al., 2012). The preparative chromatographic conditions involved a reverse phase C18 column (19 mm x 300 mm, 7 μm) and a gradient elution of 0.1% (v/v) trifluoracetic acid in water and methanol at a flow rate of 8 mL/min. Another similar study by Lo et al. (2012) demonstrated that a high purity of chrysophanol was isolated from the traditional herb, *Rheum palmatum* Linn., using preparative HPLC, equipped with a C18 column (4.6 mm x 250 mm x, 5 μm) and a gradient elution of 1% acetic acid and methanol

was used as mobile phase. Furthermore, a preparative HPLC with four C18 columns (19 mm x 100 mm, 5 μm) successfully purified the target compounds from more than one Aristolochia plants simultaneously (Zhang et al., 2010). This study also showed that even the target compounds with similar chemical structures can be isolated and purified selectively. It can be noted that preparative HPLC with selected column and mobile phases is an effective technique to purify compounds of interest selectively from the plant materials.

Other than the preparative HPLC, several techniques such as solid-phase extraction (Sowa et al., 2014), column-chromatography (Wang et al., 2014), silica gel column chromatography (Kumar et al., 2013), high-speed counter-current chromatography (HSCCC) (Shi et al., 2012) have been reported for isolating target compounds from plant materials. A study by Kumar et al. (2013) demonstrated that five terpenes from *Potentilla fulgens* were isolated and purified by the silica gel column chromatography and HPLC. Seven antioxidants were also successfully purified from *Eucommia ulmoides* Oliv. leaves using HSCCC and HPLC (Dai et al., 2013). However, the drawback of this technique is the residuals of immiscible organic solvents obtained from HSCCC may influence the further bioactivity analysis (Wang et al., 2014). Using a combination column chromatography and semi-preparative HPLC, three pure monomeric anthocyanins were isolated and purified from wild blueberries (Wang et al., 2014). Purified bioactive compounds obtained from plant materials using above-mentioned techniques were then identified by HPLC/MS.

A number of analytical tools have been widely developed for the separation and characterisation of various plant materials. For example, the HPLC with diode array detector (DAD) and electrospray ionisation mass spectrometry (ESI-MS) have been proved to isolate and characterise bioactive compounds successfully from various plant materials (Wu et al., 2014, Ferreres et al., 2014, An et al., 2013). By using negative MS modes, and a water (1% acetic acid) and methanol gradient solvent system, thirty six flavonoids and hydroxycinnamic acids were detected by HPLC-DAD-ESI/MS in the herbal tea prepared from *Grindelia ronusta* Nutt. (Ferreres et al., 2014). The compounds were identified by comparing the molecular mass and structural features of compounds that tentatively assigned from HPLC-DAD-ESI/MS data with literature data. Furthermore, eight major bioactive constituents in Gardeniae Fructus, a traditional Chinese medicine for treatment of hepatitis, jaundice, hypersonic, diabetes and hematuria, were identified using HPLC-DAD-ESI/MS by comparing the spectra produced with reference compounds (Wu et al., 2014). These findings elucidated that the profile and content of bioactive compounds in the plant materials can be identified by the combination of HPLC with UV/Vis diode array detector and MS.

However, the information in relation to the molecular weight and the nature of the substituent obtained from the UV and MS is still insufficient to adequately elucidate a compound since the specific structural features are only compared to the reference and literature data. Therefore, additional information about identification of the compound is often necessary. Nuclear magnetic resonance spectrometry (NMR) experiments can fully elucidate the target compounds by providing the complementary information based on the configuration of the substituent on the skeletal structure (Rauter et al., 2005). Generally, the proton NMR is adequate for identifying the known compound. However, further analysis such as ^{13}C-NMR and different 1D and 2D NMR are often necessary for elucidating novel compounds (Zhou et al., 2014, Sun et al., 2003). In summary, both known and novel compounds can be adequately identified according to the information of structural elucidation obtained from the combination of UV, MS and NMR (El-Seedi et al., 2013).

Table 2. Comparison of analytical and preparative HPLC

Analytical HPLC	**Preparative HPLC**
To quantify and/or identify compounds	To isolate and/or purify compounds
Target all compounds in the mixture	Target one or only a few compounds in the mixture for isolation
Sample elutes from detector to waste	Sample elutes from detector to fraction collector
Critical parameters: Resolution, peak width and peak symmetry	Critical parameters: the amount of compound produced per unit of time, purity, recovery and separation cost

Adapted from Vera & Michael (2009).

4. EVALUATION OF BIOACTIVITY AND ANTIOXIDANT ACTIVITY

Bioactive compounds can be extracted from plant materials using different extraction methods as presented in section 2. The next step is the isolation of a target bioactive compound in the crude extract in which the end product aims to a drug or a lead compound (refer to section 3). It is generally agreed that natural bioactive components in plant extracts play an important role in human health. Therefore, it is of interest to test possible bioactivities and antioxidant activities related to bioactive compounds within a crude extract or the pure bioactive compound (Figure 1) in order to allow further development of the compounds for use as nutraceuticals and/or pharmaceuticals for the treatment or prevention of numerous diseases.

4.1. Bioactivity

The therapeutic and pharmaceutical screening of extracts is very complicated as crude extracts contain complex mixtures of bioactive compounds. As a result, it is desirable to select appropriate methods to assess the bioactivity of the crude extracts or lead compound. Success in selecting appropriate screening methods for a target disease is largely dependent upon the proper design and validation of these methods. Higher throughput methodologies, with a large number of extracts or lead compounds screened quickly and cost effectively, are important. A combination of different screening methods such as chemical and biological assays is also needed to test bioactivity (Atta-ur-Rahman et al., 2001). Furthermore, the selection of the screening methods to be adopted depends on the disease type and the available knowledge about the specific plant to be studied. For instance, if a plant has a historical use for the prevention or treatment of a specific disease, a single goal screening method (a specific bioassay) can be used to predict the therapeutic activity and to extract, isolate and purify the lead compound responsible for the bioactivity. Otherwise, a battery of different evaluation techniques needs to be employed for screening bioactivity, commonly known as a bioassay-guided isolation of bioactive compounds from natural products (Atta-ur-Rahman et al., 2001). There are a number of evaluation techniques that have been used for screening potential bioactivities against certain diseases. The evaluation technique used for high throughput screening can be broadly classified into *in vitro* and *in vivo* assays. The *in vitro* assays can be carried out at molecular or cellular levels. Several methods such as

biochemical assays (spectrophotometric measurement of the end-product after an enzymatic reaction), ligand-binding assays (read-out by labelling with a tracer), and functional assays (reporter-gene and second-messenger assays) can be used to detect a reaction at the molecular level. The most common detection methods used are fluorescence-based, isotopic-labelling colourimetry and chemiluminescence assays (Mishra et al., 2008).

In the cancer field, it is important to distinguish the terms "cytotoxicity", "anti-tumour" and "anti-cancer". Cytotoxicity indicates that extracts or compounds show activity against tumour cell lines. The cytotoxic compounds may be cytostatic (stop cell growth reversibly or irreversibly) or cytocidal (kill cells). Anti-tumour compounds are effective in *in vivo* tumour systems. Anti-cancer refers to compounds that are clinically effective in human cancers. Therefore, there is a need to perform clinical trials to determine whether any anti-tumour compound has anti-cancer activity (Suffness and Douros, 1982, Atta-ur-Rahman et al., 2001). The cell-based and mechanism of action-based primary *in vitro* assays are the two common bioassay types for the screening of anti-cancer and anti-tumour activity. These bioassays have been used for investigating the pharmacological effects of crude extracts and purified/isolated compounds of interest from crude extracts. The potential effects of bioactive compounds from numerous medicinal plant extracts against cancers have been widely investigated *in vitro* and *in vivo* and these bioactive compounds are claimed to demonstrate potent anti-cancer and anti-tumour properties.

4.2. Antioxidant Activity

Screening the bioactivity of extracts or compounds can also be performed by measuring antioxidant activity using different antioxidant assays, which have been developed to evaluate the antioxidant activity of bioactive compounds in crude extracts and their complex mixtures to interact with or neutralise ROS/RNS (reactive oxygen species/reactive nitrogen species). These antioxidant assays can be classified into five different evaluation strategies (López-Alarcón and Denicola, 2013):

- The scavenging activity of stable free radicals by antioxidants: the DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2'-Azinobis-(3-ethylbenzothiazole-6-sulphonate) assays.
- The activity of the antioxidant to reduce metal ions: the FRAP (Ferric reducing antioxidant power) and CUPRAC (Cupric ion-reducing antioxidant capacity) assays.
- The ability of antioxidants to protect a target molecule exposed to a free radical source: ORAC (Oxygen radical absorbance capacity) and TRAP (Total radical-trapping antioxidant parameter) assays.
- The activity of antioxidants to prevent the oxidation of low-density lipoprotein (LDL): Cupric sulphate is commonly used as initiator of LDL oxidation and the lipid peroxidation processes.
- Nanoparticle-based assays: gold nanoparticles from an Au^{III} solution and silver nanoparticles are recently used to assess antioxidant activity of crude extracts or pure compounds.

The main advantages of these antioxidant assays are simplicity of the experimental conditions, low cost and time saving. Therefore, it is of interest to test direct antioxidant activity and potential pro-oxidant effects on different molecular targets using these assays. In addition, it is strongly recommended that at least two different methods are used to evaluate antioxidant activity in crude extracts or pure compounds due to differences between the ways in which the test systems investigate antioxidant activity (Moon and Shibamoto, 2009, Schlesier et al., 2002). For example, a particular extract could yield a high FRAP value due to containing a good metal reducing compound, however, it could be a poor scavenger of peroxyl radicals, resulting a low ORAC value. Furthermore, the crude extracts containing different bioactive compounds may have synergistic effects and thus showing a high value of antioxidant activity (López-Alarcón and Denicola, 2013).

CONCLUSION

In conclusion, it is important to extract a variety of bioactive compounds from plant materials, which have the potential to be used as nutraceuticals and pharmaceuticals for the treatment or prevention of certain diseases. In this chapter, the basic principle and mechanism of two conventional extraction methods (steam distillation and solid-liquid extraction) and four novel extraction techniques (ultrasound assisted extraction, microwave assisted extraction, supercritical fluid extraction and pressurised liquid extraction) are discussed. The applications of these conventional and non-conventional extraction techniques on the extraction of bioactive compounds from plants are also presented. The main advantages of the novel extraction methods over the conventional methods are time-saving, environmentally friendly, reduction of solvent volumes used and higher quality of the extract. However the main challenge with the novel extraction (especially SFE and PLE) is high investment costs in scaling up. A comparison of the different methods and recommendations for combined extraction techniques in future research are also made. Further isolation steps of the extract containing the bioactive compounds are also described. The two isolation techniques (ion-exchange and HPLC) are discussed in terms of the basic principle and their applications in isolation of bioactives. The ion-exchange method is much simpler and cheaper compared to preparative HPLC. However, the preparative HPLC and other techniques combined with HPLC are much more reliable and accurate. *In vitro* and *in vivo* biochemical assays play a key role for the screening and selection of a new natural plant product for the treatment or prevention of cancers, or other disease types. Components within the crude extract or the lead compounds must also be identified, while their mechanisms of action and toxicity must be established before translation into pre-clinical or clinical trials.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 7

PANCREATIC CANCER DRUGS: CASE STUDIES IN SYNTHESIS AND PRODUCTION

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ABSTRACT

Identification of bioactive compounds from natural sources represents only the first stage in the development process of some drugs. Plant and animal sources rarely produce bioactive compounds in the quantities required to meet commercial demands, thus the development of total or semi-synthetic routes to these materials becomes a core element of commercial drug production. This process brings to the table a plethora of issues that must be resolved to ensure the development of a safe, pure final product via economically feasible processes.

Herein we discuss the synthesis of three anti-cancer drugs – 5-FU (6), gemcitabine (7) and paclitaxel (8) used in pancreatic cancer treatment. The synthetic approaches undertaken differ in each case, driven by factors such as structural complexity and physicochemical properties, which influence solubility and bioavailability.

The chapter highlights the demanding nature of pharmaceutical research, covering a wide-range of issues from the exploration of reagents to maximise product outcomes in key synthetic steps, to the modification of intermediates to allow for large scale, non-chromatography based product purification.

The work illustrates the long road and many false paths that exist between initial discovery and the ultimate development of a safe and commercially viable pharmaceutical product. While technological advancements in computer-based design, synthesis and screening techniques continue to accelerate drug development, success ultimately depends upon the intellect and creativity of dedicated teams of synthetic and formulation chemists in devising innovative solutions to resolving key impasses.

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1. INTRODUCTION

Humankind has utilised Nature's storehouse of bioactive compounds present within its natural sources (particularly plant derived materials) for the treatment of disease and other ailments. This use precedes written history, with a selected number of plant species having long ceremonial histories [1]. Terrestrial plant extracts dominate the traditional medicines of most cultures across the globe, due primarily to their immediate presence and accessibility relative to other sources of bioactive molecules such as animals, microflora or marine organisms. The ancient Egyptians, Mesopotmians, Chinese and Indians all possessed extensive written documentation of the use of plant-based extracts in prescriptive treatments for a wide variety of human ailments [2]. Reliance upon natural therapies continues to the present day. The World Health Organisation estimates suggest that over half of the world's population relies almost exclusively on traditional medicines as their primary source of health care [3]. Ethnopharmacology therefore provides modern science with a valuable starting point from which to begin the search for new and more effective disease treatments through rigorous scientific assessment of the efficacy (or otherwise) of these folk treatments.

The isolation and structure elucidation of bioactive compounds from flora and fauna is now a mature science, benefiting immensely from major advances in separation technologies, e.g. HPLC, and spectroscopy, e.g. high field NMR [4,5]. This, coupled with computer-aided, structure-based drug design [6,7], combinatorial chemistry [6] and high throughput screening have significantly accelerated the discovery of new lead compounds, and had an impact on turnover rates in modern drug research.

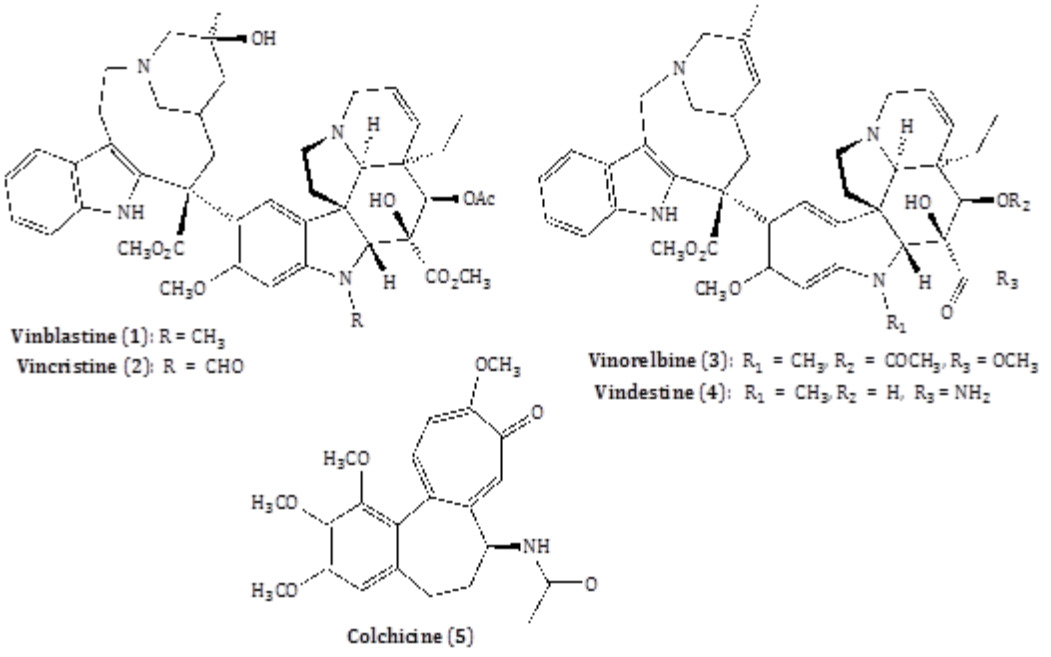


Figure 1. Chemical structures of four key Vinca alkaloids: vinblastine (1), vincristine (2), vinorelbine (3), vindestine (4) and colchinine (5).

Current estimates suggest that 40-60% of all pharmaceuticals currently available in the marketplace are still derived from natural origins [8,9]. These therapeutics include paclitaxel, gemcitabine and 5-fluorouracil, which represent three key anticancer drugs presently utilised in pancreatic cancer therapy (Figure 1). Herein, we discuss the synthetic complexities and varied synthetic strategies that have been developed to access these compounds and develop structure-activity relationship data around the primary drug core structures.

2. DRUG DISCOVERY

2.1. Cancer Treatments from Plant Sources

Plants have assumed the greatest prominence as a source of medicinal compounds, with more than 3000 plant species associated with the treatment of cancer or conditions possessing cancer-like symptoms (both in the scientific literature and ethnomedical sources) [10,11]. Scientific evaluation of a range of traditional medicines has led to the development of highly effective cancer drugs [12,13,14].

2.2. Antimitotic Agents

Cancer can, simplistically, be thought of as arising as a result of aberrant cell division and growth. This often arises due to errors in the body's machinery controlling cellular division. It is thus not surprising that the various stages of cell division: prophase, metaphase, anaphase, telophase and cytokinesis, which combined, describe mitosis, are current anti-cancer drug targets. Mitosis, the controlled cellular event leading to the formation of daughter cells, sees duplicate copies of the cellular genome moved to the opposite poles of a mitotic spindle. These spindle fibres are comprised of cylindrical, protein-based polymers know as microtubules. Microtubules form and deconstruct from the two sub-unit proteins, α- and β-tubulin, during two discreet phases of mitosis (prophase and telophase) as a result of a dynamic equilibrium state. Disruption of the mechanisms controlling this crucial sequence of events leads to the rapid and uncontrolled division of cells, otherwise known as cancer.

The ability to selectively intervene in the mechanistic elements of mitosis has been a key objective of cancer scientists and a principal assessment criterion in the *in vitro* bio-assaying of plant extracts. Tubulin-interacting antimitotic drugs can be divided into two subclasses according to their mode of action; namely compounds that inhibit tubulin polymerisation and compounds that promote tubulin polymerisation.

Early examples of microtubule inhibiting antimitotic drugs include the vinca alkaloids vinblastine (1) and vincristine (2), vinorelbine (3), vindesine (4) and colchicine (5) (Figure 1). Vinca alkaloids were derived from the Madagascar periwinkle, *Cantharanus roseus* G Don. The plant, consumed as a tea by the indigenous inhabitants of the island, was used to treat symptoms associated with diabetes. Subsequent scientific investigations, conducted to assess the validity of these claims, noted that the extracts reduced white blood cell counts and depressed bone marrow growth in laboratory animals, suggesting that the active constituents may have potential activity against blood related cancers such as lymphoma [15].

Four vinca-derived alkaloids are presently utilised in cancer treatment. Each compound possesses unique pharmacology, targeting different mechanistic aspects of cancer proliferation. Vinblastine (1) inhibits angiogenesis (new blood vessel growth) - a key step in the transition of tumours to the malignant state, making it useful as a therapeutic agent in treating a range of diseases including Hodgkin's disease, non-Hodgkin's lymphoma and breast cancer. [13,16].

Vincristine binds rapidly and reversibly to multiple tubulin dimer units, thereby preventing them from assembling into the higher tubulin protein. Vincristine is used to treat a range of ailments including acute leukemia, rhabdomyosarcoma, neuroblastoma, Hodgkin's disease, and other lymphomas. Vinorelbine (3), acts in a similar manner to vinblastine (1), exhibiting antitumour activity in patients with breast cancer as well as antiproliferative effects on osteosarcoma (bone tumour cells) [17]. It has also been shown to reduce the stability of the lipid bilayer membranes in cells. Vindesine (4), exhibits similar effects to vinblastine (1) and is used to treat melanoma and lung cancers [18].

Colchicine (5), an alkaloid isolated from the ornamental plant autumn crocus (*Colhicium autumnale*) a native of the United Kingdom, has been used to treat a range of conditions including gout, Mediterranean fever and liver cirrhosis, and was first isolated in 1820 [19]. As an antimitotic agent, 5 forms a complex with soluble tubulin, which is incorporated into the microtubule terminus, inducing a conformational change that results in a reduction in the rate of addition of new tubulin dimers, inducing spindle disassociation during metaphase [20].

2.3. Pancreatic Cancer

Pancreatic cancer is the fifth leading cause of cancer related death in Australia [21]. Despite the increasing sophistication of surgical techniques, over the past 20 years, little headway has been made in reducing pancreatic cancer mortality rates. While chemotherapy is as an important option in pancreatic cancer treatment, sustained treatment is often accompanied by the development of chemoresistance, relapse and ultimately patient death [22]. Research to identify new treatment options, more efficient drug delivery mechanisms and combinatorial drug formulations to improve treatment efficacies therefore continues in earnest.

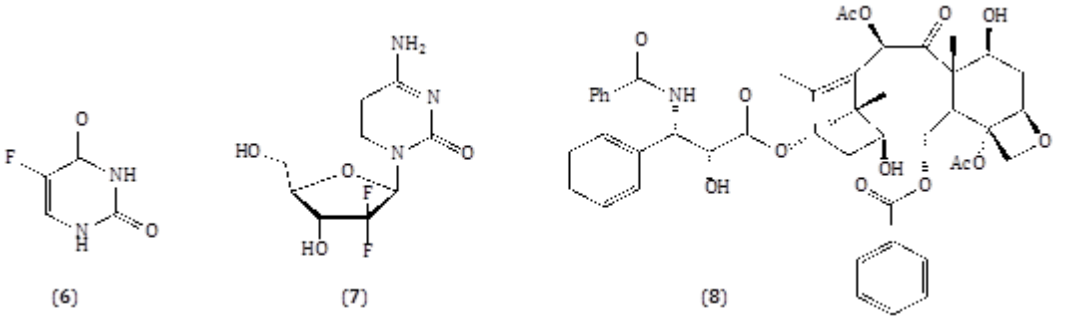


Figure 2. Chemical structures of natural product based drugs, 5-FU (6), gemcitabine (7) and paclitaxel (8), in current clinical use for the treatment of pancreatic cancer.

Chemotherapeutic treatments for pancreatic cancer have, for the past 50 years, been dominated by two therapeutic agents; 5-fluorouracil (5-FU) (6) and gemcitabine (2′-deoxy-2′,2′-difluorocytidine) (7) (Figure 2). In recent years however, combinatorial treatments involving gemcitabine and paclitaxel (8), have emerged, showing some improvement in life expectancies of some patients [23].

3. PACLITAXEL

Taxane diterpenoid-based pharmaceuticals represent one of the most successful classes of anticancer drugs yet developed, and have been at the forefront of cancer treatment over the last 25 years. Excellent clinical success has been achieved in treating a range of cancers including breast, ovarian and prostate, either as a single entity or in combinatorial treatments [24].

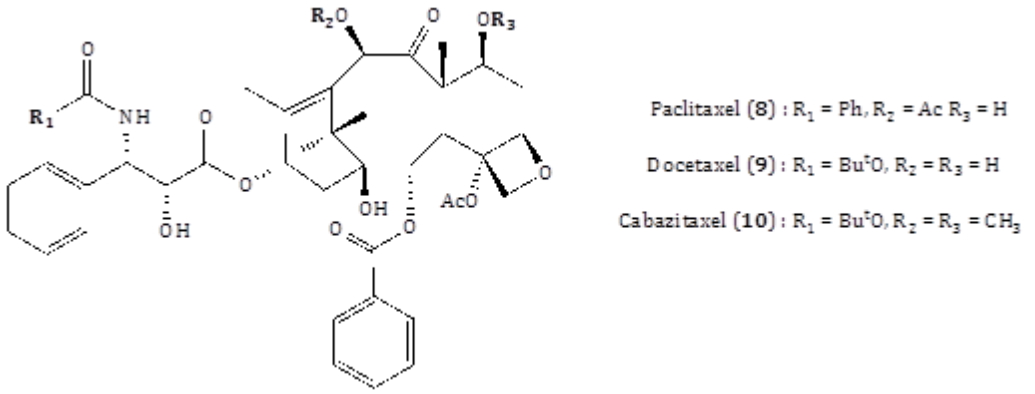


Figure 3. The chemical structures of therapeutically used taxane diterpenoid pharmaceuticals: paclitaxel (8), docetaxel (9) and cabazitaxel (10).

Paclitaxel (*Taxol*®) (8), docetaxel (*Taxotere*®) (9) and carbazitaxel (Jevtana®) (10) are three of the most widely prescribed taxane-based pharmaceuticals. Paclitaxel (8), the parent compound, occurs naturally, while 9 and 10 are semi-synthetic derivatives prepared from natural precursors containing the taxane skeleton [25]. In 2010 docetaxel sales exceeded $2.6 billion worldwide, while paclitaxel sales have declined slowly ($747m – 2005, $563m – 2006) as a consequence of patent expiration in 2000 (US), 2003 (Europe) and 2006 (Japan), and a growing generic market. Revenues from carbazitaxel, which only received FDA approval in 2010, are expected to exceed 514 million euros by 2020 [26]. Interest in and demand for taxanes remains high, with generic pharmaceutical producers driving many newer innovations in formulation, drug delivery and combinatorial therapies.

3.1. Discovery, Isolation and Identification of Paclitaxel

The discovery of the paclitaxel occurred as part of a concerted National Cancer Institute (NCI) investigation in the 1960s which saw over 100,000 extracts tested throughout the

duration of the program to identify new plant bioactives possessing anticancer properties [24,27].

In 1962, stem and bark samples from the Pacific yew (*Taxus brevifolia* Nutt.), a tree native to the US Pacific Northwest, were collected for testing. Initial *in vitro* testing of the extracts was found to be cytotoxic to KB cells [27]. Isolation and identification of paclitaxel was accomplished in 1967 with the structure confirmed and published in 1971 [28]. Formal confirmation of the structure through X-ray crystallography of paclitaxel itself was however not achieved until 1995 [29].

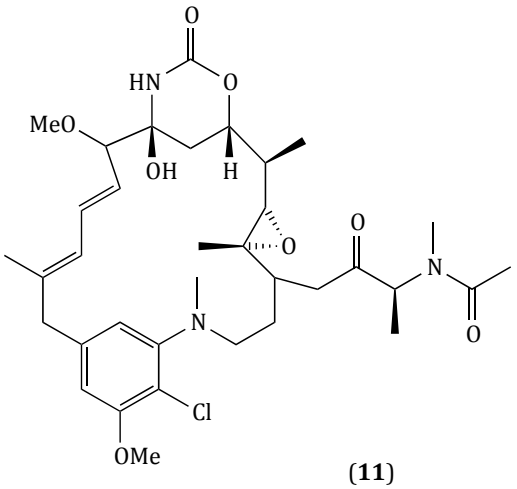
Initial *in vivo* evaluation showed paclitaxel to be modestly active against a range of rapidly growing tumour models including leukaemia cell lines and Walker 256 carcinosarcoma cells [30]. While interesting, these data were not considered particularly encouraging in the context of the mass screening studies being undertaken [31]. It was not until the introduction of new secondary screening assessment studies utilising a solid tumour model (B16 melanoma) that paclitaxel's true potency was identified, thus precipitating a decision by the NCI committee in 1977 to proceed with paclitaxel as a drug development candidate [27].

The successful transitioning of paclitaxel from a somewhat promising drug lead to a high demand commercial entity faced a number of significant hurdles. These included; a structurally complex ring system that eliminated synthesis as an economically viable supply strategy, low natural abundance in the source material (harvesting of the bark of 2500 trees is required to produce 1kg of paclitaxel) [32]; limited supplies of source material - the bark of a relatively uncommon, slow growing tree; the application of unsustainable harvesting practices to obtain the source material and the relative insolubility of paclitaxel in water, which limited formulation options, thereby impacting on bioavailability and pharmokinetic behaviour [24]. The development of techniques to overcome these issues was a central element in the eventual success of paclitaxel.

3.2. Mechanism of Action

Initial investigations of paclitaxel's mechanism of action suggested inhibitory action on the G_2-M phase of the cell cycle [33]. It was subsequently determined that paclitaxel acted in a unique fashion as a mitotic inhibitor through interactions with microtubulin structures in a manner such that they are inhibited from reverting back to tubulin [34].

Early anti-cancer agents such as vinca alkaloids (1-4), colchicine (5) and maytansine (11) (Figure 4) also target tubulin activity, but do so in a destructive manner by actively preventing the assembly of the tubulin proteins into microtubulins [35]. Paclitaxel by contrast, preferentially bound in 1:1 stoichiometry to the α□β tubulin heterodimer subunits, lowering the critical concentration of tubulin required to form microtubules and shifting the equilibrium in this dynamic state to favour microtubulin formation [36]. This disruption of the equilibrium state impacts on the cell's ability to undertake microtublin driven activities, resulting in mitotic arrest and ultimately cellular apoptosis [37].



(**11**)

Figure 4. The chemical structure of the tubulin binding agent maytansine (11).

Paclitaxel also participates in a second, non-mitotic pathway involving the protein bcl-2, which is found in both normal and transformational cell types and is thought to inhibit cellular apoptosis, regulate pancreatic morphogenesis and tissue homeostasis [38]. Paclitaxel activates Raf-1 kinase, which in turn promotes hyperphosphorylation of bcl-2, resulting in its inhibition, leading to cell death [39]. The action of newer, semisynthetic taxanes such as cabazitaxel is similar. Like paclitaxel, carbazitaxel stabilises microtubule assembly by complexing to β-tubulin [40,41]. However, in contrast to paclitaxel and docetaxel, carbazitaxel exhibits only limited affinity for multi-drug resistance proteins such as the drug efflux pump P-glycoprotein (P-gp) that, when expressed by cancer cells, can lead to acquired taxane resistance. Consequently, cabazitaxel has been successfully utilised in the treatment of docetaxel-sensitive and docetaxel-resistance cancers such as metastatic castration-resistant prostate cancer (CRPC). [26,42].

In vivo trials have established that cabazitaxel displays cytotoxicity towards pancreas P03 cells and against MIA PaCa-2 in xenograft models [43]. The lower polarity of cabazitaxel has a lower polar surface area that enables greater transport across the blood-brain barrier, and enhanced pharmacokinetics for the treatment of brain tumours [26].

3.3. Structure Activity Relationships

Exhaustive structure-activity relationships (SAR) evaluation of the paclitaxel core have been undertaken and reviewed. [27,30,44,45,46,47] Herein we focus on the key SAR findings.

3.4. Naturally Occurring Taxanes

To date about 500 taxoids have to date been identified and characterised from a variety of plant species including *baccata, T. wallichiana, T. cuspidata, T. canadensis, T. chinesensis,*

T. yunnanensis [48]. These compounds, which, by definition contain the 6 membered A ring, 8 membered B ring, 6 membered C ring and 4 membered D ring core of the taxoid skeleton and are differentiated by their substituent and functional group distribution at key ring points shown in the exemplar compounds (12 – 19), (Figure 5).

Within the naturally occurring toxoids, key modifications are present at three sites; C7, C10 and the C13 tail unit (Figures 5 & 6). C13 modifications in chain length and nature of the alkyl substituent, e.g. replacing the benzoyl group in paclitaxane, have limited effect on tubulin polymerization activity and cytotoxic behaviour, with the exception of a-branched N-acyl substituents, which show reduced activity.

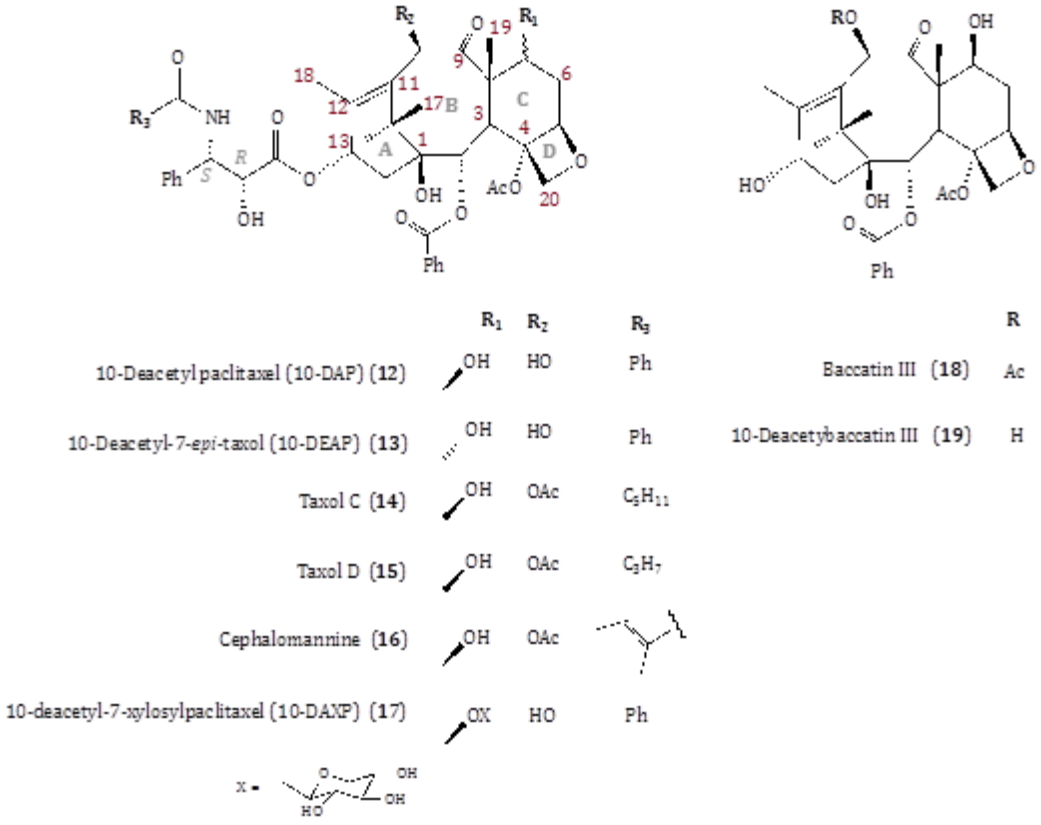


Figure 5. Selected naturally occurring taxane compounds.

Figure 6 summarises key variations at each position. C7 manipulations returned similar activity for the OH and xylosyl as did the C7-OH epimer, suggesting that neither stereochemistry nor steric bulk at this position adversely affect biological activity. A similar outcome was observed across the C10 analogues with the free –OH and ester analogues active. Modifications at C2, e.g. tigloylation showed retention of tubulin binding activity but a reduction in cytotoxicity (relative to paclitaxel). Synthetic studies modifying C2 suggest that this loss of activity is a consequence of the α-methyl substituent [49].

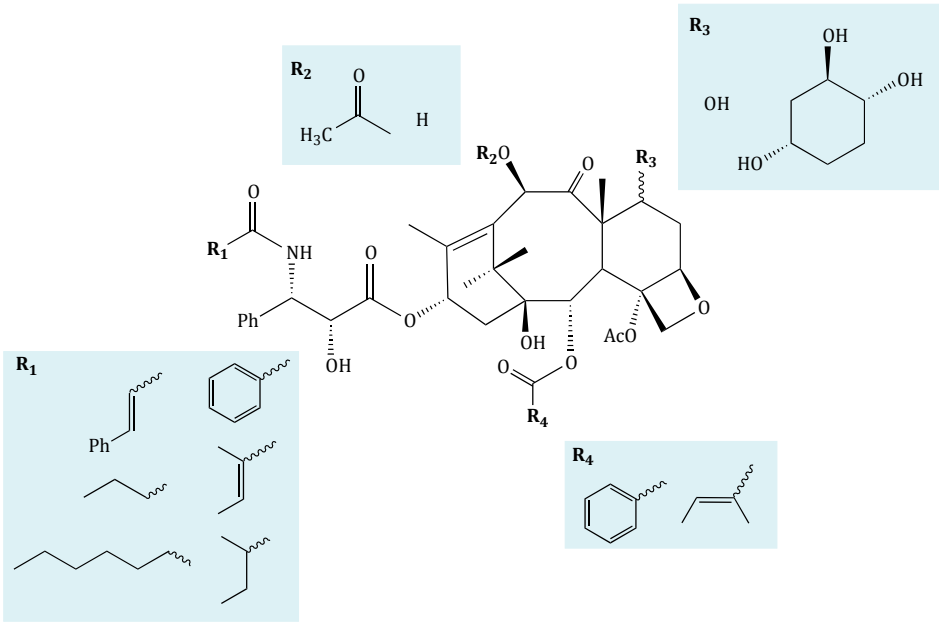


Figure 6. Selected natural derivative variations at key points within the taxane skeleton.

3.5. Synthetic Taxane Derivatives

Synthetic modification of the taxane scaffold has elucidated additional key SAR features with multiple families of semi-synthetic taxanes now reported (Figure 7). In these semi-synthetic taxanes, removal of the C2 ester moiety reduces efficacy, as does epimerisation at this centre. The steric and electronic effects of C2 benzoyl esters gives rise to varying levels of activity. Activity is reduced on introduction of aliphatic esters, but retained with alkenyl esters. Bioisosteric O→S or Se interchange within the oxetane ring and ring opened analogues show reduced activity. This suggests a possible H-bond accepting role for the oxygen atom. The introduction of pyridine or pyrrole rings reduces potency.

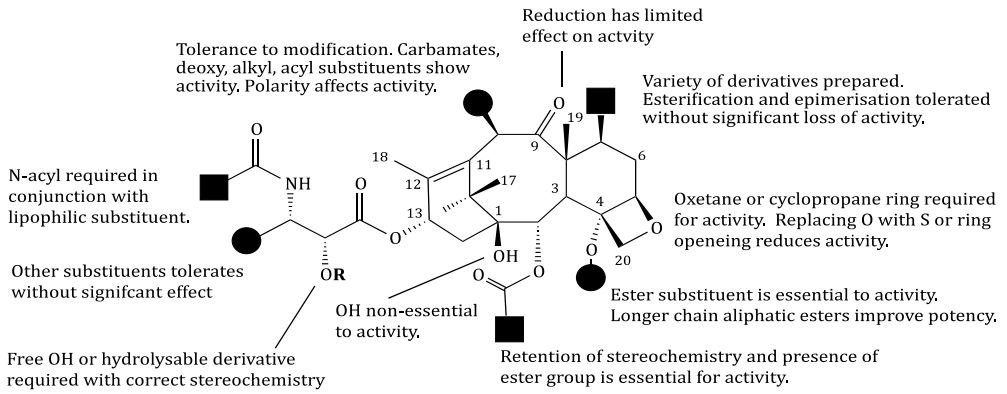


Figure 7. Summary of Structure - Activity relationships derived from synthetic taxoid studies.

The C7 substituent is the most easily altered position and has resulted in multiple chemical modifications giving deoxy, ethers, esters, carbamates, fluorination and azidated analogues. Ether analogues result in a loss of both microtubule and cytotoxic activity, particularly when polar substituents are incorporated. Thiol and thioether epimers deliver striking changes in potency, particularly between epimers. Ester derivatives generally deliver excellent microtubule activity. Dehydroxy analogues generally reduce tubulin polymerisation activity but improve toxicity. Replacement of OH with F or N_3 produces no significant change in activity. Oxidation to alkanone has limited effect. Removal of OH reduces activity in tubulin binding assay but does not affect toxicity. The C9 carbonyl moiety is typically unreactive while the cyclic C9-C10 carbonate derivative enhances microtubule activity. C9 carbonyl reduction increases tubulin-binding activity, improves water solubility with subsequent methylation further improving activity.

The C10 substituent has been successfully used to provide more favourable physicochemical attributes and is suitable for installation of enzyme cleavable groups. At C10, substituent polarity rather than steric bulk correlates well with activity and offers significant substrate tolerance with carbamate, deoxy and acetoxy substituents permitted. These data support a non-pivotal role for the C10 oxygen. Retention of the C4 ester is essential for cytotoxic activity and is an essential element in receptor binding. The C4 OH derivative is inactive while the deacetoxy derivatives exhibit significantly reduced tubulin assembly activity and cytotoxicity. Butyl and cyclopropyl ester chains exhibit greater potency, suggesting that they interact with a hydrophobic binding pocket.

Of the synthetically accessible taxane core regions, the C13 moiety has been the most extensive investigated. Evaluation of C13 modified taxanes highlighted the crucial role of the ester linkage, which is essential in the maintenance of both microtubule binding and cytotoxic activities. C13 ester chain simplification generally reduces both microtubulin binding and cytotoxic activities. Both the 2'-OH and ester linkage to the taxane A ring were essential structural elements in the maintenance of microtubule binding and cytotoxic activities. The 3'-phenyl moiety is highly substituent tolerate with incorporation of alkyl and alkenyl groups and rings heteroarene ring systems permissable. Presence of *N*-acyl group is required for activity but, as with the 3'-Ph moiety, a range of alkyl, alkenyl, arene, heteroarene substituents were tolerated. The rank order of potency for the C2', C3' diastereoimers was 2'R, 3'S > 2'R, 3'R ~ 2'S, 3'S >> 2'S, 3'R with respect to microtubule disassembly. Chain extension was detrimental to activity.

3.6. Commercial Production of Paclitaxel

Paclitaxel's clinical efficacy led to significant shortages in supplies of the drug for both ongoing research and clinical studies. Direct isolation from the bark of the uncommon and slow growing Pacific Yew affords paclitaxel yields of ~0.02% w/w, rendering the long-term supply from this source uneconomic and unsustainable [50]. In efforts to redress critical supply issues, total synthesis routes were considered and started to emerge in the literature in 1994 (Table 1). The total synthesis of the paclitaxel was an unquestionable synthetic tour-de-force, but has proved largely to be a purely academic exercise, showcasing the skill and

perseverance of the synthetic chemistry community. Seven teams have reported the total synthesis of paclitaxel (Table 1). With total synthesis ranging from 36 to 66 synthetic steps, fully synthetic commercial production remains unrealised, and perhaps unachievable. However these syntheses have been pivotal to the development of commercially viable semi-synthetic production route for paclitaxel and its derivatives.

Table 1. Successful completed syntheses of Paclitaxel

Research Group	Institution	Steps	Year
Holton et al. [52,53]	Florida State	46 (linear)	1994
Nicolaou [54,55,56,57,58]	Scripps research Institute	40 (3 conv. parts)	1994
Danishefsky [59]	Sloan-Kettering Institute	53 (2 conv. parts)	1996
Wender [60,61]	Stanford University	40 (linear)	1997
Kuwijama [62,63]	Tokyo Institute of Technology	66 (3 conv. parts)	1998
Mukaiyama [64,65,66]	Tokyo University	61 (linear)	1998
Takahashi [67]	Tokyo Institute of Technology	36 (linear)	2006

Other targeted approaches to the production of paclitaxel have included the investigation and cultivation of Yew cultivars developed for high paclitaxel (or a suitable paclitaxel precursor) content for the foundation of commercial plantation facilities, and exploring biotechnology-based means of production. These evaluations examined the chemical composition of the branches and needles and identified paclitaxel and related taxanes including baccatin III (BAC III) (18), cephalomannine (16), 10-deacetylbaccatin III (10-DAB III), (19) and 10-deacetyl-7-xylosylpaclitaxel (10-DAXP) (17). Crucially these paclitaxel precursors could be synthetically modified to yield paclitaxel, and were present in sustainably harvestable parts of Yew plants (Section 2.7) [68,69]. Table 2 shows approximate content of key taxanes (as percentage yield isolated) from selected yew species [70].

Table 2. Approximate percentage yields for isolation of selected taxanes from Yew leaves by species [71]

Taxus Species	10-DAB III	BAC III	Cephalomannine
T. baccata	0.02	0.0004	0.002
T. brevifolia	0.01	0.00078	0.00054
T. canadensis	0.002	0.00073	0.0043
T. chinensis			0.0058
T. cuspidata		0.00022	0.0015

The semi-synthesis of paclitaxel is a global operation with plant biomass harvested from commercial plantations in Italy extracted to yield 10 DAB III (16) which is shipped to Ireland for conversion to paclitaxel (via side chain addition) followed by exportation to Puerto Rico for formulation, packaging and distribution (Figure 8) [72].

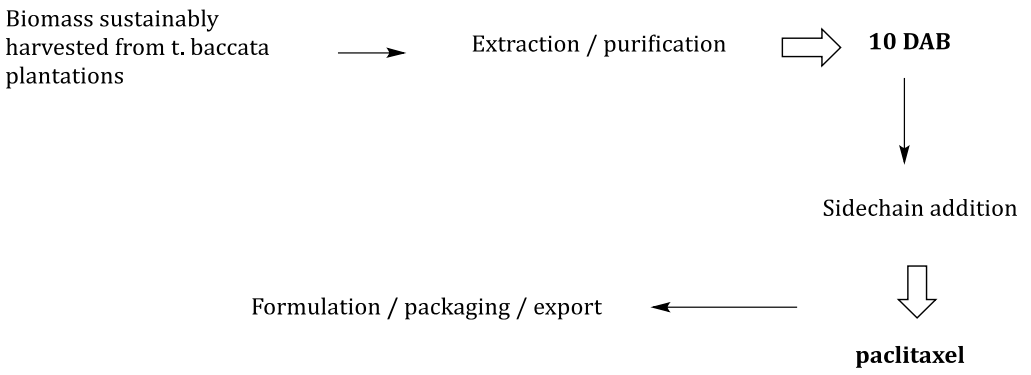
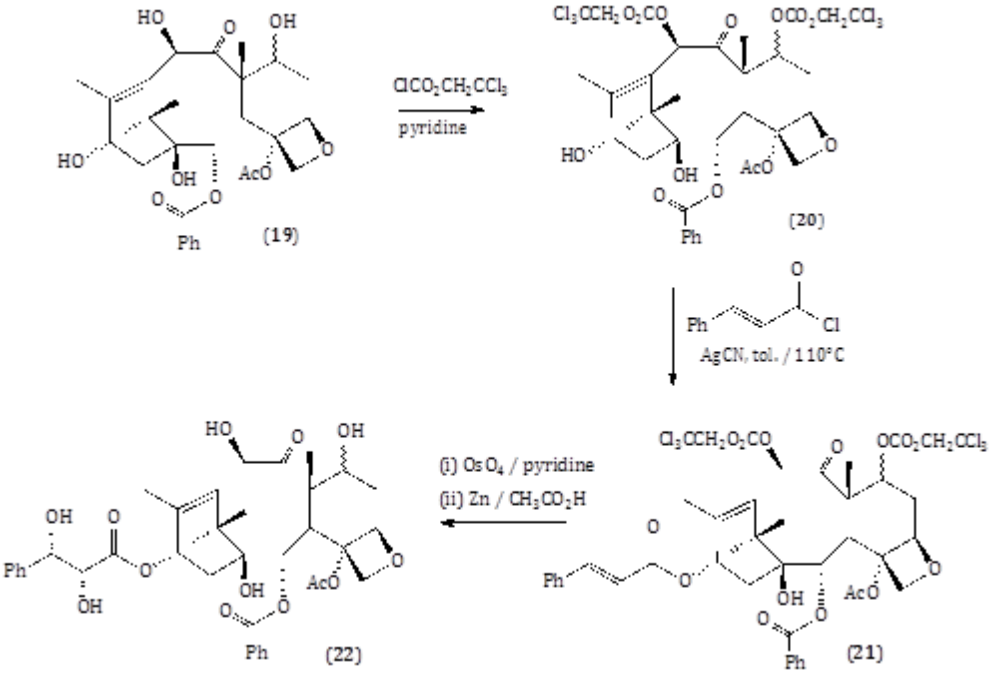


Figure 8. The paclitaxel production cycle.

3.7. Semi-Synthesis from Baccatin III

The sustainable availability of the baccatin III skeleton presented a potential means of overcoming the chronic paclitaxel supply shortage. However, despite high structural homology with paclitaxel, specific C13-OH esterification and both regio- and stereo- control of the C2' and C3' positions were synthetically challenging baccatin III modifications.



Scheme 1. Potier's synthetic strategy for the paclitaxel C13 tail unit from baccatin III (19) [74].

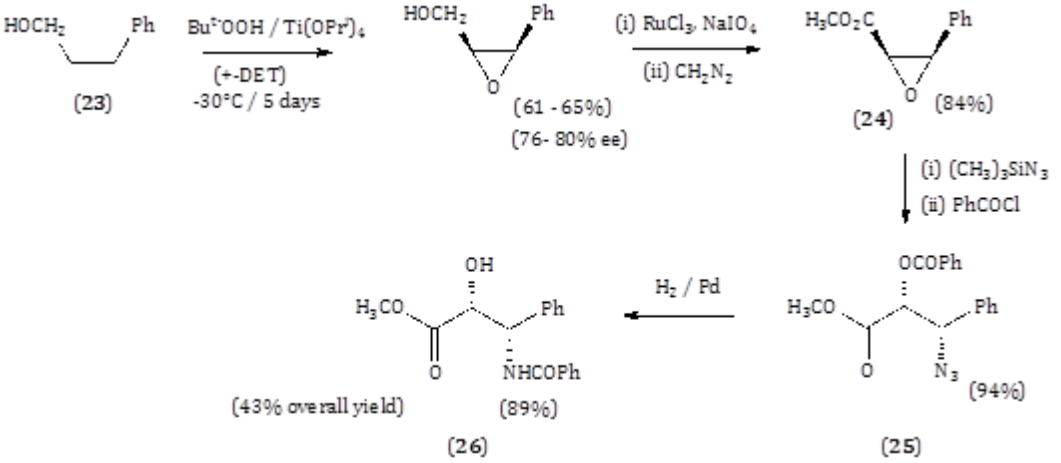
Potier's pioneering efforts in the acylation of both baccatin III and 10-deacetylbaccatin III identified the C7 and C10 –OH moieties as significantly more reactive than the C13-OH. The rank order or reactivity was, C7 > C10 >> C13, with no acylation of C1 observed

[73,74]. Potier's work laid much of the ground work for paclitaxel synthesis from deacetylbacctin III (19) and correspondingly baccatin III (18) with prior deacylation, through selective trichloroethyl chloroformate (troc) esters protection of C7 and C10 (20), enabling the installation of a cinnamoyl ester (under forcing conditions) at the C13-OH (21). Subsequent OsO_4 mediated dihydroxylated yielded a diasteriomeric product mixture (22) (Scheme 1).

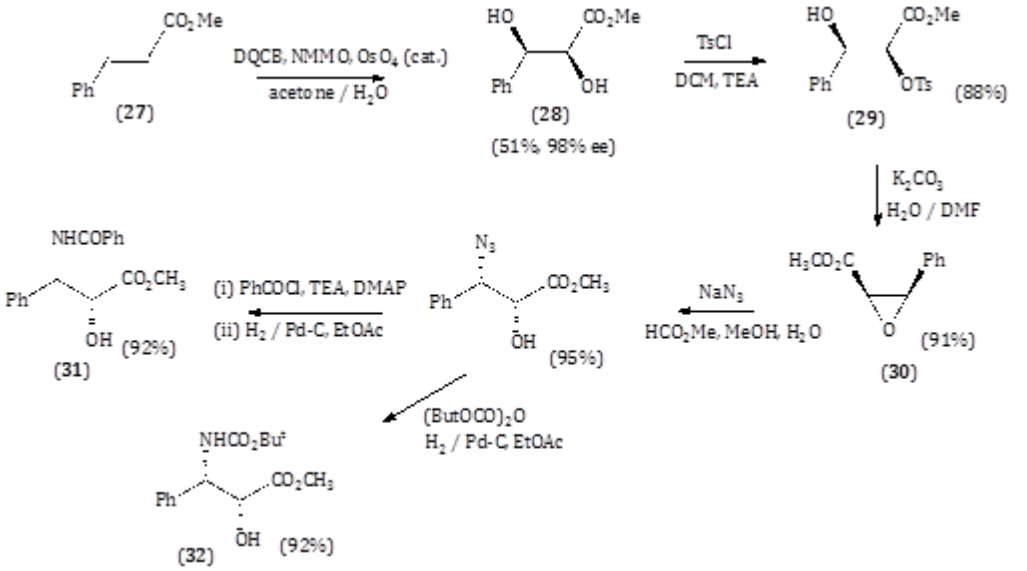
Sharpless hydroxyamination was examined as a potential means for the introduction of the C3' amino moiety to 21, but the reaction was neither regio- nor stereoselective, yielding a 1:1 mixture of 2'- and 3'- amino isomers together with their respective dihydroxylated diasteriomers (18) [75]. Addition of an amine chiral auxiliary enhanced both regio- and stereocontrol (up to 80% of the desired stereoisomer) under Sharpless conditions from the cinnamoyl ester (17) [76]. While the regio- and diastereo- isomers were less active than paclitaxel, a tertiary butyl carbamate derivative displayed significant *in vivo* anti-cancer activity and is currently marketed under the trade name Docetaxel (taxotere (RP56976)). Docetaxel is FDA approved for the treatment of a range of cancers including metastatic breast cancer, gastric cancer, head and neck tumours, hormone-refractory prostate cancer and non small-cell lung cancer [77].

Synthesis of the Paclitaxel Side Chain

Given the issues with late stage introduction of the C3'-amino substituent, a number of teams have develop asymmetric strategies to the amino tail for subsequent installation with the required stereochemistry present. Sharpless epoxidation of *Z*-cinnamyl alcohol (23)((+)-diethyl tartrate, *t*-butyl isopropoxide / $TiOPr_4$) introduced the stereo and regio-selectivity requirements to the C13-side chain in 61% yield and 78% e.e. (Scheme 2). Conversion to the epoxy methyl ester (24) followed by regio- and stereoselective ring opening with an azide nucleophile gave the azido derivative (25), which was subsequently reduced and benzoylated *in situ* by an O to N benzoyl migration (26) prior to final attachment to bacatin III [78].



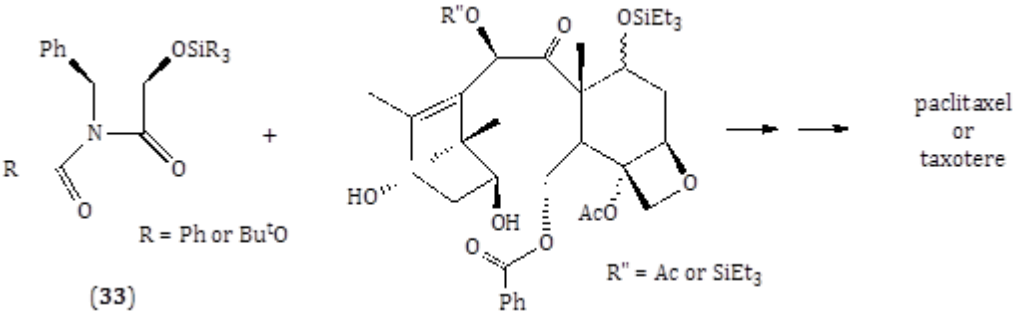
Scheme 2. Denis et al. synthesis of paclitaxel C13 side chain moiety [78].



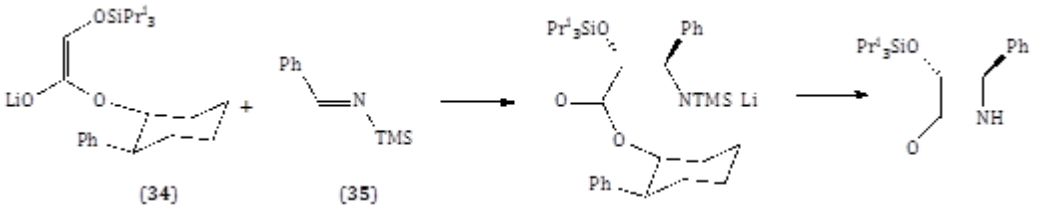
Scheme 3. Improved synthesis by Denis et al. [79] of paclitaxel and taxotere C13 side chain moieties.

A more efficient side chain synthesis can be conducted via Sharpless dihydroxylation of trans methyl cinnamate (27) affording 2S, 3R-vicinal diol (28), which could be readily purified by recrystallization (51%, >98% ee) [79]. The 2R, 3R-epoxide (30) was prepared in a diastereosomerically pure form from mono-tosyl derivative (29) in 80% yield. The previously described azide-based ring opening and benzoylation gave the paclitaxel side (31) chain in 35% overall yield. The taxotere side chain (32) was also produced in similar yield by this approach (Scheme 3).

Commercial paclitaxel production by semi-synthesis is accomplished by direct acylation of the baccatin III C13-OH group using an optically pure β-lactam (33) (Scheme 4) [27,30] with 33 capable of being produced in high efficiency (3 steps) and yield (~80%) and importantly in excellent optical purity at 100% e.e. (Scheme 5) [80,81]. The enantiomeric purity of the product is driven by a combination of Si-protecting group and chiral auxiliary within the starting material, which selectivity directs the approach of the N-TMS-imine (35) to a single face of the enolate ester (34). The key precursors are available through enzymatic hydrolysis of a racemic precursor facilitating commercial scale-up [82].



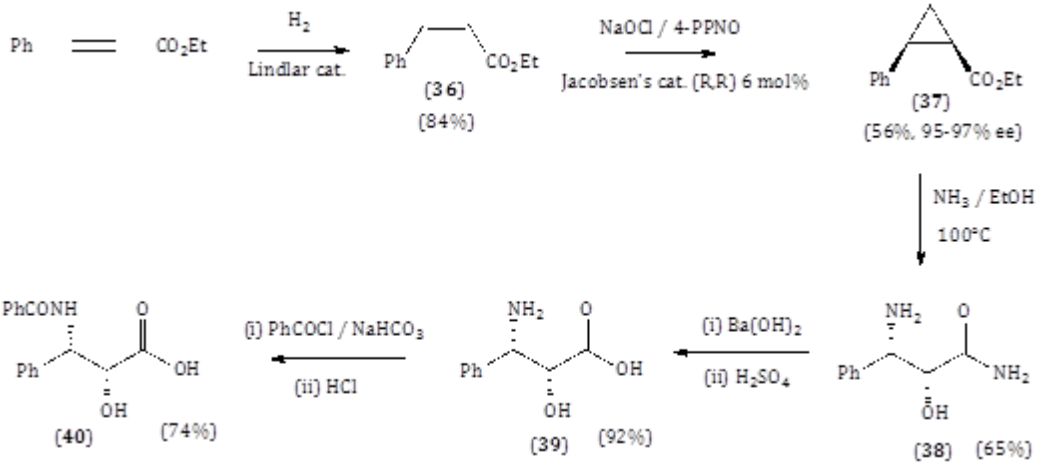
Scheme 4. Attachment of paclitaxel side chain moiety using a chiral lactam precursor [83,84,85].



Scheme 5. Ojima's stereoselctive production of lactam precursor [80].

Other paclitaxel tail synthetic approaches include chiral epoxide intermediate pathways developed by Commercon [86,87] and Jacobsen [88]. Jacobsen's approach (Scheme 6) offers opportunity for commercial development through its use of efficient catalytic procedures to generate chiral intermediates from low cost reagents. Using commercial bleach as the oxygen source, Jacobsen's catalyst (6 mol %) afforded the R, R-(+)-epoxide (37) from *cis* ethyl cinnamate (36) in 56% yield (95-97% ee). Although low levels of trans isomer formed, this could be carried through, without purification. Regioselective epoxide opening with ammonia to amide (38) and subsequent benzoylation acid (39) afforded (2R, 3S)-3-phenylisoerinamide (40), which could be purified by recrystallisation for an overall yield of 28%.

The expiry of paclitaxel production patent exclusivity has seen work on the synthetic production of paclitaxel continue [89]. Patents, particularly in relation to stereoselective production of the C13 side chain continue to be filed and granted [90,91,92]. However, the ultimate economic value of these methods over current and developing production methods for paclitaxel remains questionable.



Scheme 6. Outline of the Jacobsen synthesis of the paclitaxel side chain moiety [88].

3.8. Other Production Methods

The growing demand for paclitaxel and the emergence of generic suppliers has stimulated additional explorations towards newer, biotechnology-based production technologies. Paclitaxel production by sustainable harvesting practices has encountered difficulty in the

establishment of yew plantations due to practicalities associated with large-scale propagation from auxiliary buds, nodal, internodal and leaf explants and the long dormancy of seed embryos [93]. The situation is further complicated by seasonal variation of the paclitaxel concentration present in leaf and stem tissues [94]. Micropropagation through *in vitro* germination of zygotic embryos of a number of taxus species has been successfully achieved, although careful control of germination conditions (such as light exposure [95]) and ongoing treatment of the resultant the embryos is to required to ensure high survival rates [96].

Biotechnology-based cell culture production methods have been a major focus or research, with taxus cell cultures first being reported in 1989 [97]. Thus far, cell suspension cultures has proved the most reliable, producing taxane yields in excess of that obtained by conventional extraction of plant biomass (0.8% c.f. 0.017% dry weight from *T. brevifolia*) [93,98]. Yields of 40 mg/g of dry taxus biomass have been reported [95].

Commercial paclitaxel production is currently shared between cell culture and semi-synthesis. [30] However, direct production from cell culture offers a potentially, long-term, and environmentally sustainable production strategy for paclitaxel and associated analogues, based on overall yields alone [70,99, 93]. Isolation of paclitaxel from cell cultures is efficient with up to 90% of paclitaxel secreted directly into the culture medium [100]. Work to improve commercial scale-up to enhance the ongoing viability of this approach continues [70].

Taxomyces andreanae, the first entophytic fungus shown to produce paclitaxel was isolated from the bark of *T. brevifolia* in 1993 [101]. Subsequent investigations have revealed the presence of endophytic fungi in other taxus species as well as plants of non-taxus origin. Approximately 200 endophytic fungi spanning more than 40 genera have been catalogued, with a small number shown to synthesise paclitaxel [102]. The exact mechanisms underpinning paclitaxel biosynthesis in these fungi remain unknown. Genetic studies, and genetic manipulation of endophytic fungi, are on-going in an attempt to establish whether the production pathways of secondary metabolites such as paclitaxel are an example of convergent or parallel evolution [103].

Biotechnology approaches utilising paclitaxel-producing fungi have been investigated with only limited success because of low productivity and difficulties associated with the scale-up of these methods to commercial production volumes [104,105,106,107]. These issues have led to speculation as to whether the presence of paclitaxel in these organisms is that of a secondary metabolite or is in fact a bioaccumulation product from the host plant [108]. Investigations into the feasibility of large-scale production of paclitaxel from endophytic fungi are on-going [109,110].

Commercial production of paclitaxel and other important taxane species using sustainable harvesting practices has been achieved after exhaustive investigations to identify renewable plant sources containing compounds capable of being efficiently derivatised [111]. Taxanes such as baccatin III (18), 10-deacetyl-7-xylosylpaclitaxel (10-DAXP) (17), 10-deacetylbaccatin III (10-DAB III) (19) and cephlaomannine (16) are now routinely converted to paclitaxel [24].

3.9. Taxol Formulation

Paclitaxel is moderately soluble in organic solvents (~ 46 mM in ethanol, ~ 20 mM in methylene chloride or acetonitrile and ~14 mM in isopropanol) [112], but is poorly solubility in aqueous media. Estimates of paclitaxel solubility in water vary considerably, ranging between 0.77 and 35 mM in various studies [113]. Paclitaxel additionally displays a time-dependent solubility association. The mechanism of this behaviour is unknown, but may represent the formation of paclitaxel polymorphs [114,113]. From a formulation and manufacturing perspective this behaviour poses significant problems.

Historically, intravenous paclitaxel formulations incorporated the surfactant Cremophor EL - a synthetic polyethoxylated castor oil derivative, to overcome solubility and stability issues. Paclitaxel was typically stored in a 5 mL vial at a concentration of 6 mg mL^{-1} in a Cremaphor EL / 50% ethanol solution that was subsequently diluted 5-20-fold in normal saline or 5% dextrose immediately prior to administration, resulting in a delivered concentration of 0.35 to 1.4 mM [113]. Cremophor EL use has presented patient issues, being linked to a range of adverse patient conditions including dyspnoea, flushing, rash, chest pain, tachycardia and hypotension [115,116]. Furthermore, Cremaphor EL was also found to promote the leeching of phthalates from infusion bags, resulting in a range of conditions including anaphylaxis, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes [117]. Other issues noted include incompatibility with other prescribed drugs [118], alteration of the biochemical properties of lipoproteins [119] and non-linear pharmokinetics resulting from Cremophor EL micelle formation *in vivo* [120].

Potential alternatives to Cremophor EL include Tween 80 and Pluronic L64, which both solubilise paclitaxel without adversely affecting physical or chemical stability in solution [121]. Paclitaxel emulsions derived from natural and synthetic media including corn oil, egg phosphotidyl- choline, cholesterol, triolein and polyethylene glycol (PEG) have all been successfully formulated, exhibiting comparable or improved stability and a reduction in the undesirable side effects associated with Cremophor EL [122]. Other techniques examined include micellisation (solubilising in non-covalently bound, biodegradable, hydrophobic carriers [123,124]), liposome and non-liposome based encapsulation, cyclodextrins and slow release biodegradable implant devices designed to be used in postsurgical situations to eliminate remnant malignancies. [122,125].

Abraxane is an albumin-bound, nanoparticle form of paclitaxel. Abraxane is FDA approved for the treatment of a range of cancers including breast cancer, non-small cell lung cancer and advanced pancreatic cancer. The use of albumin offers a number of advantages as a drug carrier, particularly in relation to increasing the efficiency of delivery of hydrophobic bioactives such as paclitaxel. These include the ability to:

- Preferentially accumulate in tumour tissues through the secretion of the albumin binding protein SPARC (Secreted Protein, Acidic and Rich in Cysteine).
- Undertake high affinity, non-covalent binding to non-polar bioactives for efficient transport and release kinetics, *in vivo*.
- Assist in the passive diffusion (through receptor binding) of agents such as paclitaxel into target cells (transcytosis) [126].

Abraxane permits the use over shorter infusion times relative to Cremophor EL (30 minutes vs. 3 hours), higher dose administration (260 mg/m^2 c.f. 175 mg/m^2) [127] and elimination of any pre-medication and the use of standard infusion sets [117]. Studies show that Abraxane transportation via albumin receptor (gp60)-mediated transcytosis into tumour cells occurs four times faster than the paclitaxel Cremaphor EL formulation [128]. While Abraxane eliminates the adverse side effects associated with Cremophor EL, it also leads to more rapid elimination of paclitaxel from the bloodstream [129]. However, Abraxane does not significantly improve the pharmacokinetics profile of paclitaxel and the production costs are currently significantly greater than existing Cremophor EL formulations. This limits its use in low- and middle-income economies.

Abraxane phase III trials have shown 30% improvement in the three-year survival outcomes in metastatic pancreatic cancer [130].

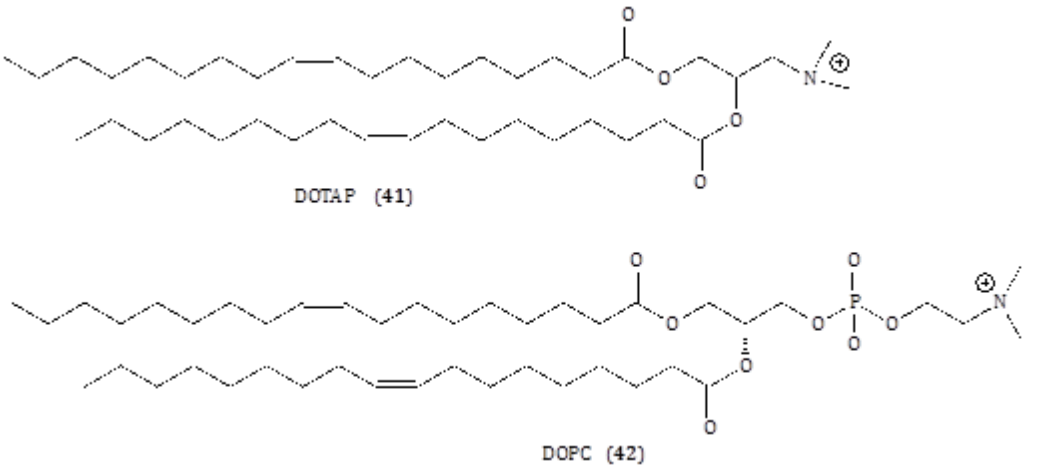


Figure 9. Structures of liposomal transfection reagents DOTAP (41) and DOPC (42).

New taxane drug formulations continue to be developed [131]. Of these, EndoTAG-I - paclitaxane in combination with the liposomal transfection agents DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl sulphate) (41) and DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) (42) (Figure 9) shows significant promise in pancreatic cancer treatment. The EndoTAG-I mechanism of action differs from other palclitaxel agents by targeting angiogenesis. EndoTAG-I acts as an endothelial cell delivery vehicle, where paclitaxel is released attacking endothelial cells during cell division, thereby inhibiting tumour blood supply, but having no impact on the endothelial cells of healthy tissue [132]. Recent phase II trials showed patients receiving medium or high dose EndoTAG-I in combination with gemcitabine had a significantly higher 12-month survival rate (36% and 33% respectively) over patients receiving gemcitabine alone (17%) [133]. Significantly, the use of EndoTAG-I allows a reduction in gemcitabine dose rates to occur without loss of anti-tumour activity [132].

3.10. Conclusion

The U.S. National Institute for Health Clinical Health website (ClinicalTrials.gov) identifies 2272 active, completed or recruiting paclitaxel clinical trials worldwide. Of these, 103 involve paclitaxel formulations targeting pancreatic cancer [134]. While progress remains somewhat limited in comparison to other, more high profile cancers, the development of new combination therapies and innovative delivery mechanisms shows significant promise. Increased understanding of the diversity of genetic factors associated with pancreatic cancer also suggests that novel more effective treatments will continue to emerge into the future.

4. Fluorinated Antimetabolites - Gemcitabine and 5-Fluorouracil

The nucleosides, deoxycytidine (43), deoxythymidine (44), deoxyguanosine (45) and deoxyadenosine (46) (Figure 10) are the fundamental building blocks required for DNA synthesis in all living systems. In addition to their structural role in DNA synthesis, deoxyribose nucleosides also possess activities associated with a range of key biochemical functions including neurotransmission, cardiovascular activity and molecular signaling [135].

The fluorinated pyrimidine analogues, 5-fluorouracil (6) and gemcitabine (7) are among the earliest examples of effective antimetabolite-based chemotherapeutic agents. Generally, antimetabolites induce cell death by targeting the S phase of cell growth. In both of the circumstances described above, drug activity relies on structural mimicry as the key to success. Critical to the success of 6 and 7 as cytotoxic agents was the H → F bioisosteric modification. Unlike the C-H bond, the C-F bond is one of the most stable found in organic compounds with a bond dissociation energy of 490 kJ mol^{-1}, rendering this bond stable to most biological reaction conditions, [136] and with antimetabolite-based chemotherapeutics, allows interruption normal cellar chemical processes, thereby enabling them to be used in a variety of cancer therapies including leukaemia, breast, ovarian and gastro-intestinal cancers [135].

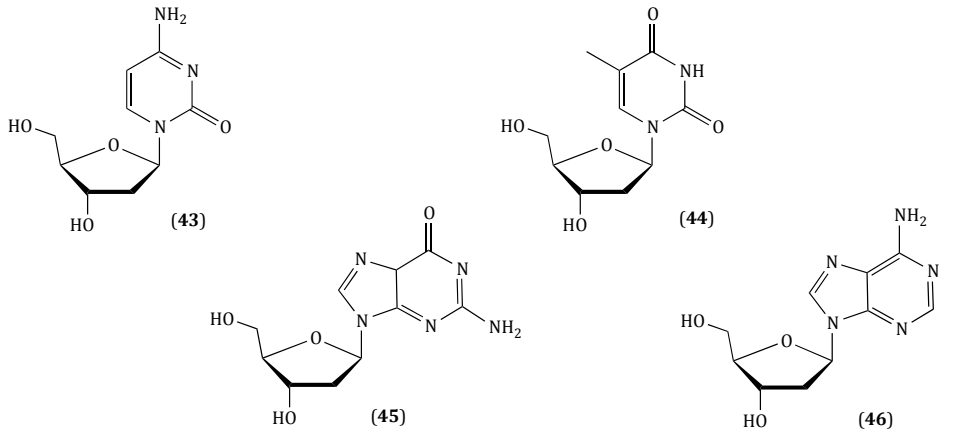


Figure 10. Chemical structures of deoxycytidine (43), deoxythymidine (44), deoxyguanosine (45) and deoxyadenosine (46).

4.1. Antimetabolite Drug Treatment

The size (1.51 Å vs. a H atom at 1.20 Å), electronic character and bonding properties of the fluorine atom offers considerable scope in drug design, permitting selective replacement of isosteric hydrogen or hydroxyl groups within either (or both) the nucleobase and sugar moieties of the nucleoside, which can significantly affect biological activity. Consequently fluorine containing nucleosides occupy an important position as structural scaffolds for the development of antiviral and anti-tumour drugs which, as DNA damaging agents, induce cell death in neoplastic cells through apoptosis [137]. This chemistry has been extensively investigated and reviewed [135,138,139,140,141]. This 'fluorine effect' has been exploited through –F modification of the ribofuranose C2'-H and -OH units of cytidine (47) yielding the anti-tumour agent gemcitabine (2'-deoxy-2', 2'-difluorocytidine) (7), with demonstrated efficacy against a range of solid tumours (Figure 11) [135,142,143].

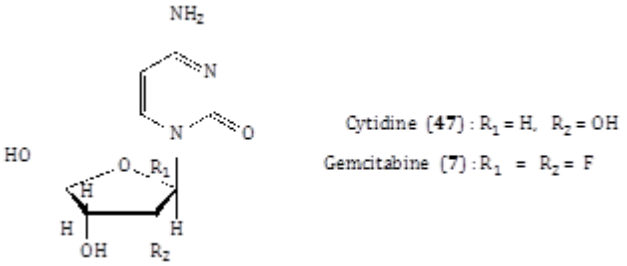


Figure 11. Chemical structures of cytidine (47) and gemcitabine (7).

Despite the success of fluorinated nucleosides in treating many forms of tumour, the systemic toxicity and susceptibility to drug resistance of these agents continues to raise questions about their long-term use and stimulates ongoing research to find safer, more acceptable alternative treatments [139].

4.2. Gemcitabine

Eli Lily's gemcitabine, trade name Gemzar, is currently used, either as a single agent or in combination with other agents, for the treatment of cancers including pancreatic, bladder and non-small cell lung carcinoma. [141] While clinically successful, Gemzar presents poor, low pH stability, requiring intravenous rather than oral administration (1000-1250 mg/m^2 per week for 3-4 weeks) and poor pharmacokinetic behaviour (low $t_{½}$). Reports highlight long-term drug storage issues, suggesting that while reconstituted solutions of the drug are room temperature stable for up to 35 days, counter-intuitively, refrigeration promotes irreversible crystallisation from solution [144].

4.2.1. Mechanism of Action

Gemcitabine (dFdC) is a cell-cycle phase specific agent, inhibiting cell proliferation in DNA synthesis at both the S phase and G1/S-phase boundary. Gemcitabine itself is a pro-drug which is activated upon monophosphorylation by deoxycytidine kinase (dCK) (Figure 12).

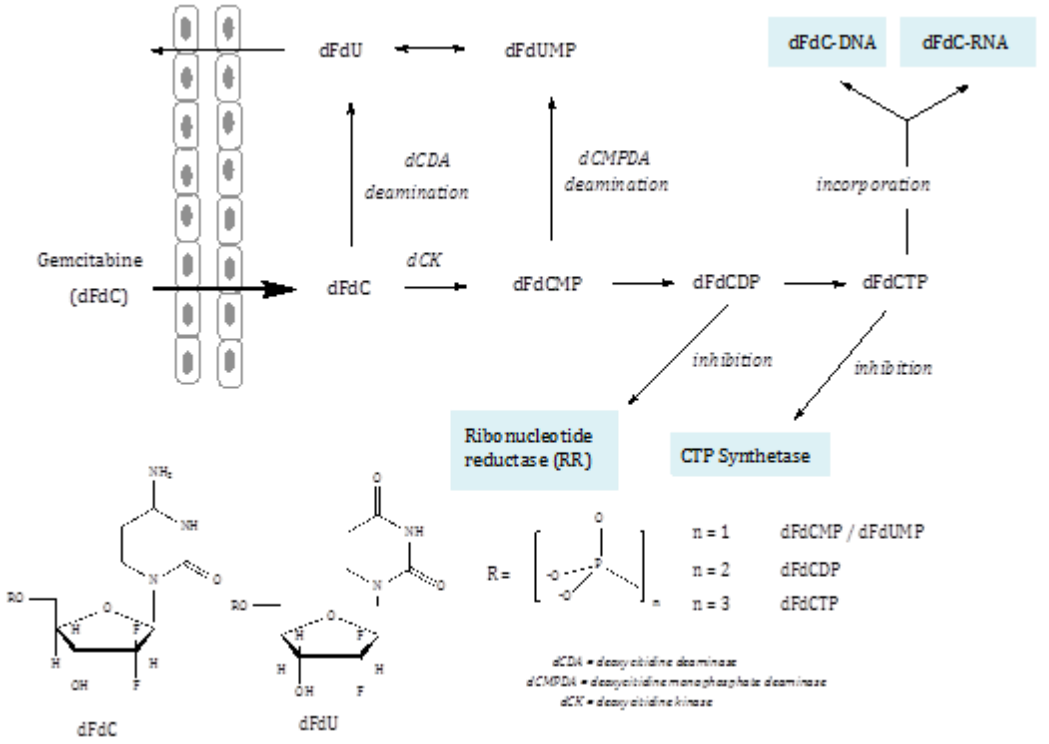


Figure 12. Metabolism and mechanisms of action of gemcitabine within the cell.

The production of gemcitabine monophosphate (dFdCMP) is the rate-limiting step for subsequent phosphorylation steps, thus controlling the pyrimidine kinases production, and intracellular concentration, of gemcitabine triphosphate (dFdCTP). dFdCDP itself inhibits the action of ribonucleotide reductase (RR), the enzyme responsible for catalyzing the production of agents such as deoxycytidine triphosphate (dCTP) which are utilised in DNA synthesis and repair. With the production of dCTP inactivated, the ribonucleotide pool diminishes, which leads to depletion of the dCK feedback inhibitor cytidine triphosphate (dCTP), which enhances gemcitabine phosphorylation.

With the ribonucleotide pool reduced, dFdCTP is incorporated into the DNA chain as a false nucleotide. This inhibits the action of DNA polymerase and associated repairing enzymes, culminating in inhibition of DNA synthesis and ultimately cellular apoptosis [145]. Gemcitabine can be inactivated via ring amino to carbonyl conversion by deoxycytidine deaminase (dCDA) and deoxycytidine monophosphate deaminase (dCMPDA). The former enzyme is the main gemcitabine deactivation and clearance pathway [146].

4.2.2. Synthesis

Initial inspection suggests that the synthesis of gemcitabine should present relatively few synthetic obstacles to commercial production (Figure 13). The two ring systems of gemcitabine may be derived from cheap and readily accessible starting materials. The three stereogenic centres (C1, C3, C4) present within the molecule are contained within the sugar moiety, allowing for considerable flexibility in synthetic design. This is further aided by the

geminal configuration of the two ring fluorine atoms at C2, which reduces structural complexity by eliminating inherent chirality.

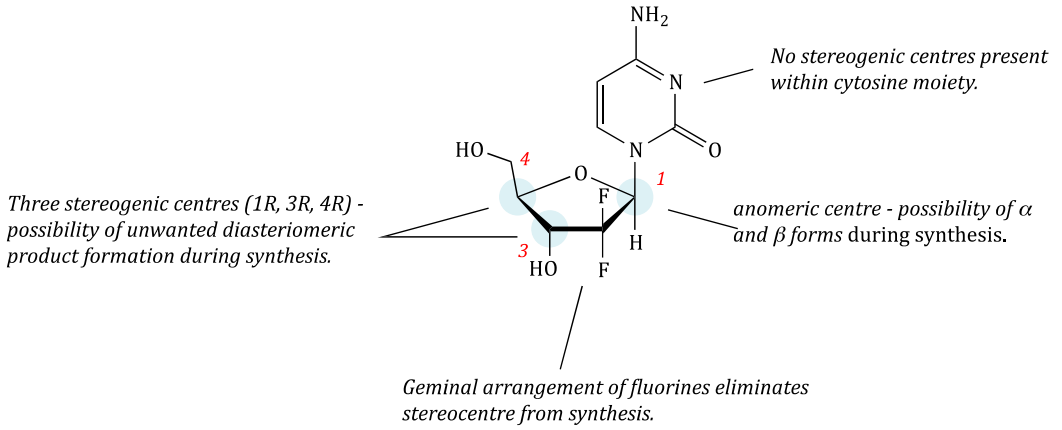


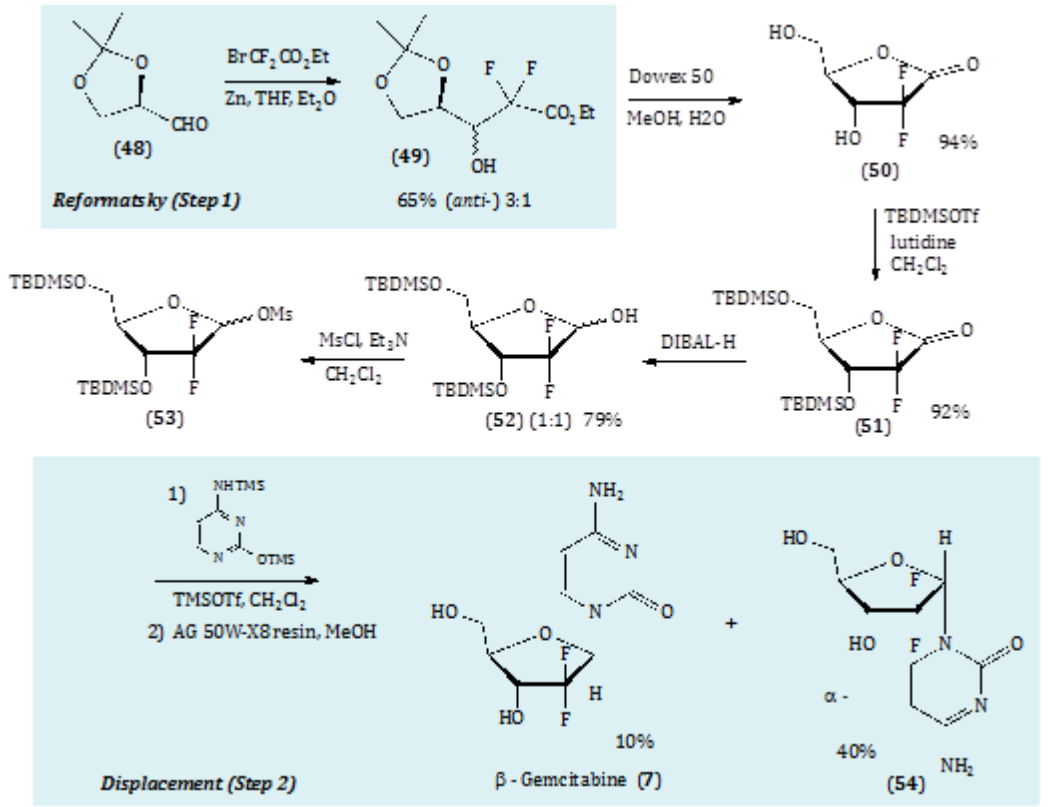
Figure 13. Summary of standout structural considerations associated with the synthesis of gemcitabine (7).

The synthesis of gemcitabine has been devised using three strategic approaches: 1) stereoselective construction of the fluorinated ribofuranose skeleton followed with subsequent attachment of the cytosine base through displacement of a suitable leaving group at the anomeric carbon centre; 2) Selective fluorination of an existing sugar moiety containing appropriate stereochemistry, followed by introduction of the base unit; and 3) synthesis of the nucleoside core followed by selective C2 difluorination.

4.2.2.1. The Hertel Synthesis

Initial approaches to gemcitabine commenced with the stereoselective synthesis of the protected difluorinated ribofuranose (55) for coupling with the TMS-protected pyrimidine core [147]. The critical element was the creation of the correct C1, C2 and C4 ribose stereochemistry. The C4 configuration was installed using enantiopure D-glyceraldehyde acetonide (48) [158]. Reformatsky coupling with ethyl bromodifluoroacetate gave a 3:1 mixture of the *anti*- and *syn*- diastereoisomers (49) (Scheme 7). The *anti*-49 possesses the required gemcitabine C3-stereochemistry [149]. Chromatographic separation afforded *anti*-51 in a 65% yield.

With pure *anti*-51 in hand, simultaneous deprotection and cyclisation to γ-lactone (50) was then accomplished using Dowex 50 resin. Silyl protection of the C3/C4-OH moieties gave 51, which on lactone reduction gave a 1:1 mixture of α and β lactol anomers (52) [150]. This anomeric mixture was converted to mesylate 53, a pivotal step as the α-F moieties significantly deactivates C1. Coupling of the protected cytidine to mesylate 53, followed by deprotection (Step 2) gave β-gemcitabine (7) and α 54 in 50% yield, as a 1:4 anomeric mixture. Despite extensive efforts by Hertel et al., all efforts to improve the yield and anomeric ratio distribution were unsuccessful [151].



Scheme 7. The Hertel synthesis of gemcitabine [147].

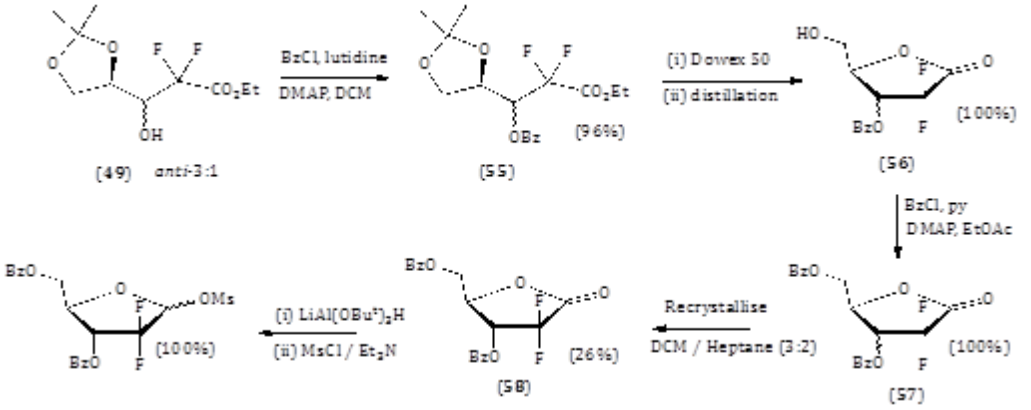
While the Hertel synthesis afforded access to gemcitabine, there were clear deficits both in terms of enantiopurity and overall chemical yield. [141] Significant efforts have been expended to improve the commercial viability of gemcitabine synthesis and these are discussed below.

Improvements to the Selectivity of the Reformatsky Reaction: Step 1.

Lin and Li [152] through a combination of ultrasonic agitation of the reaction mixture at a low temperature (12hr, 10-12°C), and using iodine as an activator, synthesised the anti-51 product in good yield (75%) and high diasteriomeric purity (98% d.e.). Moreover, the use of NaI in the nucleoside coupling step of the Hertel synthesis reversed the anomer product ratios, leading to a 2:1 ratio of the desired β-nucleoside.

Diasteriomer separation by crystallization (choice of OH protecting group).

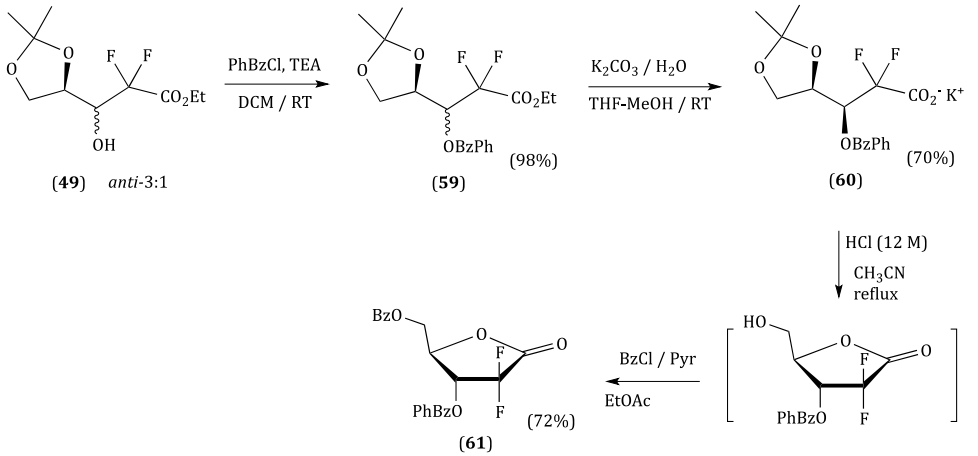
Diasteriomer formation during the Reformatsky step of the Hertel synthesis prohibits up-scaling of the reaction to commercial levels without a suitable and cost effective physical separation procedure. Hence, there has been considerable interest in developing synthetic processes employing protecting groups that yield solid products amenable to purification by crystallisation.



Scheme 8. Chou et al., [153] synthetic strategy for the production and purification of difluororibose gemcitabine precursor 58.

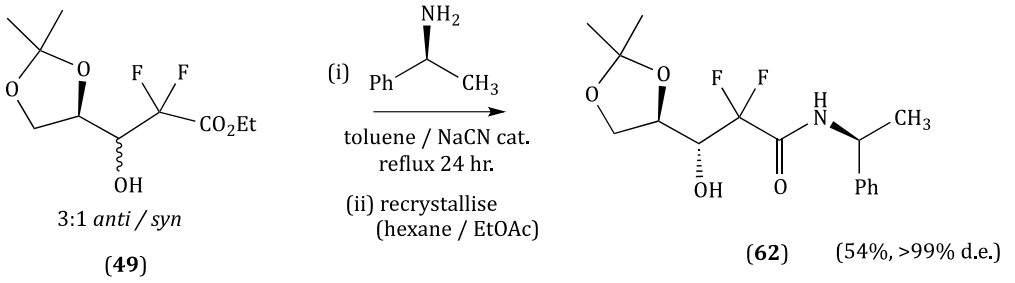
Replacement of the original the TBDMS protecting groups with benzoyl esters permitted the selective, and kg-quantity scalable, crystallisation of the desired erythro diasteriomer (58) from a 3:2 mixture of dichloromethane / heptane in a 26% yield (Scheme 8). Comparable strategies have been developed using *m*-fluorobenzoyl protecting groups to protect both alcohol moieties of the cyclised lactone product as a diastereoisomeric mixture in a single step. Erythro-58 was isolated on recrystallisation from ethyl acetate / hexane in a 46% yield and > 98% de [154]. A cinnamoyl ester strategy gave the corresponding cinnamoyl protected erthro-58 in a 43% yield and 99.3% de [155].

More recently the benzoyl ester approach has been extended to the separation of the Hertel Reformatsky ester products [156]. Taking a lead from prostaglandin synthetic approaches [157,158], Reformatsky product mixture 49 (57%, 3:1 *anti*: *syn*; Scheme 7) was protected as the *p*-phenylbenzoyl ester derivative (59) and hydrolysed to give the potassium salts, which crystallised yielding exclusive *anti*-60 (70%) (Scheme 9). Lactonisation followed by reprotection of the C4 OH afforded (61) (72% - two steps).



Scheme 9. Chang et al., [156] strategy for the separation of *anti*-Reformatsky ester products (49).

Isolation of anti-49 has been accomplished via diasteriomeric resolution of the Reformatsky ester mixture with *S*-(-)-a-methylbenzylamine, followed by recrystallisation of the crude amide mixture from hexane /ethyl acetate (54%, >99% de) (Scheme 10) [159].



Scheme 10. Park et al., [159] strategy for the separation of *anti*-Reformatsky ester products (49).

(i) Fluorination of the Carbohydrate Ring

As an alternative to the regio- and stereo- selective construction of the gemcitabine difluororibose moiety (66), the direct fluorination of a pre-formed enantiopure ribose (63) (or a related sugar) moiety was considered highly attractive. Successful stepwise introduction of two fluorine atoms at C3 succinctly bypasses issues associated with the production and separation of unwanted diasteriomeric side products as both the arabino (64) and ribo (65) monofluoro diasteriomers would be ultimately both be converted to 66 (Figure 11).

Sequential electrophilic fluorination of the TIPs-protected 2-deoxy-D-ribonolactone (67) installed the required two fluorine atoms at C3 (69) in 51% yield (2 steps). Lactone reduction (69), mesylation (70), and coupling with a protected pyrimidine, essentially as described in the original Hertel synthesis gave β-gemcitabine in 17% over 6 steps (vs. the Hertel approach at 10 steps and 10% yield). The use of the bulkier TIPS (triisopropylsilyl) protecting group was thought responsible for the higher level of β□anomer formation (1:1 vs. 1:4 with Hertel) (Scheme 11) [160,161].

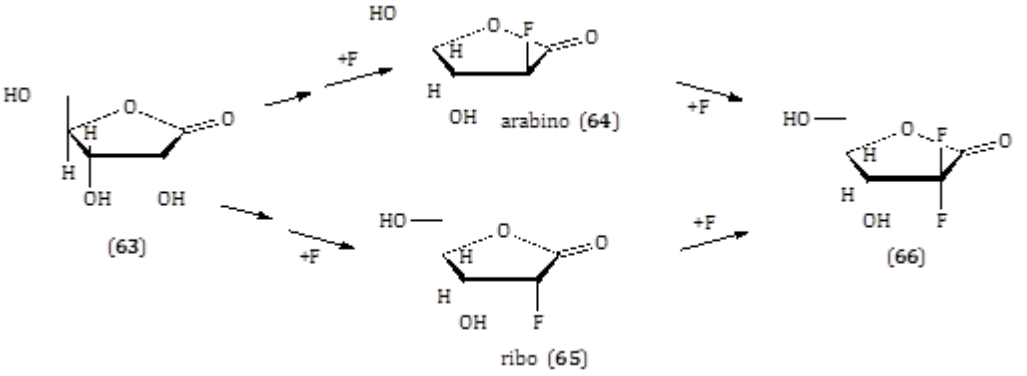
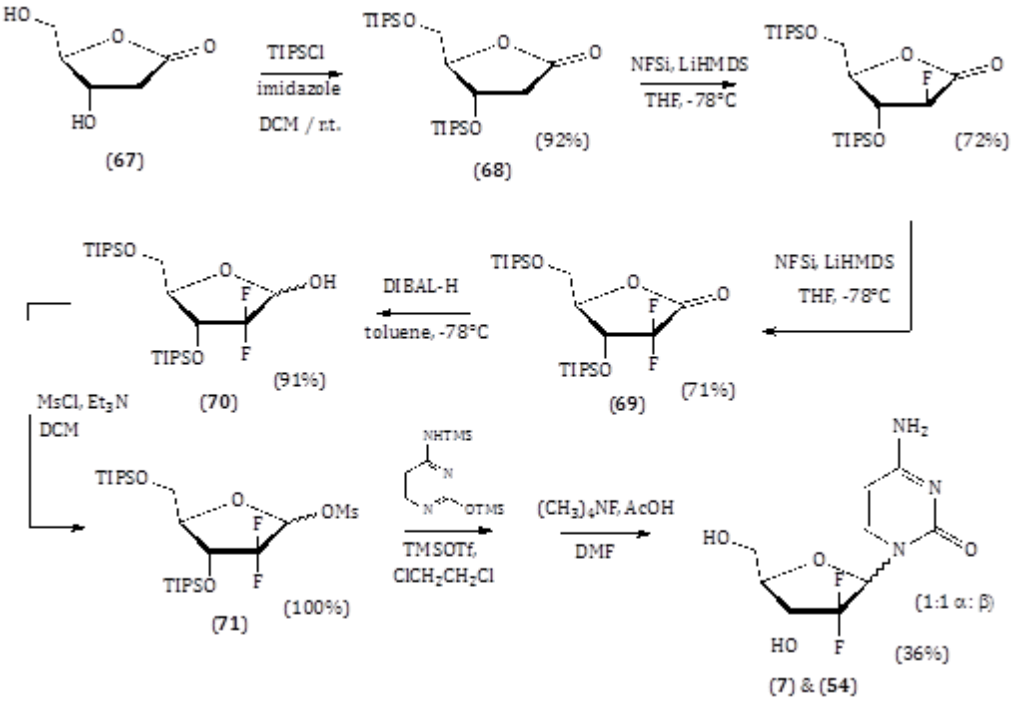


Figure 11. Synthetic strategy for the preparation of deoxyribolactone (65) by stepwise regioselective difluorination.



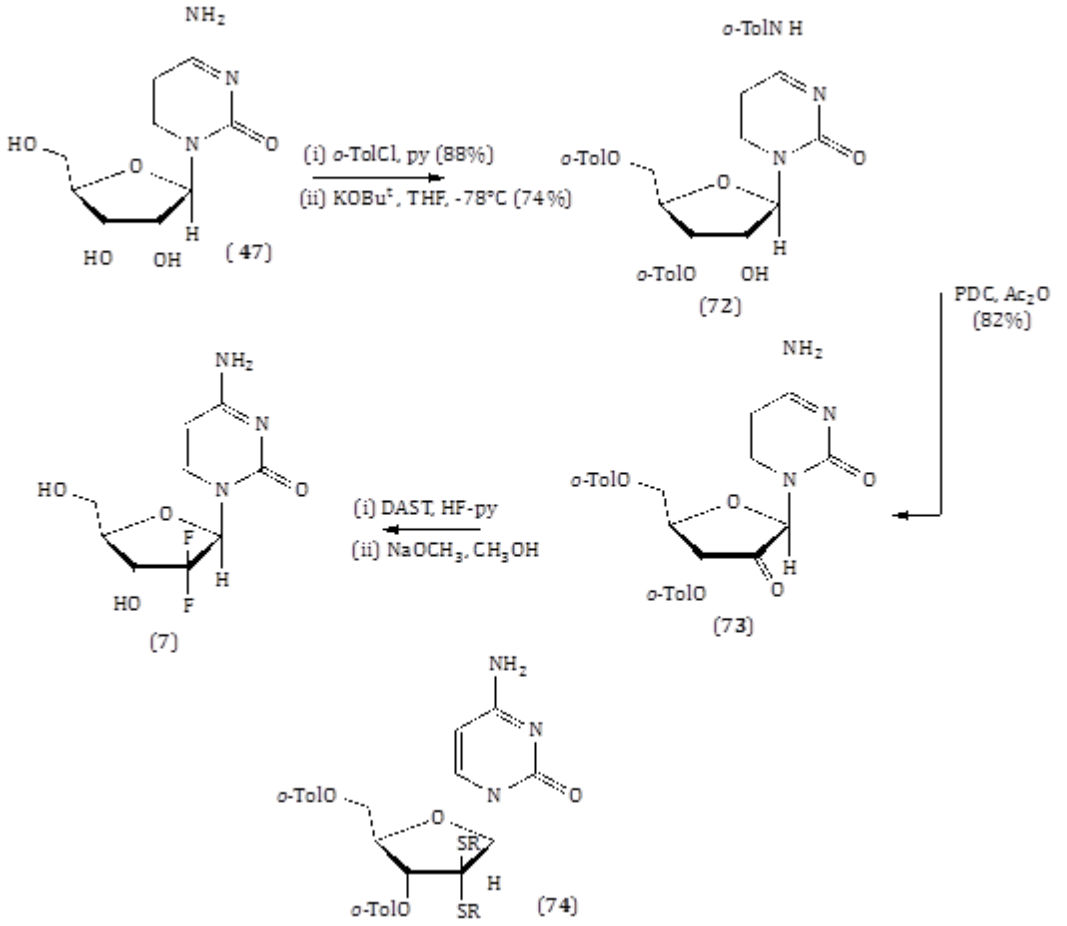
Scheme 11. Cen et al., [160, 161] synthesis of gemcitabine by regioselective difluorination of deoxyribose (67).

Difluororibose precursors are readily accessed from a range of naturally occurring carbohydrate starting materials including D-ribose, D-mannose and D-glucose. However, this chemistry is complex and beyond the scope of the discussion presented herein (see Brown et al. ref 141 for further details).

(ii) Fluorination of Nucleosides

Eli Lilly have developed a direct fluorination of a suitably derivatised cytidine to gemcitabine (Scheme 12). The success of this approach relied on development of protocols that selectively differentiates between the 2'- and 3'- hydroxyl groups on the cytidine (47) starting material and the identification and selection of protecting groups capable of surviving the reaction conditions required to difluorinate at C2'.

Conversion of 47 to the C'3 and C4' –OH protected 72 was accomplished through initial synthesis of a tri-o-touyl derivative and *in situ* regioselective C2'-deacylation using the bulky base, KO^{t}Bu [162,163]. PDC (pyridinium dichromate) oxidation of the C2'-OH afforded the corresponding ketone (73) in good yield (82%). Direct difluorination of the C2' oxo-moiety with DAST (diethylaminosulfur trifluoride) failed, even under forcing conditions, as did DAST treatment of dithioketal analogue (74), derived from 73. Gemcitabine (7) was eventually formed from 73 by the addition of a catalytic quantity of pyridine-hydrogen fluoride to the reaction mixture, which facilitated product formation following removal of the protecting groups under basic conditions (no yield reported).



Scheme 12. Kjell et al., [162, 163] synthesis of gemcitabine (7) by direct regioselective fluorination of cytidine (47).

(iii) Improving the Introduction of the Nucleoside Moiety

Arguably the most challenging problem in the synthesis of gemcitabine remains the diastereocontrol of nucleoside addition to the sugar moiety. With the exception of direct fluorination of cytidine itself, all synthetic strategies published to date afford a low overall yield of the desired β-gemcitabine (7) primarily as a consequence of poor stereocontrol, reduction and subsequent protection of the lactone precursor and the nucleoside base displacement of the leaving group. Two major factors affecting the ultimate anomer distributions are associated with the highly deactivated anomeric carbon, a consequence of two α-F moieties; and a lack of understanding of the displacement mechanism.

The original Hertel (1:4, β : α ratio) and even the Chou and Cen gemcitabine synthesis modification (1:1, β : α ratio) is clearly unsustainable from a commercial standpoint. Chou investigated the effect of temperature on anomer distribution ratios during production of the mestylate leaving group, seeking to improve production of the α-anomer to allow a S_N2 type displacement leading to β-Gemcitabine [164]. ^{19}F nmr studies on the reaction mixture showed that the concentration α-lactol in solution increased with decreasing temperature leading to an optimal ratio of 4.4:1 (α : β) at -83°C (Table 3).

Table 3. Production of anomer ratios as a function of temperature

Temperature (ºC)	Lactol anion ratio (α:β)
19	2.0 : 1
-3	2.3 : 1
-23	2.5 : 1
-43	3.0 : 1
-63	3.6 : 1
-83	4.4 : 1

Aprotic solvent conditions together with a large excess of the cytosine base were then employed to favour displacement of the α-mestylate group from the carbohydrate ring by the concerted S_N2 pathway [165]. Product yields ranged from 6 – 80% depending on reaction conditions employed with the best anomeric ratio, at 1 : 7.3 (α : β), observed at 115 ºC using 20 cytosine equivalents (Table 4).

Table 4. Effect of variations in the S_N2 coupling conditions on the gemcitabine anomer ratio outcomes

Solvent	Anomer	Cytosine (Eq).	T (°C)	Nucleoside ratio (α:β)	% Yield (β)
Xylenes	a-OMs	1.5	127	1.5 : 1	14
Xylenes	a-OMs	5	130	1 : 1.1	36
Xylenes	a-OMs	10	130	1: 2.2	50
Anisole	a-OMs	2	105	1.3 : 1	18
Anisole	a-OMs	3	105	1 : 1.3	22
Anisole	a-OMs	15	105	1 : 5.4	75
Anisole	a-OMs	20	115	1 : 7.3	80

Further enhancements in the anomer ratios to 30 : 1 were achievable through interconversion of β-mestylate to the α-mesylate through heating in the presence of an amine base. Lewis acids have been used to improve the stereoselectivity of nucleoside formation, with caesium sulfate and barium trifluormethanesulfonic acid producing excellent nucleoside anomer distributions (β : α) of 14.9 and 14.4 : 1 respectively, but in poor overall product yields (~25%). Other salts effected a higher conversion of reactants but at lower selectivity (Table 5) [166,167].

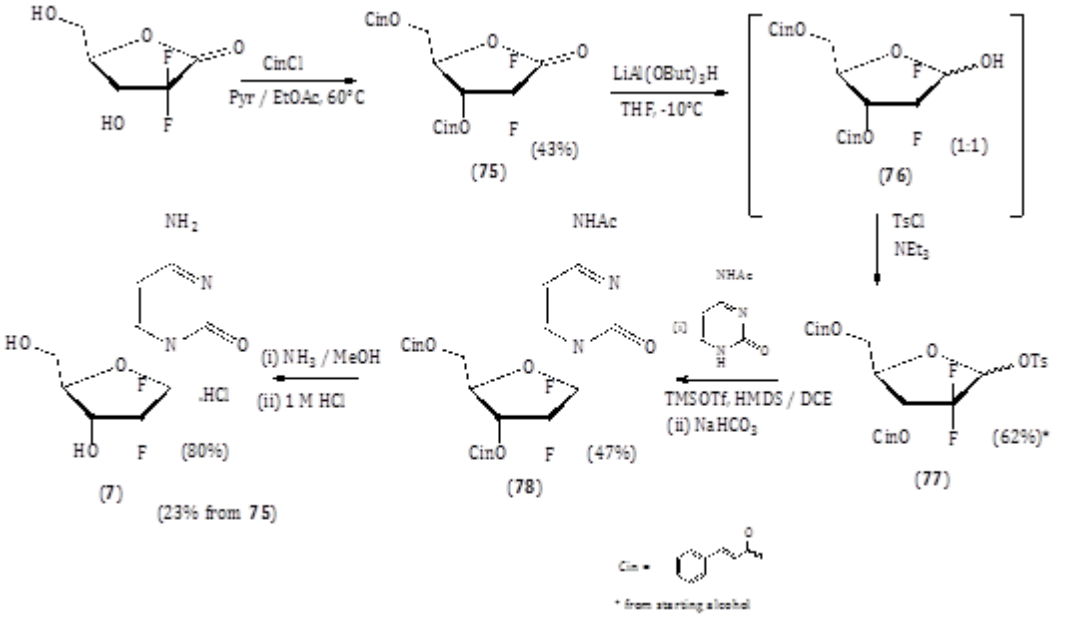
Table 5. Influence of Lewis Acid Structure on gemcitabine anomer production

Lewis Acid	Anomer Ratio (β : α)	Yield (%)
K_2SO_4	4.7 : 1	65
$(Bu)_4NTf$ *	7.1 : 1	45
$BaSO_4$	11.2 : 1	36
Cs_2SO_4	14.9 : 1	24
CsTf	7.2 : 1	65
$Ba(Tf)_2$	14.4 : 1	25
KTf	7.2 : 1	70

* Tf = Trifluoromethane sulfonic acid.

(iv) Other Leaving Groups

Tosylate, [155] halides (bromine, [156] iodine [168]), trichloracetaimidate [169] and esters have been employed to varying outcomes as alternatives to the mestylate leaving group in Hertel's original synthesis. The differing electronic and steric properties of each these reagents correspondingly exert influence over anomer distributions arising from their displacement by the cytosine base. Tosylation of a 1: 1 mixture of cinnamoyl protected lactol anomers (76) afforded (77) in a modest 62% yield, but displacement was essentially quantitative in giving (78). The complete absence of stereoselectivity suggested that this was a S_N1 process. Regardless, the resulting anomers were easily separable following deprotection and recrystallisation; with pure β-gemcitabine (7) isolated as a hydrochloride salt in 23% yield (from 75; Scheme 13).

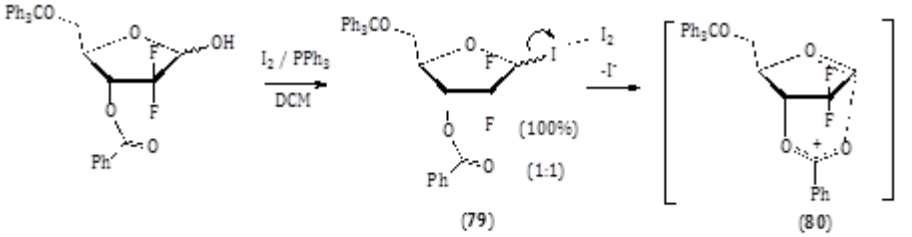


Scheme 13. Jiang et al., [155] gemcitabine synthesis by displacement of tosylate (77).

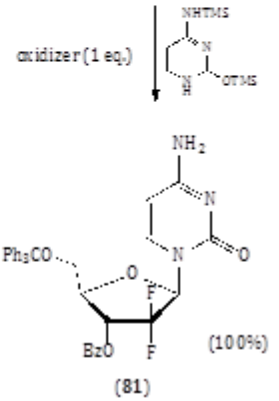
Both the α-bromo and α-iodofuranoses were formed from lactol derivatives – diphenylphosphate in the case of Br and mestylate in the case of I, although direct iodination of the lactol (I_2 / PPh_3) using iodine in the presence of a trialkylphosphine or trialkylphosphate was reported. With the bromolactone, anomeric selectivity appears to be driven by a series of S_N2-based reactions. Use of the bulky phosphate protecting group leads to preferential formation (11:1, β:α) of the corresponding β-phosphate, which is easily purified by recrystallisation from aqueous isopropanol. Concerted displacement of this phosphate by bromide ion produces the α-bromo lactol with similar selectivity (11:1, β:α). Introduction of the silyl-protected nucleoside under apolar solvent conditions (hexane) again favours stereoselective S_N2 displacement, resulting in β-gemcitabine production in 92% yield and in a 5.5:1, β:α anomeric ratio.

In contrast, iodo displacement from 79 was believed to proceed by an S_N1 pathway, facilitated by the presence of an oxidizing agent, which generates iodine in solution,

promoting loss of iodide ion from the furanose ring to form the oxonium cation 80. Selective addition of the cytosine nucleophile to the upper ring face was facilitated by the C3 benzoate ester, which both stabilised the carbocation and blocked the approach to the C1 centre from the lower face of the furanose ring (81). A range of oxidising agent / solvent combinations showed considerable variability in outcome, producing β : α anomer ratios ranging between 18 and 1.9 : 1 (Scheme 14).



Oxidizer	Solvent	Anomer Ratio
$NaIO_4$	CH_3CN	1.9 / 1
DTBP	Toluene	4.0 / 1
AIBN	Toluene	1.3 / 1
Oxone	Toluene	3.9 / 1
$K_2S_2O_8$	Toluene	5.4 / 1
$(NH_4)_2S_2O_8$	Toluene	5.9 / 1
$(NH_4)_2S_2O_8$	CH_3CN	18.0 / 1



Scheme 14. Influence of solvent conditions and choice of oxidising agent on anomer selectivity by displacement of iodo (79) [168].

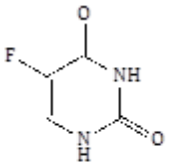
4.2.3. Conclusion

Gemcitabine has been successfully prepared via two key strategies; namely stereoselective production of the carbohydrate ring and selective fluorination of cytidine precursors. Hertel's original synthesis of gemcitabine, while successful in its ultimate goal, proved commercially impractical due to poor stereocontol and purification difficulties throughout the synthesis. Innovative solutions have been developed systematically overcoming each of these problem areas. As a consequence, gemcitabine can now be produced in large scale and good overall yield and with minimal by-product formation. While a fully stereoselective synthetic strategy for gemcitabine has yet to be developed, the expiry of patent protections and continued strong growth demand for the compound will continue to inspire researchers to overcome these issues.

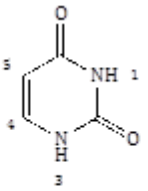
4.3.5. Fluorouracil

A number of key observations led to the conception of 5-fluorouracil (5-FU) (6), a pyrimidine analogue of the RNA nucleobase, uracil (70) (Figure 14). Early cancer activity studies noted that thymine deficiency in growing bacteria resulted in death [170]; and animal

models demonstrated that rat hepatoma tumours accumulated uracil at a significantly accelerated rate relative to normal tissue [171]. This led to the conception of 5-FU, a small molecule structurally similar to uracil, but with subtly altered chemical and electronic properties, which were subsequently shown to be effective in disrupting tumour replication mechanics. Despite first being synthesised almost 50-years ago, 5-FU remains a core weapon in the treatment of a broad range of solid tumours, including breast, gastric, pancreas, ovarian, colorectal, head and neck cancers [172,173].



5-FU: 5-Fluoro-1*H*, 3*H*-pyrimidine-2,4-dione (**6**)



Uracil: 1*H*, 3*H*-pyrimidine-2,4-dione (**82**)

Figure 14. Chemical structures of 5-fluorouracil and uracil.

4.3.6. Mechanism of Action

5-FU (6) has a multi-modal mechanism of action, affecting key cellular actions; most prominently inhibiting the action of thymidylate synthase (TS) - the 36 kDa protein responsible for the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). This is required reductive methylation of uracil's C5 H-atom, which is facilitated by 5,10-methylenetetrahydrofolate (5,10-MeFol) as the methyl donor. TS is a dimeric protein comprising two subunits, both of which possess nucleotide and folate binding sites. Crucially, this process supplies the sole *de novo* source of dTMP, which is a key contributor to cellular DNA replication and repair [174].

5-FU, like gemcitabine, requires cellular activation to elicit its biological activity. 5-FU on conversion to 5-fluorodeoxyuridine monophosphate (FdUMP), by the action of orotate phosphoribosyl-transferase, competitively binds to TS in combination with 5,10-MeFol with the same affinity as its non-fluorinated analogue (dUMP). TS enzyme inactivation arises due to an inability of 5,10-MeFol to reductively methylate the C5-F moiety (due to differences in the C-F and C-H bond dissociation energies; 492 kJ mol^{-1} for C-F c.f. 414 kJ mol^{-1} for C-H), prevents its displacement by the incoming methyl group. As a consequence, this normally transient ternary complex (83), (Figure 15) comprised of FdUMP covalently bound to both TS (via a cysteine thiol unit attached to C6) and the 5,10-MeFol reduction product (attached to C5) becomes irreversibly bound, thereby inhibiting dTMP synthesis [175].

Inhibition of the TS enzyme initiates an event cascade that sees the fall in deoxythymidine monophosphate (dTMP) and deoxythymidine triphosphate (dTTP) levels, which, by virtue of feedback loops, affects the production of a range of other deoxynucleotides. This imbalance impacts directly on DNA synthesis and repair processes resulting in single- and double- strand breakage [176].

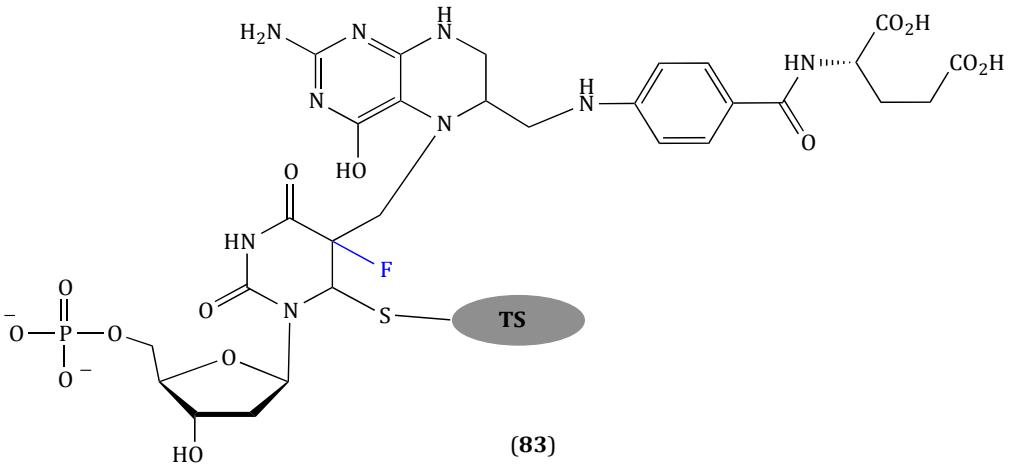


Figure 15. Ternary complex formed between 5-fluorouracil (6) and 5,10-methylenetetrahydrofolate (5,10-MeFol).

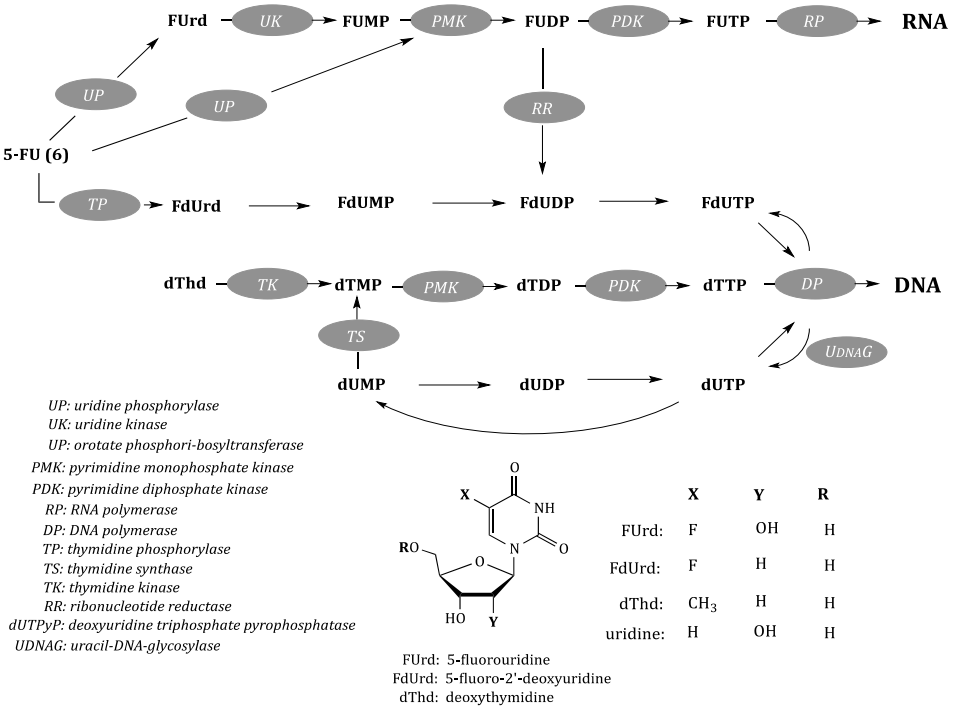


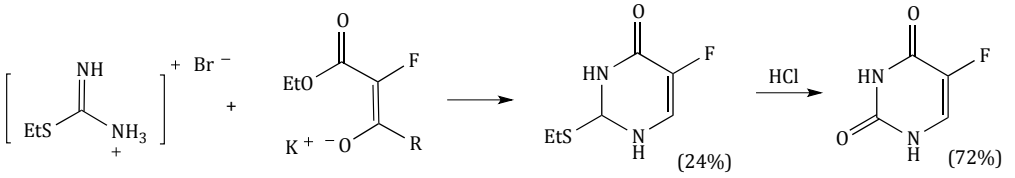
Figure 16. Intracellular metabolism of 5-FU as adapted from Grem et al., [178].

5-FU metabolites are also directly incorporated into RNA resulting in disruption to cellular replication processes. In the case of RNA, the 5-FU metabolite 5-fluorouridine-5-triphosphate (FUTP) becomes incorporated into RNA in place of its non-fluorinated counterpart uridine-5-triphosphate (UTP) resulting in inhibition of a number of RNA processes. Disruption of cellular replication through direct incorporation of 5-FU into cellular DNA has also been reported [177].

4.3.7. Synthesis

The Duschinsky Preparation

Access to the parent uracil ring was achieved using a combination of pseudourea or pseudothiourea salts (84) in the presence of an α-fluoro-β-ketoester enolate (85) (Scheme 15). Yields of the cyclised product were typically modest, presumably a consequence of the use of the unstable ketoester enolate. Thiouracil (86) was isolated in a 24% yield, which was subsequently hydrolysed in 72% yield to 5-FU (6), a 17% overall yield [179].



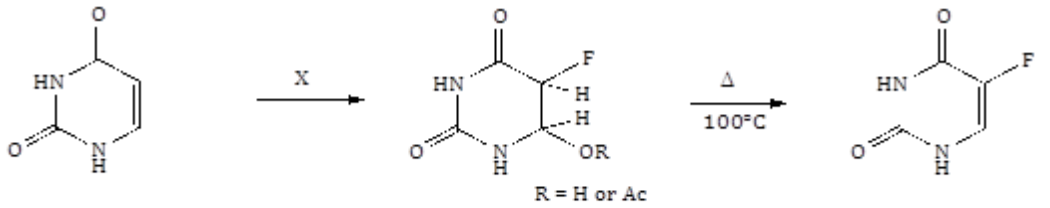
(**84**) (**85**) (**86**) (**6**)

Scheme 15. The Duschinsky synthesis of 5-FU (6) [179].

The use of ethyl fluoroacetate, a derivative of fluoroacetic acid, is also highly toxic to mammals, thereby reducing the attractiveness of this synthetic route for large-scale production of 6 [180].

(i) Electrophilic Fluorination of the Uracil Ring

Knuniants reported the selective monofluorination of uracil (82) by bubbling fluorine gas diluted in a stream of nitrogen (1:5) through an acidified uracil solution [181]. Crude 5-FU was simply recrystallised from water and washed with ether to afford pure 6 in 52-55% yield. Fluorination likely proceeds via formation of an intermediate halohydrin (87), which subsequently dehydrates to 6, possibly during drying (Scheme 16).



(**82**) (**87**) (**6**)

X	Yield	Yield*
F_2 / CH_3CO_2H/RT	80%	64%
CF_3OF / Freon 11 / -78 °C	86%	54%
CH_3CO_2H /F_2/ RT	86%	84%

*based on starting material

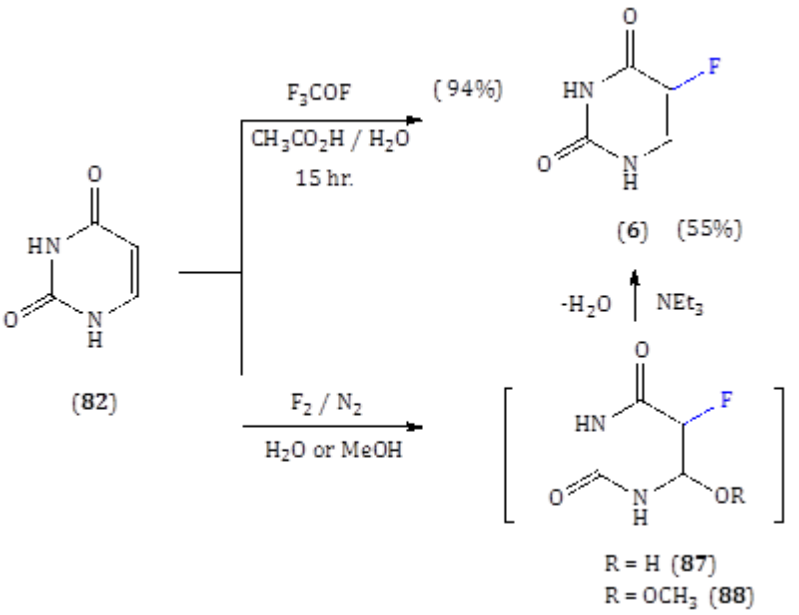
Scheme 16. Knuniants synthesis of 6 by selective monofluorination of uracil (82) [181].

The theory of halohydrin formation is supported by the isolation of 5-fluoro-6-acetoxydihydrouracil as the major fluorination product when fluorination was performed in the presence of glacial acetic acid. Conversion to 5-FU in ~80% yield was effected by heating the reaction mixture containing 5-fluoro-6-acetoxydihydrouracil to 90-95°C [182].

Both direct fluorination approaches represent considerable advancement over the Duschinsky methodh due to reduced synthetic complexity and improved yields. However the use of toxic and highly reactive agents such as fluorine gas and HF poses significant safety concerns during high volume production.

(ii) Electrophilic Fluorinating Agents

It had been previously established that uracil (82) readily underwent both nitration and halogenation at C5 [183]. Barton et al., using trifluoromethyl hypofluorite (F_3COF) as a source of electrophilic fluorine, treated uracil at low temperature to produce 5-FU in a single step reaction (94%) (Scheme 17) [184]. Interestingly, 5-FU was formed in conjunction with a second transient product, which converts to 6 upon heating. ^{19}F nmr showed a complex series of resonance peaks at δ5-6 ppm, which, in combination with a reported molecular formula of $C_4H_5N_2O_3F$ led to a tentative assignment as halohydrin (87). 5-FU was isolated as a solid by sublimation from the reaction mixture following solvent removal.



Scheme 17. Barton [184] and Robins [185] syntheses of 6 using trifluoromethyl hypofluorite as the source of electrophilic fluorine.

Robins el al., [185] confirmed the structure of 87 by adding methanol to the reaction mixture during fluorination, which allowed for the isolation of the fluoromethyl ether intermediate (88). Conversion of this product to 6 was then accomplished by treatment with triethylamine (NEt_3).

The initial electrophile addition to the C5-C6 double bond was confirmed by monitoring the reaction mixture by UV spectrophotometry (λ = 260 nm), which showed a rapid loss of

absorbance upon addition of the CF_3OF to the reaction mixture. ^1H nmr analysis of 88 showed the presence of a single geometric isomer, suggesting both a regio- and stereoselective addition process, which sees the fluorine and methoxy groups oriented *cis* at C5 and C6 respectively on the uracil ring. This relationship was subsequently confirmed by x-ray analysis [186].

While effective as a fluorinating agent, CF_3OF is considered a pseudohalogen, thus, and like fluorine gas, presents toxicity and high oxidising risks as a reagent in large-scale synthesis and is a potential explosion hazard in the presence of alcohols. With demand for fluorinated drugs rising, much research effort has been directed towards the development of safer fluorinating agents, culminating in the development of so-called NF fluorinating agents [187].

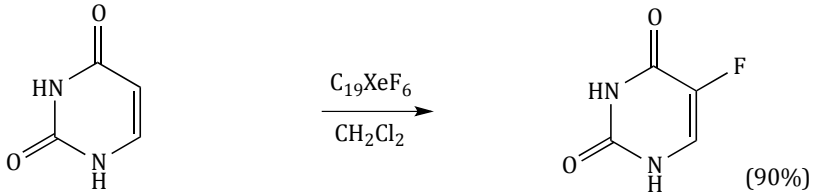
(iii) N-F Fluorinating Agents

Current generation N-F fluorinating agents represent an easily handled, selective source of electrophilic fluorine [188]. These NF reagents comprising either a neutral (R_2N-F) or quaternary (R_3N^+-F A^-) amine covalently bound to fluorine with the most common commercially available NF reagents being: *N*-fluoro-*o*-benzenedisulfonimide (NFOBS), *N*-fluorobenzenesulfonimide (NFSI) and 1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo [2.2.2]octane bis(tetrafluoroborate), (F-TEDA-BF_4) better known as Selectfluor.

Not surprisingly these NF reagents have been employed successfully in the fluorination of uracil. 5-FU (6) has been prepared on stirring Selectfluor with uracil in water at 90°C for 4 hours, and sublimation after solvent removal under reduced pressure in an 82% yield [189].

(iv) Xenon Based Fluorinating Agents

The electrophilic fluorination of uracil using graphite supported XeF_6 ($C_{19}XeF_6$) has been reported [190]. Despite a proven ability as arene fluorinating agents, the highly reactive nature of xenon fluorides has limited their entry and applicability in general organic synthesis [191,192]. As a supported reagent however, XeF_6 is a significantly milder and more stable reagent. Contrastingly, while the fluorination of uracil using XeF_2 affords only low yields of 5-FU (10%), the use of two equivalents of $C_{19}XeF_6$ at room temperature for 24 h afforded 5-FU in a 90% yield (Scheme 18) [193].



(**82**) (**6**)

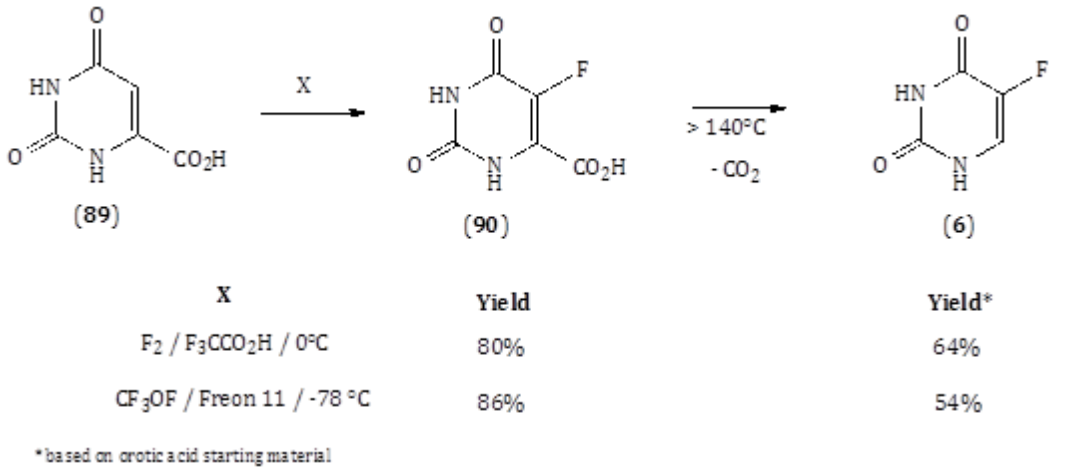
Scheme 18. Preparation of (6) using graphite supported XeF_6 as the source of electrophilic fluorine [190].

(v) Other Synthetic Approaches

The two-step synthesis of 5-FU from uracil-4-carboxylic acid (orotic acid, OA, (89)), [194] while technically identical to previously discussed preparations in terms of fluorination

procedure, offers advantage in terms of product purification at the industrial scale. Both uracil (82) and 5-FU (6) are highly polar compounds, possessing similar solubility in water, making product isolation and purification by physical means such as recrystallisation, difficult. OA (89), by contrast, is only sparingly soluble in water, allowing for easy purification of 6 and the recovery of unreacted 89 for recycling in subsequent production runs.

The use of both CF_3OF and F_2 gave good yields of intermediate acid 90, which yielded 5-FU (6) upon decarboxylation either under solvent free conditions or by heating in a high boiling point solvent (e.g. triethylene glycol dimethyl ether) (Scheme 19).



X	Yield	Yield*
F_2 / F_3CCO_2H / 0°C	80%	64%
CF_3OF / Freon 11 / -78 °C	86%	54%

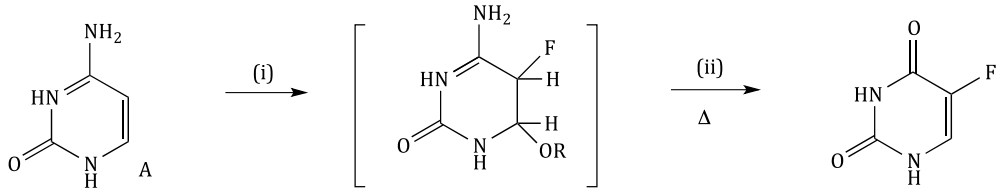
*based on orotic acid starting material

Scheme 19. Preparation of (6) from orotic acid (89) [194].

Following the early work of Robins et al., [195], Misaki, et al., [196] reported the synthesis of 6 from cytosine (91). This work extended observations made by Robins in hydrolysing 5-fluorcytosine (5-FC) to 5-FU (6). Misaki described a range of adaptations including the use of fluorine gas or fluorine fluorosulfonate (SO_3F_2) as alternate solurces of electrophilic fluorine. Fluorination at room temperature resulted in poor yields of 6, with the majority of the cytosine converted to what was described an "intermediary compound" which, though not formally identified, is presumed to be cytosine halohydrin or a related derivative (92). This problem was subsequently overcome in the post-fluorination workup by heating the reaction mixture under neutral or alkaline conditions to promote both hydrolysis of the C4 amino group and dehydration at C5-C6. While the effectiveness of the fluorination reaction appeared to be largely independent of the nature of the fluorinating agent employed, yields of 6 varied considerably with choice of workup (Scheme 20).

4.3.8. Conclusion

While the synthesis of 5-FU poses fewer synthetic challenges relative to the more structurally complex cytotoxic agents such as paclitaxel, issues associated with large-scale production such as purification and the use of toxic and highly reactive reagents must still be effectively addressed. Direct electrophillic fluorination of uracil or uracil adducts using fluorine gas has been established as the preferred industrial production method. While safer chemoselective fluorinating agents such as Selectfluor have emerged, their high cost generally restricts their use to small-scale laboratory-based syntheses.



(**91**) (**92**) R = H or OAc (**6**)

	(i)	(ii)	Yield
91	F_2 / RT / 1 hr	pH 6, 80°C / 4 hr	65%
	F_2 / RT / 1 hr	pH 10, 60°C / 4 hr	35%
	F_2 / 40-50°C / 1 hr	80°C / 4 hr	67%
	F_2, $NaHSO_3$ /RT/ 1 hr	pH 6, 80°C / 4 hr	75%
	F_2, CF_3CH_2OH / RT / 1 hr	pH 6, 80°C / 4 hr	55%
	SO_3F_2, H_2O/ RT / 75 min.	pH 8, 80°C / 3 hr	87.7%
	SO_3F_2, HF / 5°C/ 2hr.	H_2O, reflux / 2 hr	76%
91.HCl	F_2 / RT / 1 hr	pH 6, 80°C / 4 hr	66%

Scheme 20. Summary of reaction conditions and yields of 6 prepared from cytosine (91) [196].

CONCLUSION

Herein we have discussed the preparation of three anti-cancer drugs – paclitaxel (8), gemcitabine (7) and 5-FU (6) used in pancreatic cancer treatment. While the synthetic approaches undertaken vary significantly in approach, complexity and the overall level of success, the outcomes generated highlight the demanding nature of pharmaceutical research. The challenges faced in each case study vary significantly, covering a wide-range of issues from maximising the stereoselectivity of key synthetic steps to appropriately modifying the physical properties of products in order to simplify purification procedures to facilitate production up-scaling. The work illustrates the long road and many false paths that exist between the initial discovery of a bioactive natural product and the development of a safe and commercially viable pharmaceutical product. It is envisioned that with the advent of molecular phenotype guided therapeutic strategies for individualised pancreatic cancer treatment (as discussed in the Chapter of Jamieson et al.), there remains great promise that plant-derived bioactive compounds can be developed into chemotherapeutic agents with significant efficacy using a multitude of synthetic approaches. While technological advancements in computer-based design, synthesis and screening techniques continue to accelerate rates of transition in drug development, successful outcomes are ultimately reliant upon the creativity and perseverance of teams of dedicated synthetic and formulation chemists in devising practical solutions to resolving key impasses.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 8

SELECTED AUSTRALIAN FLORA AS POTENTIAL SOURCES OF ANTI-CANCER AGENTS

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ABSTRACT

Australia is a unique and diverse country, in both geography and climate, with significantly diversified plant materials. For thousands of years, indigenous Australians used the Australian flora for traditional medicines for the treatment of numerous ailments indicating that specific Australian flora may possess key bioactive compounds with potent health promoting properties. Recently, investigators have identified bioactive compounds within Australian plant materials, and several studies have linked their extracts and isolated compounds with the prevention or treatment of cancers, including pancreatic cancer. This chapter outlines the use of Australian flora as traditional medicines, discusses the composition and anti-cancer properties from selected Australian plants and proposes a trend for future studies of their purported health, and anti-cancer benefits.

1. INTRODUCTION

Australian flora plays an important role in the Aboriginal community as they have been used as foods and medicines for thousands of years (Roberts et al., 1990), and today they still contribute socially and economically to the Aboriginal community (Vuong et al., 2014b). In addition, Australia is a unique and diverse country, in both geography and climate. As an island continent and the world's sixth largest country, located between the Indian and Pacific Oceans, Australia experiences diversified weather throughout the country. For example, it is

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generally warm throughout the year in the northern states but it is cold in winter in the southern states (White, 1994). With such diversity in climate and geography, Australian flora has developed unique survival characteristics to adapt to such conditions, and thus they may contain key bioactive compounds for the prevention and/or treatment of cancers (Mohanty and Cock, 2012).

Numerous plant-derived compounds have been identified, isolated and tested *in vitro* and *in vivo* against different types of cancer; of which many compounds are currently in preclinical development (Cragg and Newman, 2005). Approximately 45% of all anti-cancer drugs have been derived from plant materials (Newman and Cragg, 2012), indicating that plant-derived compounds play a significant role in the development of therapeutic agents for cancer treatment. Of note, only limited species of Australian plants have been screened for their bioactivity and anti-cancer properties. As such, there is great potential for the investigation of plant bioactive compounds from Australian native plants for the development of novel anti-cancer therapies. This chapter outlines the use of Australian flora as traditional medicines, discusses the composition and anti-cancer properties of selected Australian flora and proposes a trend for future studies. By discussing the traditional use of Australian flora, describing their initial link with their purported anti-cancer properties, especially for pancreatic cancer, we intend to show the diversification and potential use of Australian flora for the development of novel therapeutic agents against cancer, particularly pancreatic cancer.

2. TRADITIONAL USE OF AUSTRALIAN FLORA

The Australian Indigenous community has used numerous Australian floras as folk medicines for many years (Table 1), with the *Eucalyptus* an important example. Being the home of *Eucalyptus*, Australia has more than 800 *Eucalyptus* species (Hasegawa et al., 2008, Konoshima and Takasaki, 2002). *Eucalyptus* has been traditionally used for the treatment of various ailments such as colds, flu, fever, aching, sores, internal pain, and inflammation (Smith, 1991, Kumar and Lamidhar, 2011). *Eucalyptus* leaves, stem and bark are brewed in water and the decoction has been traditionally used as a wash to treat colds, flu and fever. A small amount of decoction can be sipped to treat these ailments. The decoction can be also used as an antiseptic wash for sores, cuts and any infected skin problems (Barr et al., 1993). Furthermore, the liquid is also known for effectively relieving aches and pains, which are associated with influenza, fever or rheumatism (Smith, 1991).

Leaves from other Australian native flora such as *Avicennia marina, Brachychiton diversifolius, Calytrix brownii, Cymbopgon obtectus* and *Eremophila alternifolia* have been traditionally used for pain and congestion relief, treatment of fever, colds, coughs and sores. For example, young leaves of *A. marina* are chewed and applied on the areas of punctures caused by the sting of a stone-fish to relieve the pain (Bandaranayake, 1998, Tsai et al., 2013). The leaves of the *C. brownii* are boiled and the steam is inhaled to relieve the congestion of the nasal and bronchial passages (Smith, 1991). Young or mature leaves from *B. diversifolius, C. obtectus* and *E. alternifolia* are crushed and then brewed, and the decoction has been used to treat fever, colds, coughs and sores (Brand and Cherikoff, 1985).

Fruits from Australian floras have not only been used as foods, but have also been used for the treatment of different aliments (Vuong et al., 2014b). For instance, the fruit pulp of *Adansonia gregorii* has been taken to treat gastric disturbance (Lowe, 1998); while, the fruit pulp of *Citrullus colocynthis* can be applied directly to treat skin disorders such as scabies or tinea (Habs et al., 1984). Immature *Cochlospermum fraseri* fruits have been used for the treatment of skin infections by directly applying the crushed pulp to localised areas of infections (Barr et al., 1993); whereas, ripe fruits of *Scaevola sericea* have been used for the treatment of redness or sores of the eye (Lassak and McCarthy, 1983).

The traditional use of Australian flora for the treatment of various ailments reveals that these materials might hold key bioactive compounds associated with numerous health benefits. Several floras have been studied for their bioactive compounds and health benefits, especially for their purported anti-cancer properties. However, Australian flora is significantly under investigated. As such, there is great potential in the investigation of bioactive compounds from Australian flora for utilisation as prospective supplements or anti-cancer agents, especially for pancreatic cancer where current therapies show very limited efficacy and novel therapies are urgently needed.

3. ANTI-CANCER COMPOUNDS FROM AUSTRALIAN FLORA

Bioactive compounds derived from plant material have been described in previous chapters of this book. The biodiversity of bioactive compounds provide great potential for investigation of their key bioactive compounds and anti-cancer activity (Vuong et al., 2014b). The fact that there has been no reduction in the incidence, nor improvements in the mortality rates of pancreatic cancer, as discussed in the earlier Chapter of Jamieson et al., necessitates urgent investigations for new therapeutic options for patients with this deadly disease. Therefore, investigation of the key bioactive compounds from the plant materials could help to identify novel preventive and/or therapeutic agents for this devastating cancer. Furthermore, these novel agents can be used as scaffolds, or lead compounds, for the development of effective drugs for treatment of pancreatic cancer (Vuong et al., 2014b).

Australia has diverse environmental and soil conditions, and as such Australian plant materials are also diversified in terms of their species and bioactive compounds. Although numerous plants have been used as traditional medicines, the scientific research on Australian flora is very limited (Vuong et al., 2014b), with only few studies performed to identify Australian flora with anti-cancer activity. In this section, information about the physicochemical nature and anti-cancer properties of selected Australia native plants are described to implicate their potential use as novel therapeutics, and to propose the direction for future studies.

Table 1. Botanical name of selected plants and their parts traditionally used as remedies for aliments by Indigenous communities in Australia (Vuong et al., 2014b)

Latin name / Family	Aboriginal name	Parts used	Preparation and traditional use	References
Acacia estrophiolata F.Muell. / Mimosaceae	Athenge	Bark, root, gum	Brewing with water and solution is applied on affected areas to treat infected skin lesions, scabies burns and wounds.	(Lister et al., 1996)
Adansonia gregorii F.Muell. / Bombacaceae	Jamulang	Fruit pulp	Raw fruit powdery pulp is eaten to reduce gastric disturbance.	(Lowe, 1998)
Avicennia marina (Forsskal) Vierh. / Verbanaceae	Maanyarr	Leaves	Young shoots are chewed and mixed with saliva applied to site of puncture to relive pain caused by the sting of stone-fish/sting-rays.	(Bandaranayake, 1998, Tsai et al., 2013)
Brachychiton diversifolius R. Br. / Sterculiaceae	Nanungguwa	Leaves	Leaves are crushed and infused. Liquid is used for washing over the body to reduce fevers of unknown origin.	(Brand and Cherikoff, 1985)
Buchanania obovata Engl. / Anacardiaceae	Mangkarrba	Petioles, mid-vein of leaves	Scraping to remove outer layer, heating then inserting into tooth cavity for toothache.	(Barr et al., 1993)
Callitris intratropica R. Baker & H.G. Smith / Cupressaceae	Gangi	Bark	Bark is boiled with water and used to wash over the whole body to relieve abdominal pain from diarrhoea.	(Barr et al., 1993)
Calytrix brownii (Schauer) Craven / Myrtacea	Alunkwaluwa	Leaves	Young leaves are crushed and boiled in water. Steam is inhaled to relieve congestion of the nasal and bronchial passages.	(Smith, 1991, Rasoanaivo et al., 2013)
Capparis umbonata Lindley / Capparaceae	Burnayingmi	Bark	Bark is chopped or pounded and then boiled in water until red in colour. The solution is then applied on affected area such as chicken pox, boils, scabies, muscle and joint pain.	(Smith, 1991)
Carissa lanceolata R. Br. / Apocynaceae	Manigudja	Root	Young roots without the bark are chopped, crushed and boiled with water. Solution is used to rub onto the chest as part of treatment.	(Smith, 1991)

Latin name / Family	Aboriginal name	Parts used	Preparation and traditional use	References
Cassia notabilis F. Muell. / Caesalpiniaceae	Kampijung	Leaves and twigs	Leaves and twigs are crushed and boiled with water. The liquid is used to wash the body to reduce fever associated with colds and flu.	(Barr et al., 1993)
Citrullus colocynthis (L.)Schrader / Cucurbitaceae	--	Fruit	The pulp of the ripe fruit is directly applied to scabies or tinea.	(Habs et al., 1984, Ayyad et al., 2012, Tannin-Spitz et al., 2007)
Cochlospermum fraseri Planchon ssp. Heteronemum (F. Muell.) Poppendieck / Bixaceae	Kalijpa	Fruit	Unripe fruit is broken and directly applied to localised skin infections.	(Barr et al., 1993)
Croton arnhemicus Muell. Arg. / Euphorbiaceae	Ngarrik	Inner bark	Inner bark is boiled with water and the decoction is used to wash affected areas to relieve headache and joint swelling.	(Smith, 1991)
Cymbopgon obtectus S. T. Blake / Poaceae	Linytji	Leaves	Leaves are chopped and boiled with water and the liquid is taken to treat colds and coughs.	(Barr et al., 1993)
Diospyros maritima Blume / Ebenaceae	Glumunyu	Fruit	Fruit is placed on hot ashes and heated gently until soft and black, then mashed with water and applied on tinea-form lesions.	(Palombo and Semple, 2001, Kuo et al., 1997)
Eremophila alternifolia R. Br. / Myoporaceae	Irmangka irmangka	Leaves	Leaves are sun-dried and then infused in boiling water to treat colds, fever, internal pain and severe illness.	(Goddard and Kalotas, 2002)
Eucalyptus tetrodonta F. Muell. / Myrtaceae	Gadayka	Inner bark, leaves	Inner bark or leaves are brewed with water then used to treat sores and scabies.	(Locher and Currie, 2010)
Eucalyptus kino / Myrtaceae	Mijilypa	Gum	Kino is dissolved in water and then used to wash on cuts or sores.	(Locher and Currie, 2010)

Table 1. (Continued)

Latin name / Family	Aboriginal name	Parts used	Preparation and traditional use	References
Flueggea virosa (Roxb. Ex Wild.) Voigt ssp. Melanyhesoides (F. Muell.) Webster/ Euphorbiaceae	Kudjung	Seeds	Seeds are boiled with water and liquid is applied to pruritic skin conditions.	(Isaacs, 2002, Gan et al., 2006, Zhao et al., 2011)
Morinda citrifolia L. / Rubiaceae	Gununyi	Fruit	Soft ripe fruit is eaten raw as a remedy for coughs, colds and sore throat.	(Isaacs, 2002, Brown, 2012)
Nymphaea macrosperma Merr. & Perry / Nymphaeaceae	Kanyngurniny	Fruiting capsule	After peeling off the fleshy layer, the fruiting capsule is eaten raw to stop diarrhoea.	(Barr et al., 1993)
Scaevola sericea Vahl / Goodeniaceae	Yilyarra	Fruit, stem	Ripe fruit is squeezed and the juice dropped directly into eyes to relieve redness and soreness. Fruit can be mashed and applied to bites and stings. Stem is extracted in water and used as an anti-cancer agent.	(Lassak and McCarthy, 1983)
Strychnos lucida R. Br. / Loganiaceae	Yerrweyi	Fruit	Fruit is mashed and brewed with water. Liquid is used to wash localised skin infections.	(Isaacs, 2002)
Syzygium suborbiculare (Benth.) Hartley & Perry / Myrtaceae	Narrani	Fruit	Fruit is boiled and mashed into liquid, which is then taken to relieve bronchial congestion and colds. Fruit and seed are chewed to relieve the toothache.	(Lim, 2012)

3.1. *Eucalyptus* (Myrtaceae)

Australia is the home of the genus *Eucalyptus*, with approximately 800 species of *Eucalyptus* identified (Hasegawa et al., 2008, Konoshima and Takasaki, 2002). *Eucalyptus* belongs to the Myrtaceae family and several species have been distributed to different countries around the world, mainly for timber and paper production (Konoshima and Takasaki, 2002). Different parts of the *Eucalyptus* such as leaf, bark and stem have been used to produce essential oils, which contain many types of volatile compounds and have been widely used in the pharmaceutical and cosmetic industries. Besides the volatile compounds, numerous triterpenoids, flavonoids, tannins and other non-volatile compounds have been identified from the *Eucalyptus* (Kumar and Lamidhar, 2011). These compounds have been linked with various health benefits, including the prevention of cancers (Takasaki et al., 2000, Tian et al., 2012)

Numerous studies *in vitro,* and several studies *in vivo,* have been conducted to identify the link between the oils and extracts from different parts of the *Eucalyptus* with anti-cancer benefits; however, limited information has been published on the link between the *Eucalyptus* oils or extracts and cancer in human clinical trials. Anti-proliferative and cytotoxic effects of eucalyptus oils and extracts have been examined and the results have revealed positive effects against several cancer cell lines. Essential oils extracted from *E. globulus*, *E. torquata*, *E. sideroxylon* and *E. benthamii* have been demonstrated to inhibit the nuclear translocation of NF-kappa B induced by LPS in leukemic monotype THP-1 cells, and also exhibit cytotoxic effects on the human breast adenocarcinoma cell line (MCF7), Jurkat (J774A.1), and HeLa cell lines (Ashour, 2008, Doll-Boscardin et al., 2012, Zhou et al., 2003). However, the cytotoxic effects were different depending of the parts of the plants used for extracting the oil, possibly due to different compositions of volatile compounds (Ashour, 2008).

Photo courtesy of Mr Deep Jyoti Bhuyan.

Figure 1. Leaf of the *Eucalyptus robusta*.

Aqueous and organic solvent crude extracts from different *Eucalyptus* species have been reported to exhibit cytotoxic and anti-proliferative effects *in vitro*. For example, crude extracts from *E. citriodora*, *E. globulus*, *E. maiden* and *E. camaldulensis* were shown to inhibit growth of colon (SW-620, SW480), liver (HEP-2), ovary (OVCAR-5, A2780), prostate (PC-3), cervix (HeLa), neuroblastoma (IMR-32), lung (HOP-62, A-549), breast (MCF7, MDA-MB-231), and gastric (BGC-823, KE-97) cancer cells (Bhagat et al., 2012, Mota et al., 2012, Islam et al., 2012). Bhagat et al. (2012) further tested the effects of crude extracts from *E. citriodora* and demonstrated that the growth of Ehrlich ascites carcinoma was also suppressed.

In our recent study, we found that aqueous crude extract prepared from *Eucalyptus robusta* leaves significantly exhibited growth of cancer cells; however, there were differences in efficacy across the panel of the tested cell lines. The crude ER extract showed the greatest toxicity against colon, lung, neuroblastoma, glioblastoma and ovarian cancer cells. The *E. robusta* crude extract was also tested on three different pancreatic cancer cell lines and we demonstrated that cell viability decreased by 86%, 62% and 47% respectively, when compared to untreated control cells. These data, demonstrate the potential of the bioactive compounds within the crude *E. robusta* extract to be further purified and investigated for their anti-pancreatic cancer properties (Vuong et al. *Unpublished*).

Several individual volatile compounds such as 1,8-cineole, α-pinene, terpinen-4-ol, and γ-terpinene have been isolated and tested against several cancer cell lines and the results showed positive anti-cancer efficacy (Doll-Boscardin et al., 2012, Murata et al., 2013). In addition, several non-volatile compounds such as euglobal-G1 from *E. grandis*, and resveratrol, piceatannol, macrocapal G from *E. maiden* have been shown to inhibit growth of several cancer cell lines *in vitro* (Takasaki et al., 2000, Tian et al., 2012). Other non-volatile compounds such as cypellocarpins and chromene glucoside isolated from *E. cypellocarpa* were also found to reduce tumour growth *in vivo* (Ito et al., 2000). These initial findings revealed that volatile and non-volatile compounds from *Eucalyptus* species possess activity for their development into anti-cancer agents. With more than 800 different *Eucalyptus* species (Kumar and Lamidhar, 2011) and numerous unidentified volatile and non-volatile compounds, thus there is a great potential for development of anti-pancreatic cancer therapeutic agents from *Eucalyptus*.

3.2. *Scaevola Spinescens* (Goodeniaceae)

Scaevola spinescens (maroon bush, currant bush, or fanflower) belongs to Goodeniaceae family and is native to Australia. It has been traditionally used by the Aboriginal community for the treatment of various ailments such as colds, stomach ache, urinary problems, boils, sores and rashes (Ghisalberti, 2004). The first claim for the cancer curing capacity of this plant was reported in 1946 in Western Australia, where a patient claimed that he was cured following continued ingestion of an aqueous extract of the *S. spinescens* root bark combined with ashes of the desert poplar *Codonocarpus cotinifolius* (Ghisalberti, 2004). Currently there has been limited research undertaken to elucidate the phytochemical profile and anti-cancer properties of *S. spinescens* (Kerr et al., 1996).

Photos courtesy of Jeanie Crago.

Figure 2. *Scaevola spinescens* (Goodeniaceae).

In our recent study (Vuong et al., *Unpublished*), we have tested the link of various *S. spinescens* extracts with growth inhibitory activity of different cancer cell lines and found that *S. spinescens* extracts inhibited growth of cancer cells but their efficacy differed between cancer type. The crude acetone extract demonstrated the greatest growth inhibition against all cell lines, while subsequent dose response analyses showed the acetone extract exhibiting the greatest effects against ovarian, glioblastoma, prostate and breast cancer cells. Moreover, the *S. spinescens* extract was up to 9-fold more potent at inhibiting growth in the MCF-7 breast cancer cells than in those derived from other tumour types and was 6-fold more potent in breast cancer cells than in normal breast cells. Approximately 75% of all breast cancers are hormonally dependent and the MCF-7 cell line is representative of such estrogen-dependent breast carcinomas (Harvey et al., 1999, Musgrove and Sutherland, 2009). Anti-estrogen therapy targeting the synthesis of estrogen and the estrogen receptor are the mainstay treatment options for hormonally dependant breast cancers. Various plant-derived polyphenolic phytoestrogens are known to alter estrogen dependent signalling pathways and reduce cancer growth (Liu et al., 2012). Potentially the selectivity of *S. spinescens* extract towards estrogen-responsive breast cancer cells may lie within the extracted total phenolic compounds. Thus it is clearly necessary to further purify and investigate *S. spinescens* for its anti-cancer activity.

3.3. Lilly Pilly (*Syzygium Paniculatum*, Myrtaceae)

The lilly pilly (*Syzygium paniculatum Gaertn.*) belongs to the family Myrtaceae and is a small to medium-sized tree endemic to coastal New South Wales (NSW), Australia (Hyland, 1983). The lilly pilly fruits are produced between January and May and are in the form of purple berries (12-25 mm diameter). When ripe, the fruits are shiny and possess fleshy distal calyx lobes (Floyd, 2008). They have a pleasantly sour apple-like flavour and can be eaten fresh or made into jams (Floyd, 2008). Several studies have determined the bioactive compounds contained in the fruits. Quijano-Celis et al. (2013) analysed volatile constituents

in the oil extracted from lilly pilly fruits and found a total of 155 individual volatile compounds, of those terpenes (α-pinene – 32.8%, (Z)-β-ocimene – 21.8%, limonene – 6.9% and α-terpinol 5.1%) dominated the oil profile. Another study by Longo et al. (Longo et al., 2007) analysed the anthocyanin composition, identifying only one compound – malvidin 3,5-diglucoside in the fruit extract (325.9 mg / kg).

In addition, Vuong et al. (2014a) further determined total phenolic compounds and the secondary metabolites, flavonoids and proanthocyanidins, and found that the lilly pilly ethanolic extract had a total phenolic content of 96 mg GAE/g, which is equivalent to approximately 25 mg GAE/g of the dry weight (DW) of the fruit. In comparison with other fruits, lilly pilly fruits have higher total phenolic content than other fruits such as the apple, apricot, kiwi and peach (5, 17, 9 and 18 mg GAE/g DW, respectively) (Ishiwata et al., 2004). The lilly pilly ethanolic extract contained flavonoids and proanthocyanidin levels of 52 and 29 mg CAE/g, which accounted for 54% and 30% of the total phenolic content, respectively. In addition, the lilly pilly extract was found to possess potent antioxidant capacity and exhibited growth inhibition of the pancreatic cancer cell lines MiaPaCa-2 and ASPC-1, while having little effect on the normal human pancreatic ductal epithelial (HPDE) cells. Therefore, the lilly pilly fruits might contain potent bioactive compounds with anti-pancreatic cancer activity and more vigorous investigations are required to determine mechanisms of action (Vuong et al., 2014a).

3.4. Kakadu Plum (*Terminalia Ferdinandiana*, Combretaceae)

The Kakadu plum (*Terminalia ferdinandiana*, Combretaceae), also known as the billy goat plum, grows in the Northern Territory and Western Australia (Gorman et al., 2006). When ripe it is yellow-green, plump and sweet to eat (Barr et al., 1993). The fruit is fibrous and is usually used in the form of a powder (Konczak et al., 2010). It is generally used to make sauces, jams, preserves, as well as in cosmetic products (Cock and Mohanty, 2011). For medicinal purposes, it is traditionally used by Aborigines for the treatment of skin sores and debility (Barr et al., 1993).

The Kakadu plum is a substantial source of vitamin C, with levels at 71.32 μmol/g fresh weight (FW). This content of ascorbic acid is approximately 900 times higher than that in the same fresh weight of blueberries and 1000 times higher than that in brush cherries (Netzel et al., 2007). In Kakadu plum dry weight (DW), ascorbic acid accounted for about 5.5 %, which is 11 times higher than that in oranges, grapefruit or lime (0.5 % DW), which are well known for their abundant source of ascorbic acids in the plant world (Cock and Mohanty, 2011).

Ascorbic acid is known to have cancer preventive potential due to its redox properties. Acting as a radical scavenger, it can reduce oxidative stress; thus can be protective during the initiation and promotional stages of carcinogenesis (van Poppel and van den Berg, 1997). Furthermore, ascorbic acid has been reported to improve the efficacy of several chemotherapeutic agents such as procarbazin, asparaginase, vinblastine and gemcitabine (Verrax and Buc Calderon, 2008). Recently, Espey et al. (2011) demonstrated that combining pharmacological levels of ascorbic acid with gemcitabine results in a synergistic cytotoxic response in wide panel of pancreatic tumour cell lines, including for gemcitabine resistant cells. Further, they showed that the gemcitabine-ascorbic acid combination treatment had an

improved effect on the inhibition of growth of pancreatic tumour xenografts, when compared to gemcitabine alone suggesting that ascorbic acid has the potential to be used as an adjuvant to other chemotherapeutic strategies, particularly as it is safe, with few side effects (Espey et al., 2011, Cullen et al., 2011).

Source: http://www.rbgsyd.nsw.gov.au/education/Resources/bush_foods/Syzygium_paniculatum.

Figure 3. The lilly pilly (*Syzygium paniculatum*).

Source: http://dharmakesuma.blogspot.com.au/2013/04/kakadu-plum-vitamin-c-mega-no-1-dunia.html

Figure 4. The Kakadu plum (*Terminalia ferdinandiana*, Combretaceae).

Kakadu plum has been shown to contain high levels of total polyphenols, accounting for approximately 158 mg/g dried weight (DW) (Konczak et al., 2009). The level of total polyphenols in the Kakadu plum is in a medium range in comparison to the levels found in green tea (119-252 mg/g DW) (Astill et al., 2001), and is significantly higher than found in other native Australian fruits, such as lemon aspen, riberry and quandong (10, 24 and 33 mg/g DW, respectively) (Konczak et al., 2010). Kakadu plum has been shown to possess high scavenging and antioxidant properties (Konczak and Roulle, 2011), exhibiting strong activity against human promyelocytic leukaemia LH-60 cells (Tan et al., 2011). Therefore, Kakadu plum could be considered a potential source of novel bioactive agents for the prevention and treatment of cancers, including pancreatic cancer.

3.5. Quandong (*Santalum Acuminatum,* Santalaceae)

The quandong (*Santalum acuminatum,* Santalaceae), also known as the native peach, is native to Central Southern Australia. The quandong fruit ripens in the spring and is an important staple for the Australian Aboriginal community (Konczak et al., 2010). The fruit is a 15-25 mm wide drupe with striking, shiny red skin. Its flesh is firm, approximately 3-5 mm thick and surrounds the edible stone (Brand-Miller and Holt, 1998). The fruit can be used in cooking to prepare sweet or savoury dishes, and its kernel has been traditionally used by Aborigines to relieve the pain of swelling/bruises, sprains and backache (Ahmed and Johnson, 2000, Barr et al., 1993). The kernel is ground into paste, then mixed with water or saliva and finally the liniment is rubbed into the affected region to relieve pain (Barr et al., 1993).

The quandong fruit is a rich source of nutrients and fibre. Its flesh contains 17 % carbohydrates and 4 % fibre, while its roasted kernel contains 63 % fat with energy of 2920 kJ per 100 g (Brand-Miller and Holt, 1998). Polyphenol levels in the quandong fruit are approximately 33 mg/g DW (Konczak et al., 2009), however it possesses a very high antioxidant capacity. Although the level of polyphenols in quandong was found to be 5 fold lower than that of Kakadu plum (33 and 158 mg/g DW, respectively), its oxygen radical absorbance capacity (both hydrophilic and lipophilic) was only slightly lower than that of Kakadu plum (2027 and 2511 μmol Trolox equivalents (TE)/g DW, respectively) (Konczak et al., 2009).

Source: http://www.outbackchef.com.au/products/quandongs-santalum-acuminatum-75g-cannister/132/1.

Figure 5. The quandong (*Santalum acuminatum,* Santalaceae).

Studies on the composition of bioactive components in the quandong fruit are limited. A preliminary study reported that the fruit contained cyanidin-3-glucoside, rutin, and kaempferol (0.13, 0.53 and 0.61 mg/g FW, respectively) (Konczak et al., 2010), while it was also found to contain vitamin E and malic acid (0.013 and 19.1 mg/g FW, respectively) (Konczak and Roulle, 2011, Konczak et al., 2010). There is no published data linking the quandong fruit with the prevention of cancer however, the fruit contains various bioactive components: cyanidin-3-glucoside, rutin, and kaempferol, which have been linked to the prevention of cancers of the breast, lung, ovary and pancreas (Zhang et al., 2005, Chen et al., 2006, Luo et al., 2011).

As described previously, the levels of polyphenols and some identified bioactive compounds were relatively low in the quandong fruit, however its antioxidant capacity is quite high indicating that there may be more powerful bioactive compounds within the quandong, which have yet to be identified. Thus the potential of the quandong fruit for investigations into their bioactive compound composition and their potential link to cancer prevention/treatment is warranted.

3.6. Davidson's Plum (*Davidsonia Jerseyana* and *Davidsonia Pruriens*, Davidsoniaceae)

There are two types of Davidson's plum, including the *Davidsonia jerseyana*, which grows in sub-tropical rainforest in northern NSW; and the *Davidsonia pruriens*, which grows in north east Queensland (CSIRO, 2006). The Davidson's plum belongs to the Davidsoniaceae family and is a small rainforest tree up to 15 m tall (Ahmed and Johnson, 2000). The Davidson's plum has a plum like fruit with a diameter of 3-6 cm. The fruit has a brilliant burgundy colour, has a sour taste and has been used for making jams, sauces, vinegars, dressings, ice cream, drinks, and for stewing (CSIRO, 2006, Ahmed and Johnson, 2000).

Source: http://www.rainforestbounty.com.au/index.php/newsandmore/blog/summer-harvest-summer-flavours/.

Figure 6. Davidson's plum (*Davidsonia jerseyana*).

The fruit of the Davidson's plum has a polyphenol content of approximately 50 mg/g DW, which is lower than that of the Kakadu plum but higher than the quandong fruit (158 and 33 mg/g DW, respectively) (Konczak et al., 2009). Although, the antioxidant activity of the Davidson's plum is lower than that of the Kakadu plum or quandong fruit, it still possesses high antioxidant activity. Several bioactive components including: delphinidin sambubioside, cyanidin sambubioside, petunidin sambubioside and peonidin sambubioside have been identified in the Davidson's plum (Konczak et al., 2009). In addition, the Davidson's plum was found to have the highest level of anthocyanins in comparison with other native fruits, such as the Kakadu plum, quandong, riberry or the desert lime (Konczak et al., 2009).

Some of bioactive components identified in Davidson's plum have been recently reported to exhibit anti-tumour effects. Delphinidin sambubioside has been shown to induce apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathways (Hou et al., 2005), while anthocyandins were demonstrated to inhibit tumour development in mouse models of colon cancer (Apc^{Min} mice) and reduce proliferation of human colon cancer cells (Kang et al., 2003). Anthocyanidins have also been reported to inhibit the growth of colon cancer (Zhao et al., 2004) and pancreatic cancer (Zhang et al., 2011) cells. In our recent study, we found that the crude ethanolic extract from Davidson's plum exerted significant growth inhibition on pancreatic cancer cell lines, while having little effect on normal human pancreatic ductal epithelial cells (Vuong et al. *Unpublished*), warranting further investigations into its anti-cancer activity.

3.7. Illawarra Plum (*Podocarpus elatus*, Podocarpacease)

The Illawarra plum (*Podocarpus elatus*) belongs to the Podocarpacease family and is native to temperate, sub-tropical eastern New South Wales and Queensland (CSIRO, 2006). The Illawarra plum is known as an evergreen conifer and grows to 5-30 m tall, preferring to grow in areas with an annual rainfall of 800-1500 mm (CSIRO, 2006, Ahmed and Johnson, 2000). The fruit, which measures 2 to 3 cm in diameter, is the major product used from the tree, and is a blue-black colour, is fleshy and plum-like and has an inedible seed attached to the outside of the fruit (CSIRO, 2006, Ahmed and Johnson, 2000). The fruit has been used for its "fruit-type" flavour in sweet and savoury products, as well as in making jams or conserves.

Source: http://www.prosperitywithnature.com.au/bushfood-plants/15-illawarra-plum.html.

Figure 7. Illawarra plum (*Podocarpus elatus*).

The Illawarra plum fruit was found to contain 68 mg/g FW of polyphenols, and this level is significantly higher than that of other Australian native fruits, such as the Davidson's plum, riberry, finger lime, and the molucca raspberry (17, 13, 11, and 22 mg/g FW, respectively) (Netzel et al., 2007). Similarly, antioxidant capacity of the Illawarra plum was shown to be greater than most other Australian native fruits (Netzel et al., 2007), suggesting that the fruit may have potential for the prevention or treatment of cancers.

Recently, Tan et al. (2011) investigated the anti-proliferative and pro-apoptotic activity of the Illawarra plum on a panel of cancer and normal cell lines and showed that the fruit extract exhibited greater anti-proliferative and pro-apoptotic activity in cancer cell lines than in normal cells (Tan et al., 2011). Another recent study also demonstrated that the Illawarra plum could inhibit telomerase, increase histone deacetylase activity and decrease proliferation of colon cancer cells (Symonds et al., 2012). However, further investigations are required to determine the efficacy of bioactive components from the Illawarra plum on other types of cancers, including pancreatic cancer.

3.8. Riberry (*Syzygium Leuhmannii,* Myrtaceae)

The riberry (*Syzygium leuhmannii*) belongs to the Myrtaceae family and is native to sub-tropical and tropical New South Wales and Queensland (CSIRO, 2006). The riberry is a rainforest tree between 4 and 30 m in height, while the riberry fruit is the main component used from the tree. The riberry fruit is up to 13 mm long and 4 mm in diameter, and is available from December to February. The fruit has a pink or red colour, is pear-shaped and has a spicy clove flavour, and it has been used to make jams and chutneys (Ahmed and Johnson, 2000). Recently, the whole fruit has been used as partial ingredients to make ice cream, chocolates and sauces for meat dishes (CSIRO, 2006).

Source: http://www.allnatives.com.au/trees/various/riberry-lily-pilly-syzygium-luehmannii.

Figure 8. Riberry (*Syzygium leuhmannii*).

The riberry contains lower levels of total phenolic compounds than other Australian native fruits such as the Davidson's plum, Illawarra plum and the Kakadu plum (Netzel et al., 2007). However, the riberry possesses a high total antioxidant activity with oxygen radical absorbance capacity of 817 μmol TE/g DW (Konczak et al., 2009). Several bioactive components have been identified from the riberry fruit including cyanidin 3-galactoside, cyanidin 3-glucoside, rutin, and quercetin (Konczak et al., 2010). These bioactive components have been linked with prevention of certain cancers. For example cyanidin 3-galactoside, in combination with the bilberry extract mirtoselect, was found to show potential for the prevention of colorectal cancer (Cooke et al., 2006), while rutin has been shown to inhibit the proliferation of murine leukemia cells and promotes the immune response *in vivo* (Lin et al., 2009). Despite limited data reporting the anti-cancer association of the riberry, there remains great potential for the discovery of novel anti-cancer agents following further investigations into the bioactive composition of the riberry.

4. PROPOSED TREND FOR FUTURE STUDIES ON AUSTRALIAN FLORA

Plants in Australia are potentially great sources of novel anti-cancer agents, including for pancreatic cancer (Vuong et al., 2014b). However, the major challenge is the diversity of plant materials. Therefore, priority should be given to the plants, which have been linked with therapeutic properties. Inheriting the knowledge from traditional use of plants as herbal medicines is essential to choosing the right starting materials for rigorous investigation. In Australia, the Aboriginal people have rich knowledge and experience in traditional medicines, which are extremely valuable for the selection of starting materials (Tan et al., 2010). In Figure 9 we propose a trend for future investigations of the anti-cancer activity of Australian flora.

Management and preparation of plant materials for further experimental processes are also important. As the plant kingdom is diversified, selected plant materials should be carefully authenticated by qualified experts to assure the types and classification of the materials. Preparation of plant materials is the next important step. As plant-derived bioactive compounds are sensitive to heat and light (Pandey and Rizvi, 2009), sample collection, transportation and storage need to be properly conducted to minimise the degradation of the bioactive compounds, especially for samples collected from long distances. Drying the samples is an extremely important step but is usually neglected. Drying is not only associated with the production cost, but it can significantly affect the stability of plant-derived bioactive compounds (Manach et al., 2004). Medicinal plant materials are usually dried under shade or under the sun for a few days and a significant loss of bioactive compounds can occur during this process due to enzymatic activity (Mahesh and Satish, 2008). Therefore, optimal drying conditions for each type of materials are important to reduce production costs and minimise the degradation of the bioactive compounds.

Optimisation of extraction to achieve maximal yield of bioactive compounds from the plant materials into the solvents for further isolation and purification is also important (Vuong et al., 2010). Optimum extraction conditions not only assists to infuse high levels of bioactive compounds, but also introduces sufficient composition of bioactive compounds into the solvents. Using improper extraction conditions could lead to false conclusions and

interpretations of content. There are eight different factors that might affect the extraction efficiency of the bioactive compounds. Extraction temperature, duration, ratio of solvent-to-samples, particle size, type of solvent, pH of solvents, number of extraction times, and methods of extraction (microwave assisted extraction, supercritical fluid extraction, ultra high pressure, ultrasonic assisted extraction, as highlighted in the earlier Chapter of Kha and Nguyen) have been reported to significantly affect the extraction efficiency of bioactive compounds from plant materials (Vuong et al., 2010). As different plant materials have various bioactive compounds with different physical and chemical properties, it is a must for future studies to establish optimal extraction conditions for each type of plant material.

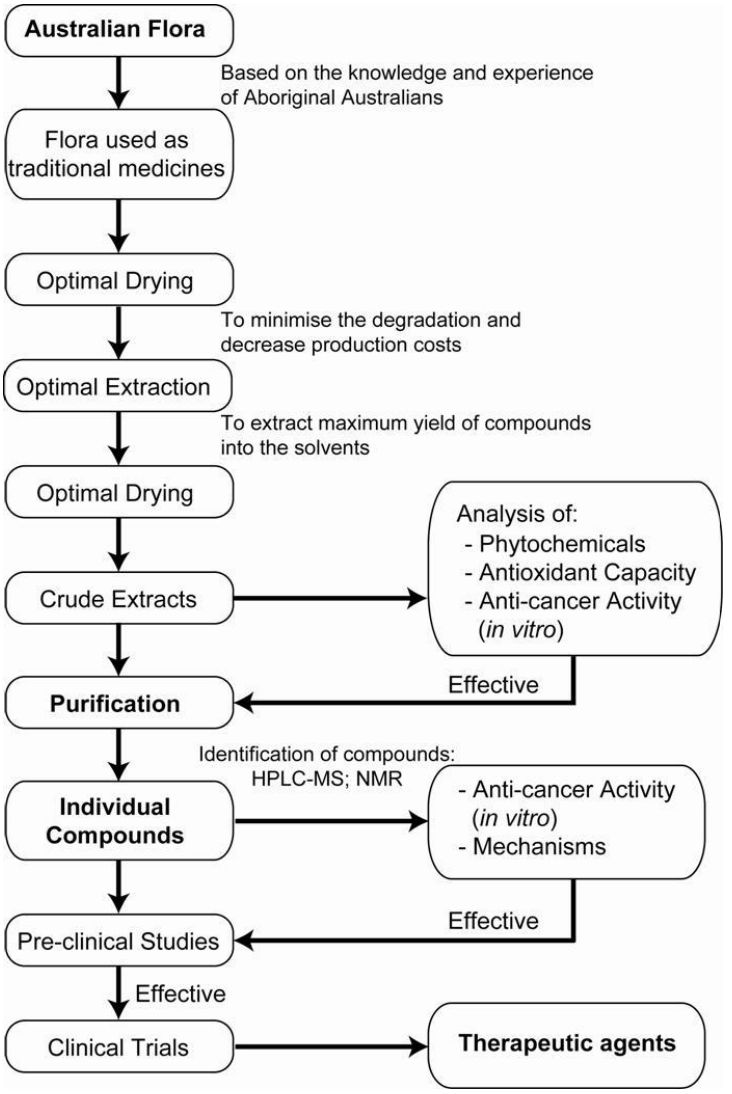

Figure 9. Proposed trend for screening, extraction and isolation of bioactive compounds from Australian flora for investigation of novel therapeutic agents for prevention and/or treatment of pancreatic cancer.

As different plant extracts and bioactive compounds may exhibit different health properties (Link et al., 2010), the preparation of crude extracts from plant materials for the preliminary screening their effect against pancreatic cancer cells *in vitro* is important to reduce the cost and time of further steps. If the plant crude extracts exhibit initial potent effects, further isolation and purification of groups of, or individual compounds are recommended for rigorous bioassay guided investigation. These active compounds can be identified using modern techniques such as UHPLC-MS and NMR. The mechanisms of these compounds can then be elucidated using state-of-the-art molecular biology techniques, with an aim of identifying compounds with efficacy against mechanisms identified from NGS studies. These promising bioactive compounds can also be chemically modified to improve their biological activity and then further assessed in pre-clinical animal models, then translted into early stage clinical trials.

CONCLUSION

Australian flora has great potential for the discovery of novel anti-cancer agents, with the hope that novel compounds will demonstrate efficacy against the deadly pancreatic cancer. Australia possesses such a rich, and diverse population of plant materials, which cannot be found anywhere else in the world, of which many have not been studied for their physicochemical properties let alone for their anti-cancer activity. It is clear that Australian native plants and fruits contain key bioactive compounds with demonstrated anti-cancer activity, and these investigations need to be escalated to find better therapeutic agents for diseases with no current effective cures. With the advantages of an enriched knowledge on the traditional use of plants as medicine from the Australian Indigenous population, and the diversification of plant materials, there are enormous opportunities for investigation of novel anti-cancer compounds from Australian flora.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 9

VIETNAMESE MEDICINAL PLANTS AS POTENTIAL ANTI-CANCER AGENTS

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ABSTRACT

Plants have been used as traditional drugs since ancient times and have remained an important source of new drugs and new chemical entities for entering into the pharmaceutical market. Today, many modern drugs in clinical use are derived from plants. The search for bioactive compounds from medicinal plants is often guided by ethnopharmacology and ethnotherapy, which leads to investigations of both crude medicine and plant-derived pharmaceutical agents. We highlight in this chapter the treatment experienced by Vietnamese people and the research of Vietnamese scientists on plants used as anti-cancer drugs and their active principles. Further, we provide the medicinal plants used in Vietnamese Ethnomedicine for the prevention and treatment of cancers, as well as the phytochemical, pharmacological and clinical studies on herbal medicines that are considered anti-cancer agents. Some developed anti-cancer drugs and promising natural compounds are also described.

1. INTRODUCTION

Plants have been used as medicines by man for thousands of years (Evans, 2002; Gurid-Fakim, 2006). A large number of plant species have been used as drugs in traditional

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medicine worldwide, and a number of plant-derived chemotherapeutic agents have been developed and translated into clinical use (Foye, Lemke, Williams, 1995; Gurid-Fakim, 2006). Historically, plants and their natural compounds were the most consistently successful source of drug leads (Harvey, 2000; Newman and Cragg, 2007). Today, this tremendous chemical diversity still remains an important source for the discovery and development of new candidate drugs, new synthetic drugs and drug analogues (Butler, 2005; Saklani and Kutty, 2008).

Vietnam, located in Southeast Asia, is a tropical country with different climatic and geographical features throughout its various regions. The country therefore has been granted an abundance of diverse natural resources, including a great variety of plant species (Ho, 1997). For a thousand of years, the Vietnamese people, especially in the countryside and the mountains, have used indigenous medicinal plants for the protection and treatment of illness, as well as for the maintenance of good health (Loi, 2012; Chi, 2012). Vietnam therefore possesses an age-old traditional system of medicine, a precious heritage handed down from times immemorial. Surveys have revealed that among the 12,000 species of plants in Vietnam, approximately 4000 have been used as medicinal plants in an ethnomedicine context (NIMM, 2010). For the past 30 years, the use of plants in Vietnamese ethnomedicine has involved the isolation and characterization of active constituents. Many bioactive compounds have been found from this natural source on the basis of their ethnomedical information and/or assessment of their biological activities (NIMM, 2004; Thuong 2014). Some of these active constituents are in preclinical studies, and some are in clinical use (Ho, 2006; Moi, 2005; Moi, 2009; NIMM, 2013).

In this chapter, we highlight the treatment by ethnic Vietnamese people and the research conducted by Vietnamese scientists on plants used as anti-cancer drugs and their active principles. We aim to provide the readers scientific information of the phytochemical, pharmacological and clinical studies on the Vietnamese medicinal plants. Further, we aim to inform the readers the therapeutic uses of medicinal plants for treating cancers in traditional Vietnamese medicine.

2. TRADITIONAL USE OF PLANTS AS ETHNOMEDICINE IN VIETNAM

It is estimated that approximately 80% of the population of Vietnam relies on traditional and folk medicines (MOH, 2012). Since ancient times, Vietnamese people have domesticated Traditional Chinese Medicine (TCM) and developed it to be an important medical system, which has contributed many benefits to healthcare in Vietnam (Duc, 1995; Loi, 2012). Although Traditional Vietnamese Medicine (TVM) has evolved under the shadows of TCM, Vietnamese people have created their own traditional system of medicine, a precious heritage handed down from times immemorial (Duc, 1995; Loi, 2012). Today, traditional medicine in Vietnam (generally known as Đông Y in Vietnamese) is separated into two major categories, including Traditional Vietnamese Medicine (called Southern Medicine, or Thuoc Nam in Vietnamese) and Traditional Chinese Medicine (Northern Medicine, or Thuoc Bac). Both of these categories are of oriental origin (Loi, 2012).

The drugs used in TVM (Đông Y) for the prevention and treatment of illness is based on some fundamental theories, which have existed for generations. The oldest and most widely

accepted theory is Yin and Yang (Âm Dương) (Loi, 2012), which proposes the existence of the important balance between opposite states Yin (cold, inactive, deficiency, interior, dark) and Yang (hot, active, excess, exterior, light). Yin diseases correspond to symptoms of cold, inactivity, deficiencies, and interior/within the body. In contrast, Yang conditions refer to hot, active, excess and exterior/outside. Another well-known theory in Đông Y is the Five Elements (Ngũ hành) (Loi, 2012). Everything, such as organs in the body, traditional drugs, and diseases could be divided into five element groups of Water, Wood, Fire, Earth, and Metal. Based on these natural elements, Đông Y physicians keenly relate these same concepts to our health. The drugs of the practice and clinical trials in Đông Y are guided by these theories and often given in a formula, which includes one or more ingredients and taken by decoction. Sometimes a formula can be taken in the form of powder, pill, extract, and even raw materials (Loi, 2012).

Most of the drugs used in TVM are plant-derived medicines, and minor ingredients are mushroom, animals and minerals (Loi, 2012). However, there is no report that characterizes exactly how many plant species are used in TVM. The Vietnamese Pharmacopoeia 2010 (VP 2010), the national document that defines the quality of drugs, describes 314 monographs of plant materials and remedies that are widely applied as drugs in Đông Y (MOH, 2010). These origins of oriental drugs listed in VP 2010 are mainly derived from both Vietnam (Thuốc Nam) and China (Thuốc Bắc), and rarely from other neighbouring countries. As well as TCM, TVM ingredients generally involve many processes of collection, preparation, formulation and practice (HUM, 1999; Loi, 2012).

Another important medical system in Vietnam is Vietnamese ethnomedicine (VEM) (NIMM, 2010; NIMM, 2013). Among the population of ninety million, more than sixty million Vietnamese people live in the countryside and mountainous regions, including a large number of ethnic minority groups that are scattered in the forest highlands. Fifty-three Vietnamese ethnic minority groups are living in these regions, which are poor and have certain difficulties in gaining access to modern medicines (Tây Y). For several thousands of years, these people have relied on locally available medicinal plants for medical protection and the treatment of illnesses as well as health maintenance with minimum processing. Most knowledge and experience were passed unselfconsciously from one generation to the next. Therefore, medicinal plants have made a tremendous contribution to the national health and development from the very beginning to now. Approximately 4,000 of the 12,000 species of plants in Vietnam have been used as medicinal plants in ethnomedicine (Ho, 1997; NIMM, 2004; NIMM, 2010; NIMM, 2013). People have used ingredients in ethnomedicine as remedies or formulas based on individual cultures and theories by practitioners and physicians all over the country, or by people themselves (NIMM, 2010; NIMM, 2013). Vietnamese people have used medicinal plants to treat many disease groups, such as infection and influenza, bronchial asthma, fever, cough, inflammation, pain and rheumatism, postpartum, allergy, hypertension and cardiovascular diseases, obesity and diabetes, cancers, as well as immunotherapy and other health benefits (NIMM, 2013).

There have been many publications on medicinal plants used in traditional and folk medicine in Vietnam. Perhaps the oldest literature on Vietnamese medicinal plants and its uses is the "*Miraculous Efficiency of Southern Medicine*" (Nam Dược thần hiệu) written in the 14th century that described 580 indigenous drugs of Vietnam (Tue Tinh, 1960). The author of this famous book, Tuệ Tĩnh, has been referred to as the Founder of Traditional Vietnamese Medicine. With the idea that "Vietnamese drugs cure Vietnamese people", he offered and

practised traditional medical services using plants grown and cultivated in Vietnam. In the 20th century, there have been many books reporting on the knowledge and experience of the use of Vietnamese medicinal plants. The two typical treatises are "*Vietnamese Medicinal Plants, Ingredients, and Remedies*" and "*Dictionary of Vietnamese Medicinal Plants*". The first book was written by Do Tat Loi, which illustrates more than 720 medicinal plant species, providing each scientific name, botanical characteristics, distribution and origin, processing and preparation, active principles, pharmacological effects, traditional and modern therapeutic uses (Loi, 2012). The second treatise describes 3107 medicinal plant species, listed alphabetically (Chi, 2012). Each document medical knowledge systems and therapeutic uses of a great number of plant species used in both VTM and VEM. However, many plant ingredients in both these books are of Chinese origin (called Thuốc Bắc), and do not exist in Vietnam. The National Institute of Medicinal Materials (NIMM) of Vietnam, has published several books of healing practice and experience with medicinal plants obtained during projects and surveys from all over the country (NIMM, 1990; NIMM, 1993; NIMM, 1999; NIMM, 2004; NIMM, 2013). Some of these books describe Vietnamese indigenous medicinal plants only, with an emphasis on native plants of Vietnam (NIMM, 1993; NIMM, 1999; NIMM, 2013).

3. MEDICINAL PLANTS FOR THE TREATMENT OF CANCER IN VIETNAMESE ETHNOMEDICINE

In 2008, the Vietnam National Cancer Hospital estimated that there were over 150,000 new cases with about 75,000 deaths of cancer per year. The rate is indicated to increase over time with the development of the country. In the year 2000, the ratio of the incidence of cancer in males was 141.6 per 100,000, increasing to 181.3 per 100,000 in 2010. In females, the incidence increased from 101.6 per 100,000 to 134.9 per 100,000 (MOH, 2012). The most common cancers are lung, stomach, liver, and colorectal cancers for males; and breast, stomach, uterine, and liver cancers for females (VNCH: http://www.benhvienk.vn). Recently, the Vietnam National Cancer Hospital estimated that the number of new cases and deaths of cancers in 2013 were 200,000 and 100,000, respectively. Cancer is now the major leading cause of death in Vietnam (MOH, 2010). Therefore, in 2010 the Vietnamese Government initiated the "National Strategy for Cancer Control" with the major aim to reduce the cancer incidence and mortality rate, and to improve the quality of life for patients with cancer (VNCH: http://www.benhvienk.vn). The Vietnam National Cancer Hospital, and Centers for Oncology of National Hospitals all over the country contribute principally to the tasks of this project (Nguyen, 2006; VNCH://www.benhvienk.vn). The prevention and treatment of cancers in the Vietnamese Public Health system relies upon both Western Medicine and Traditional Vietnamese Medicine, however, the methods of chemotherapy, radiation therapy and surgery are mostly applied (Nguyen, 2006).

Although prevention and treatment of cancers by medicinal plants and remedies are not populated in the Public Health system, Vietnamese people use these tools for the treatment of these diseases. Many of the 4000 medicinal plants species have been used for the prevention and treatment of cancers in Vietnam ethnomedicine (NIMM, 2013; Hoai, 2013; Phan, 2002; Duc, 1997).

Table 1. Medicinal plants used as anticancer drugs in Viet Nam ethnomedicine

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
1	*Acanthopanax gracilistylus*	Araliaceae	Ngũ gia bì hương	Stem bark	Cancers, Immunotherapy Gastric cancer, Immunotherapy	NIMM, 2013 Phan 2002
2	*Acanthopanax aculeatus*	Araliaceae	Ngũ gia bì gai	Stem bark	Immunotherapy Gastric cancer, Immunotherapy	NIMM, 2013 Phan, 2002
3	*Adenosma caeruleum*	Scrophulariaceae	Nhân trần	Aerial parts	Liver cancer	NIMM, 2013;
4	*Adenosma indianum*	Scrophulariaceae	Bồ bồ	Aerial parts	Liver cancer	NIMM, 2013
5	*Aglaonema costatum*	Araceae	Vạn niên bụi	Root	Gastric, liver cancers	NIMM, 2013
6	*Allium sativum*	Liliaceae	Tỏi	Bulbs	Cancer prevention and treatment Gastrointestinal, liver, oesophagus, lung cancers	NIMM, 2013 Phan, 2002
7	*Aloe vera*	Asphodelaceae	Lô hội, nha đam	Leaf	Cancer prevention and treatment	Phan, 2002
8	*Amorphophallus rivieri*	Araceae	Khoai nưa, nưa	Rhizome	Liver cancer and lymphoma	Duc, 1997
9	*Ampelopsis heterophylla* var. *hancei*	Vitaceae	Dâu dây, dây mề gà	Aerial parts	Cancers	Duc, 1997
10	*Andrographis paniculata*	Acanthaceae	Xuyên tâm liên	Whole plant	Gastric cancer Breast, gastrointestinal, liver, oesophagus, tongue cancers	NIMM, 2013 Phan, 2002

Table 1. (Continued)

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
11	*Angelica dahurica*	Apiaceae	Bạch chỉ	Root	Cancer prevention and treatment, Immunotherapy	NIMM, 2013; Phan, 2002
12	*Angelica acutiloba*	Apiaceae	Đương quy	Root	Leukemia, liver, breast, colon, pharyngeal and uterus cancers, Immunotherapy	NIMM, 2013; Phan, 2002
13	*Anemone rivularis*	Ranunculaceae	Phong quì bờ	Whole plant	Lung, uterus cancers Gular cancer	NIMM, 2013 Phan, 2012
14	*Anodendron paniculatum*	Apocynaceae	Dây duy, ngà voi	Root Stem bark	Tumors	Hoai, 2013
15	*Anoectochilus roxburghii* *Anoectochilus albolineatus* *Anoectochilus sikkimensis*	Orchidaceae	Lan kim tuyến, lan gấm, cỏ nhung	Whole plant	Cancer prevention, Immunotherapy	NIMM, 2013
16	*Aphanamixis polystachya*	Meliaceae	Gội nước	Stem bark	Tumors	Phan, 2002
17	*Anthocephalus cadamba*	Rubiaceae	Gáo	Stem bark	Liver cancers	NIMM, 2013
18	*Arctium lappa*	Asteraceae	Ngưu bàng	Root, Seed	Cancer prevention and treatment. : uterine, colon, gular cancers	Phan, 2002
19	*Argimonia eupatoria*	Rosaceae	Long nha thảo	Whole plant	Breast, colon, Uterus cancers Cancer prevention	NIMM, 2013; Phan, 2002

No	*Species*	Family	Vernacular name	Part used	Indication	References
	Plants					
20	*Artabotrys hexapetalus*	Annonaceae	Móng rồng	Stem, root	Carcinoids	NIMM, 2013
21	*Asparagus cochinchinensis*	Asparagaceae	Thiên môn	Radix	Breast and lung cancers	Phan, 2002
22	*Bombax ceiba*	Bombacaceae	Gạo	Stem and root bark	Lung, liver and gastrointestinal cancers	NIMM, 2013; Phan, 2002
23	*Canarium sp*	Burseraceae	Trám hồng	Stem bark	Tumors	NIMM, 2013
24	*Carica papaya*	Caricaceae	Đu đủ	Leaf	Cancers	NIMM, 2004 Duc, 1997
25	*Celastrus hindsii*	Celastraceae	Dây gối	Aerial parts	Cancers	NIMM, 2013
26	*Cirsium japonicum*	Asteraceae	Đại kế	Whole plant	Liver cancers	NIMM, 2013; Phan, 2002
27	*Codonopsis javanica*	Campanulaceae	Đẳng sâm	Root	Leukemia, Immunotherapy	NIMM, 2013 Phan, 2002
28	*Corchorus capsularis*	Tiliaceae	Đay	Seeds	Liver cancer	NIMM, 2013
29	*Coscinium fenestratum* *Coscinium usitatum*	Menispermaceae	Vàng đắng	Stem, root	Liver cancer	NIMM, 2013
30	*Costus speciosus*	Costaceae	Mía dò	Rhizome	Liver and kidney cancers	NIMM, 2013
31	*Coptis teeta* *Coptis chinensis*	Ranunculaceae	Hoàng liên chân gà	Root	Colon, skin cancers	Phan, 2002 NIMM, 2013
32	*Crinum latifolium*	Amaryllidaceae	Trinh nữ hoàng cung	Leaf	Prostate, breast, ovarian cancers	Nguyen, 2014

Table 1. (Continued)

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
33	*Crinum asiaticum*	Amaryllidaceae	Náng hoa trắng	Leaf	Prostate and bladder cancers Breast cancer	NIMM, 2013 Phan, 2002
34	*Cudrania tricuspidata*	Moraceae	Mỏ quạ	Whole plant	Cancers	NIMM, 2013 Phan, 2002
35	*Curcuma longa*	Zingiberaceae	Nghệ	Rhizome	Cancer prevention and treatment	NIMM, 2013; Phan, 2002
36	*Curcuma zedoaria*	Zingiberaceae	Nghệ đen, Nga truật	Rhizome	Uterine, colon, liver, bladder, gastrointestinal and skin cancers	NIMM, 2013 Phan, 2002
37	*Dasymaschalon rostratum var. glaucum*	Annonaceae	Nhãn chày, Mao quả có mỏ	Leaf	Leukemia, Bone cancer, Tumors	Hoai, 2013
38	*Dioscorea bulbifera*	Dioscoreaceae	Củ dại	Root	Gastric and oesophagus cancers	Duc, 1997
39	*Ehretia asperula*	Boraginaceae	Xạ đen	Aerial parts	Cancers, Immunotherapy	NIMM, 2013
40	*Elephantopus scaber*	Asteraceae	Cúc chỉ thiên	Whole plant	Liver cancer, Tumors	Phan, 2002
41	*Elephantopus mollis*	Asteraceae	Cúc chỉ thiên mềm	Whole plant	Liver cancer, Tumors	Phan, 2002
42	*Ficus pumila*	Moraceae	Trâu cổ	Fruit	Cancers, Immunotherapy Breast, colon, liver, uterine cancers	NIMM, 2013 Phan, 2002
43	*Ficus vasculosa*	Moraceae	Đa bông	Leave, root	Breast, colon, liver cancers	NIMM, 2013; Phan, 2002
44	*Fragaria indica*	Rosaceae	Dâu đất, Dâu dại	Whole plant	Breast, gastric cancers	Phan, 2002

No	*Species*	Family	Vernacular name	Part used	Indication	References
	Plants					
45	*Glechoma longituba*	Lamiaceae	Rau má lông	Whole plant	Kidney, bladder, prostate, liver and pancreatic cancers	NIMM, 2013; Phan, 2002
46	*Glycine max*	Fabaceae	Đỗ tương	Seed	Cancer prevention, Immunotherapy	Phan, 2002
47	*Gomphandra tonkinensis*	Icacinaceae	Bổ béo	Root	Liver cancer	NIMM, 2013
48	*Gynostemma laxum*	Curcubitaceae	Cổ yếm lá bóng	Whole plant	Cancers, Immunotherapy	NIMM, 2013
49	*Gynostemma pentaphyllum*	Cucurbitaceae	Giảo cổ lam	Whole plant	Cancers, Immunotherapy	NIMM, 2013; Phan 2002
50	*Gynostemma pubescens*	Cucurbitaceae	Thất diệp đởm	Whole plant	Cancers, Immunotherapy	NIMM, 2013
51	*Hedyotis diffusa*	Rubiaceae	Bạch hoa xà thiệt thảo	Whole plant	Leukemia, Cancers HIV/AIDS Immunotherapy Colon, liver and lung cancers	NIMM, 2013; Phan, 2002 Duc, 1997
52	*Hedyotis pressa*	Rubiaceae	An điền sát	Wh	Tumors	Hoai, 2013
53	*Hedyotis tenelliflora*	Rubiaceae	Bòi ngòi hoa nhỏ	Wh	Cancers	Phan, 2002
54	*Helixanthera parasitica*	Loranthaceae	Chùm gửi kí sinh	Wh	Gastric cancers	Hoai, 2013
56	*Hydnophytum formicarium*	Rubiaceae	Bí kỳ nam	Stem	Liver cancer	Phan, 2002
57	*Hydrocotyle nepalensis*	Apiaceae	Rau má lá to	Wh	Liver cancer	Phan, 2002
58	*Hydrocotyle sibthorpioides*	Apiaceae	Rau má mơ	Wh	Liver cancer	Phan, 2002
59	*Hovenia dulcis*	Rhamnaceae	Khúng khéng	Fr	Liver cancer	NIMM, 2013; Phan, 2002

Table 1. (Continued)

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
60	*Lactuca indica*	Asteraceae	Bồ công anh Việt Nam	Arial parts	Lung, oeophagus, gastrointestinal, nose cancers	Phan, 2002
61	*Leea rubra*	Leeaceae	Gối hạc tía	Root	Tumors	Hoai, 2013
62	*Livistona chinensis*	Arecaceae	Cọ	Fruit	Nasal, oesophagus, pharyngeal cancers	Duc, 1997
63	*Lonicera japonica*	Asclepiadaceae	Kim ngân	Arial parts, flower	Prostate cancer	Phan, 2002
64	*Mahonia japonica*	Berberidaceae	Hoàng liên ô rô	Root, stem	Liver, lung cancers	Phan, 2002
65	*Micromelum minutum*	Rutaceae	Kim sương	Leaf, stem	Neck cancer, Tumors	Hoai, 2013
66	*Morinda longissima*	Rubiaceae	Nhó đông	Root	Liver cancer	NIMM, 2013
67	*Morinda officinalis*	Rubiaceae	Ba kích, cây ruột gà	Root	Bone, brain, and prostate cancers, Immunotherapy	Phan, 2002
68	*Morus alba*	Moraceae	Dâu tằm	Root, stem, leave, fruit	Cancer prevention and treatment	Phan, 2002
69	*Momordica cochinchinensis*	Cucurbitaceae	Gấc (màng hạt)	Aril	Cancer prevention and treatment	Phan, 2002
70	*Momordica cochinchinensis*	Cucurbitaceae	Gấc (hạt)	Seed	Leukemia, brain, breast, bone, gastric cancers	Phan, 2002
71	*Momordica charantia*	Cucurbitaceae	Mướp đắng	Fruit	Cancer prevention and treatment	Phan, 2002

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
72	*Morinda citrifolia*	Rubiaceae	Nhàu	Fruit, root	Cancers, Immunotherapy	Phan, 2002
73	*Morinda officinalis*	Rubiaceae	Ba kích	Root	Cancers, Immunotherapy Bone, brain, prostate cancers	NIMM, 2013 Phan, 2002
74	*Morinda cochinchinensis*	Rubiaceae	Ba kích lông	Root	Cancers, Immunotherapy	NIMM, 2013
75	*Naravelia laurifolia*	Ranunculaceae	Bạch tu lá quế	Root	Tumors	Hoai, 2013
76	*Panax bipinnatifidus*	Araliaceae	Tam thất vũ diệp	Rhizome and Radix	Cancers, Immunotherapy	NIMM, 2013
77	*Panax noto-ginseng*	Araliaceae	Tam thất	Radix	Cancers, Leukemia, Immunotherapy	Phan, 2002 NIMM, 2013
78	*Panax stipuleanatus*	Araliaceae	Tam thất hoang	Rhizome and Radix	Cancers, Immunotherapy	NIMM, 2013
79	*Panax vietnamensis*	Araliaceae	Sâm Việt Nam	Rhizome and Radix	Cancer prevention and treatment, immunotherapy	NIMM, 2013
80	*Pandanus tectorius*	Pandanaceae	Dứa dại	Root	Liver cancer	NIMM, 2013
81	*Paramignya trimera*	Rutaceae	Xáo tam phân	Stem, Root	Cancers	Reputed
82	*Paris spp.*		Bảy lá một hoa	Root	Breast, gastric, lung, colon, oropharyngeal cancers Lung cancer	Ethnomedicine Duc, 1997
83	*Phellodendron amurense*	Rutaceae	Hoàng bá	Stem and root bark	Cancers	Phan, 2002
84	*Phyllanthus urinaria*	Euphorbiaceae	Chó đẻ răng cưa	Aerial parts	Liver cancer	NIMM, 2013
85	*Phyllanthus amarus*	Euphorbiaceae	Diệp hạ châu đắng	Aerial parts	Liver cancer	NIMM, 2013

Table 1. (Continued)

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
86	*Phyllanthus reticulatus*	Euphorbiaceae	Phèn đen	Aerial parts	Liver cancer	NIMM, 2013
87	*Podophyllum tonkinense*	Berberidaceae	Bát giác liên	Rhizome	Cancers Breast cancer and lymphoma	NIMM, 2013 Duc, 1997
88	*Polygonum cuspidatum*	Polygonaceae	Cốt khí củ	Rhizome	Liver cancer	Phan, 2002
89	*Polygonum multiflorum*	Polygonaceae	Hà thủ ô đỏ	Root	Cancers, leukemia Immunotherapy	Phan, 2002
90	*Polygonum perfoliatum*	Polygonaceae	Thồm lồm gai	Wh	Kidney, liver cancers	Phan, 2002
91	*Plumbago zeylanica*	Plumbaginaceae	Bạch hoa xà	Wh	Leukemia, cancers	Phan, 2002 NIMM, 2013
92	*Prunella vulgaris*	Lamiaceae	Hạ khô thảo	Aerial parts	Breast, bone, neck, liver and gastrointestinal cancers	Phan, 2002
93	*Pseuderanthemum palatiferum* *Pseuderanthemum bracteatum*	Acanthaceae	Xuân hoa, hoàn ngọc, con khỉ	Aerial Parts	Cancers, Immunotherapy	NIMM, 2013
94	*Rorippa indica*	Brassicaceae	Cải hoang	Whole plant	Liver, lung cancers	Phan, 2002
95	*Salacia chinensis*	Celastraceae	Chóc máu	Stem, Root	Cancers	NIMM, 2013
96	*Salacia cochinchinensis*	Celastraceae	Chóc máu nam	Stem, Root	Cancers	NIMM, 2013
97	*Salvia miltiorrhiza*	Lamiaceae	Đan sâm	Root	Liver, gastric cancers	Phan, 2002

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
98	*Sarcandra glabra*	Chloranthaceae	Sói rừng	Whole plant	Pancreatic, liver, gastric and colorectal cancers	Phan, 2002 NIMM, 2013
99	*Sausurus chinensis*	Saururaceae	Hàm ếch	Whole plant	Bladder, prostate, renal, liver cancers	Phan, 2002
100	*Schefflera canaensis*	Araliaceae	Đáng	Stem bark	Tumors	Phan, 2002
101	*Schisandra sphenanthera*	Schisandraceae	Ngũ vị tử nam	Fruit	Liver cancer, Immunotherapy	Phan, 2002
102	*Scoparia dulcis*	Scrophulariaceae	Cam thảo đất	Whole plant	Lung cancer	Phan, 2002
103	*Scutellaria baicalensis*	Lamiaceae	Hoàng cầm	Root	Cancers	
104	*Scutellaria barbata*	Lamiaceae	Bán chi liên, Hoàng cầm râu	Whole plant	Breast, liver, lung and colon cancers	Duc, 1997; NIMM, 2013; Phan, 2002
105	*Selaginella doederleinii*	Selaginellaceae	Quyển bá xanh lục	Whole plant	Liver and lung cancers Lung, nasal and oropharyngeal cancers	NIMM, 2013 Duc, 1997
106	*Selaginella tamariscina*	Selaginellaceae	Quyển bá trường sinh	Whole plant	Cancers	NIMM, 2013
107	*Smilax* spp	Smilacaceae	Thổ phục linh	Rhizome	Gastric, colon, nasal, gular and cervical cancers	Duc, 1997; Phan, 2002
108	*Solanum procumbens*	Solanaceae	Cà gai leo	Whole plant	Liver cancer	NIMM, 2013; Phan, 2002
109	*Solanum nigrum*	Solanaceae	Lu lu đực	Whole plant	Tumors, breast cancer	Phan, 2002
110	*Taraxacum spp*	Asteraceae	Bồ công anh Trung Quốc	Whole plant	Lung, oeophagus, gastrointestinal, nose cancers	Phan, 2002
111	*Torenia benthamiana*	Scrophulariaceae	Tô liên	Whole plant	Gastric cancers	Hoai, 2013

Table 1. (Continued)

No	Species	Family	Vernacular name	Part used	Indication	References
	Plants					
112	*Trapa bicornis*	Trapaceae	Củ ấu	Fruit	Breast, gastric, uterine cancers	Duc, 2012
113	*Tylophora spp.*	Asclepiadaceae	Đầu đài	Ari	Leukemia, skin	Phan, 2002
114	*Uvaria cordata*	Annonaceae	Bồ quả lá to	Stem	Tumors	Hoai, 2013
115	*Uvaria grandiflora*	Annonaceae	Bù dẻ tía	Leaf, Stem	Tumors	Hoai, 2013
	Fungi					
116	*Cordyseps sinensis*	Hypocreaceae	Đông trùng hạ thảo	Whole	Brain, liver cancers, Immunotherapy	Phan, 2002
117	*Ganoderma lucidum*	Ganodermataceae	Nấm linh chi	Whole	Liver cancer, Cancer prevention, Immunotherapy	NIMM, 2013 Phan, 2002
118	*Ganoderma neo-japonicum*	Ganodermataceae	Linh chi đầu rắn	Whole	Liver cancer, Cancer prevention, Immunotherapy	NIMM, 2013
119	*Ganoderma applanatum*	Ganodermataceae	Nấm linh chi cổ	Whole	Cancer prevention, Immunotherapy	NIMM, 2013
120	*Lentinus edodes*	Pleurotaceae	Nấm hương	Whole	Leukemia, lung, gastrointestinal and uterine cancers	Phan, 2002

Habitual plants with their botanical names and uses are listed in Table 1 (NIMM, 2013; Hoai, 2013; Phan, 2002; Duc, 1997). All of these plants are found in Vietnam, most of these are used ethnomedically without scientific research, and some have been studied at a preclinical level. Only a few of these plants have been clinically studied for their anti-cancer activities. Many of these herbs are used similarly with the Chinese Traditional Medicine (Chang, 1992).

4. MEDICINAL PLANTS IN VIETNAM AS POTENTIAL ANTI-CANCER AGENTS

4.1. Plant-derived Anti-Cancer Agents in Preclinical Development

4.1.1. ent-Kaurane Diterpenoids from Croton tonkinensis

The plant *Croton tonkinensis* Gagnep. (family Euphorbiaceae), vernacularly known as "khổ sâm bắc bộ" or "khổ sâm cho lá", is a native plant to North of Vietnam ("Tonkin" means "bắc bộ", North of Vietnam). The leaf of this plant is traditionally used for the prevention and treatment of stomach ache and malaria, but not for cancer (Loi, 2012; Chi, 2012). However, in a recent study by Thuong et al (2011), a preliminary screening assessment revealed that the methanol extract of these leaves displayed significant cytotoxicity activity against various cancer cell lines *in vitro* (Thuong et al, 2011). Bioassay-guided investigation of the active principles of this plant led to the isolation of more than 30 of natural *ent*-kaurane diterpenoids (C*e*KDs) from the plant *Croton tonkinensis* (family Euphorbiaceae) (Thuong et al., 2009; Thuong et al., 2011; Thuong et al., 2012; Dao et al., 2010; Dao et al., 2011).

The cytotoxic activities against various cancer cell lines of isolates were examined, and were compared with the chemotherapeutic agents camptothecin and palitaxel as positive controls (Thuong et al., 2011; Thuong et al., 2012; Thuong et al., 2014c). Among them, only C*e*KDs with the 15-oxo-16-ene moiety (Figure 1) exhibited significant inhibitory effects on cell growth, when compared with compounds without this moiety, which displayed less than 10-fold weaker activity (Thuong et al., 2011; Thuong et al., 2014a; Kou et al., 2007). The results summarized in Table 2 clearly indicate that C*e*KDs **1**-**6** significantly suppressed the survival of cancer cells and exhibited comparable activities, showing IC_{50} values of 0.48-0.83 μM and 1.17-2.19 μM against Caco-2 and LS180 cells, respectively. Two *ent*-kauranoid dimmers (**11** and **12**; Figure 1) were also isolated from this plant and found to exhibited strong cytotoxicity against some cancer cell lines (Thuong et al., 2012). There were significant differences in growth inhibitory activity between C*e*KDs **1**-**6** bearing the moiety 15-oxo-16-ene and compounds **7**-**10** without this structure. Therefore, this 15-oxo-16-ene (CH_2=C−C=O) moiety appears to be necessary for the strong cytotoxic activity of C*e*KDs, similar to previous reports (Lee et al., 1971; Lee et al., 1977).

Further studies indicated that active C*e*KDs suppressed cancer cell growth by inducing apoptosis of the colorectal cancer cell lines Caco-2 and LS180 (Thuong et al., 2014a). To investigate the mechanisms by which C*e*KDs prompt cancer cells to undergo apoptosis, Thuong et al (2014) found that active C*e*KD induced the activation of ERK and JNK, but the inactive ones induced that of ERK, but not that of JNK. It thus appears that JNK played an important role in the apoptotic activity of the active compounds. The dual-specificity JNK

kinase MKK4 was activated in both colorectal cancer cells treated with the active C*e*KD, but MKK7 was not activated. Further, the active C*e*KD (**1**), but not the inactive one (**8**), enhanced the generation of intracellular reactive oxygen species (ROS) in both cells. C*e*KD-induced cell apoptosis and ROS generation, as well as JNK activation, were inhibited by the antioxidant *N*-acetyl-L-cysteine. Therefore, ROS stimulated the phosphorylation of JNK mediated by MKK4 and played a critical role in C*e*KD-induced apoptosis in colorectal cancer cells. The moiety 15-oxo-16-ene may attack cysteine residues in redox sensor proteins of cancer cells and lead to the generation of ROS, which would play a critical role in the activation of MKK4 and subsequent JNK activation (Thuong et al., 2014a). Another study on the molecular mechanism of C*e*KD induced-apoptotic action in human hepatocellular carcinoma SK-HEP1 cells indicated that activation of AMP-activated protein kinase is responsible for compound **1**-induced anti-cancer activity including apoptosis (Sul et al., 2013). Our results suggest that *e*KDs with the moiety 15-oxo-16-ene from the plant *C. tonkinensis* significantly enhance apoptosis in cancer cell lines. This group of natural constituents should be further considered as potential agents for the discovery and development of anti-cancer drugs.

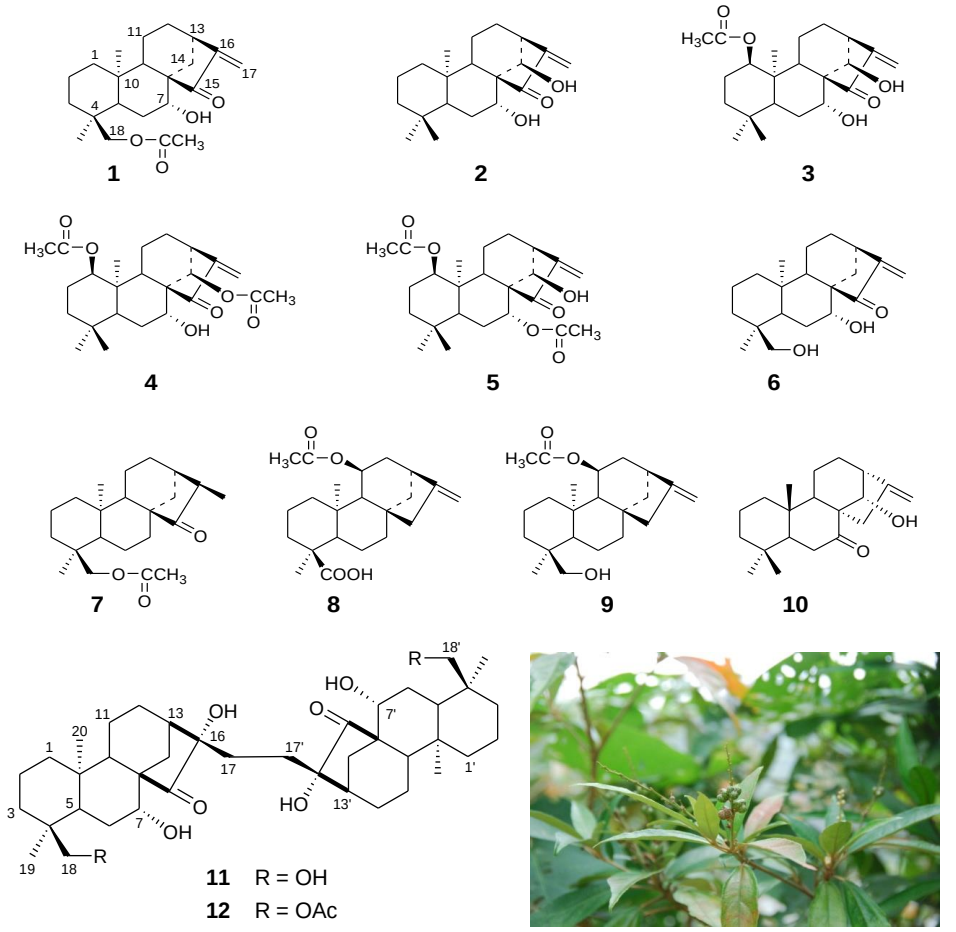


Figure 1. Cytotoxic compounds from the plant *Croton tonkinensis.*

Table 2. Inhibitory effects of diterpenoids from *Croton tonkinensis* on colorectal cancer cell proliferation (Thuong et al., 2014a,c)

Compound	IC_{50} (μM)[a]	
	Caco-2	LS180
ent-18-acetoxy-7*β*-hydroxykaur-15-oxo-16-ene (**1**)	0.59 ± 0.18	1.38 ± 0.13
ent-7*β*,14*α*-dihydroxykaur-15-oxo-16-ene (**2**)	0.83 ± 0.27	1.50 ± 0.28
ent-1*α*-acetoxy-7*β*,14*α*-dihydroxykaur-15-oxo-16-ene (**3**)	0.48 ± 0.10	2.19 ± 0.25
ent-1*α*,14*α*–diacetoxy-7*β*-hydroxykaur-15-oxo-16-ene (**4**)	0.61 ± 0.30	1.38 ± 0.36
ent-1*α*,7*β*-diacetoxy-14*α*-hydroxykaur-15-oxo-16-ene (**5**)	0.63 ± 0.25	1.30 ± 0.04
ent-7*β*,18-dihydroxykaur-15-oxo-16-ene (**6**)	0.83 ± 0.04	1.17 ± 0.33
ent-(16*S*)-18-acetoxykaur-15-one (**7**)	>10[b]	>10
ent-11*α*-acetoxykaur-16-en-18-oic acid (**8**)	>10	>10
ent-11*α*-acetoxykaur-16-en-18-ol (**9**)	>10	>10
14*α*-hydroxykaur-16-en-7-one (crotonkinin A, **10**)	>10	>10
Camptothecin[c]	0.05 ± 0.01	0.19 ± 0.06
Betulinic acid[c]	>10	>10

[a] Values are mean ± SD from three separated experiments; [b] The inhibitory effects did not reach 50% at 10 μM; [c] Reference compounds.

4.1.2. Pristimerin from Salacia cochinchinensis

A methanol extract of the roots of the Vietnamese plant *Salacia cochinchinensis* Lour. (Celastraceae) also displayed a remarkable suppressive effect on cancer cell growth (Lee et al., 2013; Thuong et al., 2014c). The plant *Salacia cochinchinensis* is also a native species in Central Vietnam, called "Chóc máu Nam bộ" (cochinchinen means "Nam bộ"). The ethnic group Vân Kiều uses the root and stem of this species for the treatment of rheumatism, pain and asthenia (NIMM, 2013). Bioassay-guided phytochemical studies on this methanol extract led to the isolation of an active principle pristimerin (**13)**, which is a major constituent in the material (Figure 2). This compound, a quinonemethide triterpenoid, exhibited significant cytotoxic activity against all tested cancer cells (Table 3), including breast cancer cells (MCF-7, MCF/Adr, MCF7/Tam, SKBR3), colorectal cancer cells (Caco-2, LS180, Lovo), gastric (NCI-N87), liver (HepG2), lung (A549), ovarian (OVCAR-8) and uterine (HeLa) cancer cells.

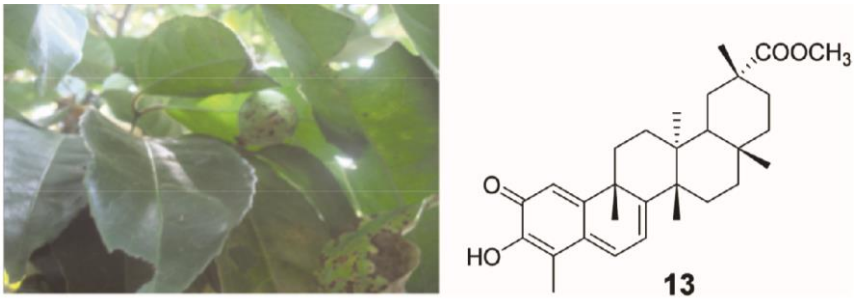


Figure 2. The plant *Salacia cochinchinensis* and the active compound Pristimerin.

Since pristimerin (**13)** significantly inhibited proliferation in dose- and time-dependent manners in many cancer cell lines, we and colleagues studied the molecular target of apoptosis-induction by this compound in the SKBR3 human breast cancer cell line. It was found that pristimerin suppressed the epidermal growth factor receptor 2 (HER-2) protein and mRNA expression, and downregulated fatty acid synthase (FASN) expression. The results indicate that pristimerin is a novel HER2-downregulated compound that is able to decrease fatty acid synthase and modulate the Akt, MARK, and mTOR signalling pathways to influence metastatic and apoptosis. The compound therefore should be considered for further evaluation as a new chemotherapeutic agent for treatment of cancers (Lee et al., 2013).

Table 3. Cytotoxic activity of pristimerin isolated from *S. cochinchinensis* (Thuong et al., 2014c)

Cell Line	Inhibitory effect (IC_{50}, μM)		
	Pristimerin	**Adriamicin**[a]	**Camptothecin**[a]
MCF-7	0.68	0.63	-
MCF/Tam	1.54	0.54	-
MCF/Adr	2.32	21.66	-
NCI-N87	2.12	1.07	-
HepG2	2.18	0.56	-
A549	2.82	0.74	-
OVCAR-8	1.17	0.61	-
Hela	0.73	0.11	-
Caco-2	0.28	-	0.05
LS180	0.79	-	0.19
Lovo	0.23	-	0.08
SKBR3	2.4	-	-
MDA-MB-435	0.55	-	-
K562	3.2	-	-

[a]Adriamycin and camptothecin were used as positive controls.

4.1.3. Meso-Dihydroguaiaretic acid from Myristica fragrans

Four lignans, including *meso*-dihydroguaiaretic acid (**14)**, macelignan, fragransin A_2 and nectandrin B, were isolated from the seeds of *Myristica fragrans* (Figure 3; Vietnamese nutmeg, "Nhục đậu khấu"), belonging to the family Myristicaceae (Thuong et al., 2014b). The four isolates were tested for their cytotoxic activity against eight cancer cell lines, including H1299, H358, H460, Hela, HepG2, KPl4, MCF7, and RD. The results showed that compound (**14)** exhibited the most potent cytotoxicity, with IC_{50} values of 10.1-27.7 μM. This compound also showed antitumour activity in allogeneic tumour-bearing mice model. When compound **14** was orally administered to the allogeneic tumour-bearing mice, the sizes of solid tumours were significantly reduced and the life spans of the tumour bearing mice were elongated (Thuong et al., 2014). This finding suggests that the major constituent in Vietnamese nutmeg *meso*-dihydroguaiaretic acid possesses promising anti-cancer properties. The mechanism of antitumour action of this compound remains inconclusive and in need of further investigation.

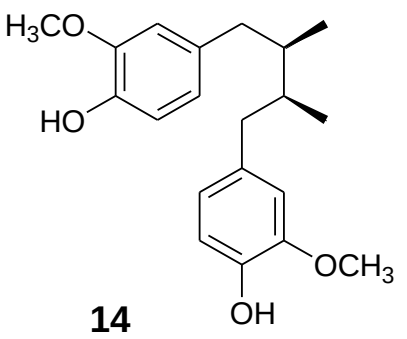


Figure 3. The seeds of *Myristica fragrans* and *meso*-dihydroguaiaretic acid.

4.1.4. Promising plants and their active principles

Many natural compounds from Vietnamese medicinal plants have been studied for their cytotoxicity against various different human cancer cell lines (Figure 4). In our program searching for anticancer agents from Vietnamese medicinal plants, we screened plant extract for their citotoxic activities against many human cancer cell lines, for example A549, HeLa, HepG2, K562, L-1210, MCF-7, NCI-N87, and OVCAR-8 (Thuong, 2014c). Some candidate plants have been selected for phytochemical and biological investigation. Parthenin (**15**) from *Parthenium hyoscyamus* (Cúc liên chi dại) and a *Crinum ensifolium* (Náng hoa trắng) exhibited significant cytotoxicity against eight cancer cell lines (Khoi et al., 2011). Three diterpenoids tanshinone I (**16**), tanshinone IIA (**17**), and cripotanshinone (**18**), major principles of the *Salvia miltiorrhiza* (Đan sâm), are found to display interesting suppressive effects on non small cell lung cancer cells (Thuong et al., 2014c). The triterpenoids ursolic acid (**19**), oleanolic acid (**20**), and betulinic acid (**21**) were isolated from the same medicinal plant *Tetracera scandens* (Chặc chìu). Other triterpenoids corosolic acid (**22**), maslinic acid (**23**), and 24-hydroxyursolic acid (**24**) were obtained from *Lagerstroemia speciosa* (Bằng lăng), *Leea rubra* (Gối hạc), and *Diospyros kaki* (Hồng), respectively. All these compounds (**19**-**24**) are of interest in our program's search for anti-cancer agents from nature (Thuong, 2014c). Flavonoids such as baicalein (**25**) and chrysin (**26**) from *Oroxylum indicum* (Núc nác) and 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (**27**) from *Cleistocalyx operculatus* (Vối), and a lignan (**28**) from *Leea rubra* are also obtained and considered in our program (Thuong, 2014c). Numerous other scientific groups are searching for anti-cancer agents from natural products in Vietnam and also found many promising cytotoxic lignans (**29**-**36**) from Vietnamese medicinal plants: two major lignans secoisolarisiresinol (**29**) and isotaxiresinol (**30**) from *Taxus yunnenensis* (Thông đỏ) inhibit tumor necrosis factor-α-dependent hepatic injury induced by D-galactosamine/liposaccharide in mice (Banskota et al., 2004); the compound isolated from *Bursera tonkinensis* (Rẫm bắc bộ) 4′-demethyldesoxypodophyl-lotoxin (**31**) showed significant cytotoxicity against human mouth epidermoid carcinoma (KB), human colon cancer (Col2) and human prostate cancer (LNCaP) cell lines (Jutiviboonsuk et al., 2005); three compounds from *Aglaia perviridis* (Gội xanh), including rocaglaol (**32**), 4′-demethoxy-3′-4′-methylenedioxyrocaglaol (**33**), and 4′-demethoxy-3′-4′-methylenedioxyrocaglate (**34**) were found to have remarkable cytotoxicity against human

colon cancer cell line (HT-29) and normal colon cell line (CCD-112CoN) as well as significant NF-kB (p65) inhibitory activity (Pan et al., 2013); some arylnaphthalene lignans from the Acanthaceous *Justicia patentiflora*, for example 7-*O*-*β*-D-quinovopyranosyl-diphyllin (**35**) and 7-*O*-*β*-L-fucopyranosyldiphyllin (**36**), exerted potent citotoxic effect on KB, HCT116 (colon cancer cell) and MCF-7 cell lines (Susplugas et al., 2005). The group leaded by Kadota tested the methanol, methanol-water (1:1) and water extracts from seventy-seven Vietnamese medicinal plants for their anti-proliferative activities against human fibrosarcoma cells (Ueda *et al.*, 2002). Some extracts from the plant samples *Caesalpinia sappan* (Tô mộc), *Catharanthus roseus* (Dừa cạn), *Coscinium fenestratum* (Vàng đắng), *Eurycoma longifolia* (Bách bệnh), *Hydnophytum formicarum* (Bí kỳ nam) and *Streptocaulon juventas* (Hà thủ ô trắng) exhibited considerable anti-proliferative activities. Further examination indicated that the extract of *C. fenestratum* showed selective anti-proliferative activity against lung carcinoma and/or lung metastatic cell lines, while those of *H. formicarum* and *S. juventas* showed selective activity against the uterine and lung human tumour cell lines (HeLa and A549, respectively). Characteristic morphological change and DNA fragmentation indicated the anti-proliferative activity to be due to the induction of apoptosis. The active constituents of *S. juventas* were characterized as cardenolides (Ueda *et al.*, 2003a; Ueda *et al.*, 2003b; Han *et al.*, 2010), of which the compounds **37** and **38** are principles. Many cycloartane-type triterpenes with cytotoxic activity were isolated from *Combretum quadrangulare* (Trâm bầu), and methyl quadrangularate D (**39**) were the most potent compound (Banskota et al., 1998). Recently, the group of Dr. Kinghorn has been interested in cytotoxic constituents from plants collected in Vietnam. Many compounds have been discovered by this group, including quassinoids bruveantin (**40**) and brucein A (**41**) from *Brucea javanica* (Pan et al., 2009), rotenoids cis-(6ab, 12ab)-hydroxyrotenone (**42**) and rotenone (**43**) from *Indigofera spicata* (Perez *et al.*, 2013). Plumbagin (**44**) from the plant *Plumbago zeylanica*, a traditional herb used to treat cancer, was found to exhibit significant cytotoxicity against the breast cancer cells (MCF-7) and Bowes melanoma cells (Nguyen et al., 2004). A series of compounds of new class with the structure miliusanes, derivatives of miliusate (**45**) and miliusol (**46**) were discovered from *Miliusa sinensis* by the group of Dr. Fong (Zhang et al., 2006). They exhibit significant cytotoxic activity in cancer cell line panels comprising KB, Col-2, LNCaP, Lu-1, MCF-7 and HUVEC cells, and represent a new promising class of anti-cancer agents (Zhang *et al.*, 2006). An anthraquinone, chrysophanol bianthrones (**47**) and its derivatives from *Rhamnus nepalensis* also displayed potent cytotoxic effects on KB cells (Mai *et al.*, 2001).

Using an *in vitro* angiogenesis assay, Nam and colleagues screened 58 Vietnamese medicinal plants for antitumour activity (Nam et al., 2003). Some plant samples exhibited strong anti-angiogenic activity on *in vitro* tube formation of human umbilical venous endothelial cells (HUVEC), including the herbs of *Ephedra sinica* (Ma hoàng), leaves and stems of *Ceiba pentandra* (Gòn), seeds of *Coix lachryma-jobi* (Ý dĩ), rhizomes of *Drynaria fortune* (cốt toái bổ), fruits and stems of *Illicium verum* (Đại hồi) and stem barks of *Bombax ceiba* (Gạo). Bioactivity-guided fractionation and isolation on methanol extract of the stem barks of *Bombax ceiba* afforded lupeol (**48)** as an active principle, which showed marked inhibitory activity on HUVEC tube formation at 50 and 30 mg/mL but did not affect the growth of tumour cell lines such as SK-MEL-2, A549, and B16-F10 melanoma (You et al., 2003).

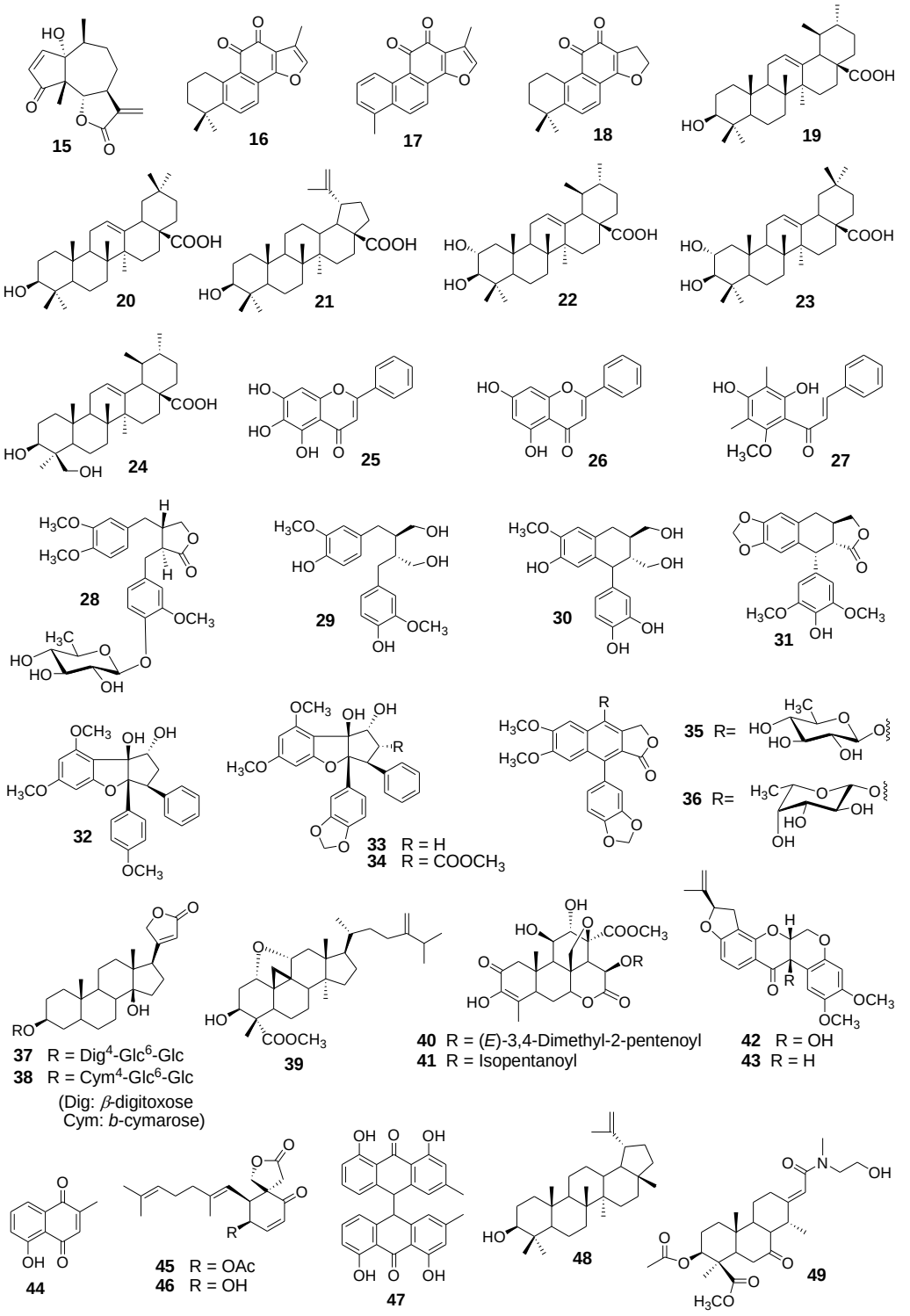


Figure 4. Cytotoxic compounds isolated from Vietnamese medicinal plants.

The water fraction, the remaining water phase from the methanol extract of *Ephedra sinica* after extraction with ethyl acetate and butanol, has been found to have anti-angiogenic, anti-invasive and antitumour activities (Nam et al., 2003). At the non-cytotoxic concentration 30 mg/mL, this fraction inhibited the tube formation induced by human umbilical venous endothelial cells and the invasion of B16F10 melanoma cells through a matrix membrane by more than 90%. The inhibitory activity of this fraction at 30 mg/kg/day on the growth of a tumour mass in BDF1 mice inoculated with B16-F10 murine melanoma cells was comparable to that of adriamycin (ADR) administered at 2 mg/kg/day. The results suggested that the antitumour activity of the water fraction from *Ephedra sinica* might be attributed to its anti-angiogenic and anti-invasive activities. However, the active constituents of this fraction have not yet been discovered. More recently, a cassaine diterpenoid alkaloids 3β-acetyl-norerythrophlamide (**49**) isolated from the medicinal plant *Erythrophleum fordii* was reported to exert potent inhibitory effect on the capillary-like structure formation of human umbilical vein endothelial cells (Hung et al., 2014).

4.2. Plant-derived Anti-cancer Agents in Clinical Development and Clinical Use

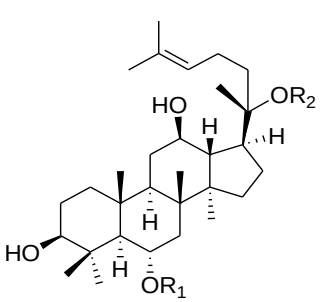
Many anti-cancer agents from plants have been discovered and approved for clinical use. Some typical examples are paclitaxel from the bark of *Taxus brevifolia*, vinblastine and vincristine (Vinca alkaloids) from periwinkle plant *Catharanthus roseus*, camptothecin from Chinese tree *Camptotheca acuminata*, and podophyllotoxins from podophyllum plant *Podophyllum pentatum*. Despite possessing medicinal plant sources of anti-cancer agents such as *Taxus wallichiana*, *Catharanthus roseus*, and *Podophyllum tonkinense* (Thuong, 2014c), Vietnam has to import these agents and their analogs for the clinical treatment of cancers. Except for these drugs, some Vietnamese medicinal plants are used clinically to prevent and treat cancers.

4.2.1. Saponins from Panax vietnamensis (Vietnamese gingseng)

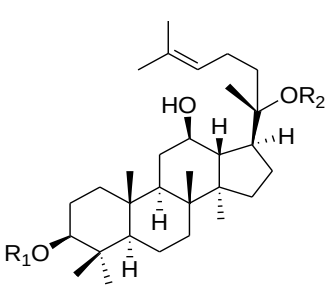
Ginseng is generally referred to any one of several species belonging to the genus *Panax* of the family Araliaceae, distributed in Eastern Asia and North America. Some well-known are the Korean ginseng (*P. ginseng*), American ginseng (*P. quinquefolius*), Japanese ginseng (*P. japonicas*), Himalayan ginseng (*P. pseudoginseng*), notoginseng (*P. notoginseng*), and Vietnamese ginseng (*P. vietnamensis*). The principle constituents of ginseng are characterized as triterpenoid saponins, which are classified into damaran-type and oleane-type saponins. Ginsengs have been thought to promote yang in the body, thus have been used in traditional medicine as tonic and stimulant herbs in oriental medicine (Loi, 2012). Nowadays, many studies have demonstrated that ginsengs exert many valuable pharmaceutical activities (Attele et al., 1999), including anti-cancer activities (Nag et al., 2012).

The *Panax vietnamensis* Ha et Grushv. is the most famous ginseng variety and is the most important among Vietnamese endemic plant species (Nguyen, 1989; NIMM, 2004). This herb is native to the 1700 m mountainous Ngoc Linh area, located in Central Vietnam, and is therefore called Vietnamese ginseng. The rhizome and root of this plant has been used as a tonic and *"life-giving"* drugs by the ethnic minority group Sedang, who have lived in this area for hundred of years. Vietnamese chemists have previously demonstrated that *P.*

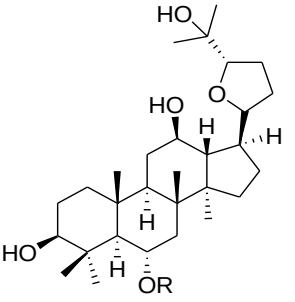
vietnamensis contains many damaran- and oleane-type saponins, which are mostly similar to saponins from other *Panax* species (Nguyen et al., 1993; Nguyen et al., 1994a; Nguyen et al., 1994b; Tran et al., 2001). The major saponins of Vietnamese ginseng include majonoside R2 (MR2), ginsenoside Rg1 and ginsenoside Rb1 (Figure 5), of which the ocotillol saponin majonoside R2 (MR2) is representative for this species, as it constitutes >5% of dried weight of the root and radix (Nguyen et al., 1993; Nguyen et al., 2007). Previous studies indicated that both the extracts from *P. vietnamensis* and its principle MR2 display various interesting biological activities, including anti-hyperlipidermia (Nguyen et al., 2007), anti-stress (Nguyen et al., 2007), anti-depresive and anxiolytic (Huong et al., 2005), hepatoprotective (Nguyen et al., 2000; Tran et al., 2001; Tran et al., 2002), neuroprotective (Yobimoto et al., 2000; Huong et al., 2005), and anti-cancer activities (Konoshima et al., 1998; Konoshima et al., 1999). The compound MR2 also showed potent anti-tumour-promoting activity in two-stage carcinogenesis on mouse skin induced by two different types of promoters TPA (12-O-tetradecanoylphorbol-13-acetate) or fumonisin B1 (Konoshima et al., 1998), as well as by nitric oxide (NO)/TPA or peroxynitrite/TPA (Konoshima et al., 1999). These observations support that Vietnamese ginseng and saponin MR2 might be used as chemopreventive agents for certain cancers. Therfore, *P. vietnamensis* has been used by Vietnamese people for the therapy of various types of cancers, as well as many other diseases. No clinical study of this herb against cancers has been reported so far, however the Vietnamese people believe that the ginseng may help them from illness and death.



50
Ginsenoside Rg1
(R_1 = R_2 = Glc)



51
Ginsenoside Rb1
(R_1 = Glc^6-Glc; R_2 = Glc^2-Glc)

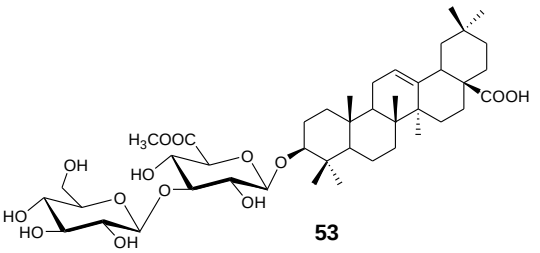


52
Majonoside R2
(R = $OGlc^2$-Xyl)

Fig 5. *Panax vietnamensis* and its major constituents.

4.2.2. Compounds from other Panax species

Two other *Panax* species, *P. stipuleanatus* (tam thất hoang) and *P. bipinnatifidus* (tam thất vũ diệp, tam thất lá xẻ; Figure 6), are also found in Vietnam. These types of ginseng grow widely on Hoàng liên Mountain in the West-North of Vietnam, which is from 1500 to 3400 meters high. Only a few studies on the chemical constituents and biological activities of two these species have been reported, however, Vietnamese people use it as ginseng for the treatment of cancers as well as other diseases (Nguyen et al., 2007; NIMM, 2013). Our analyses indicate that chemical constituents of *P. stipuleanatus* rhizomes are similar to those of Vietnamese ginseng *P. vietnamensis*, containing Rb1, MR2, Rg1 and other ginsenosides (Thuong et al., 2014c).



53

Panax vietnamensis (Sâm Việt Nam) | *Panax stipuleanatus* (Tam thất hoang) | *Panax bipinatifidus* (Tam thất vũ diệp)

Fig 6. Three *Panax* species found in Vietnam.

However, some research groups indicated that oleanane-type saponins are major constituents of two these *Panax* species (Liang et al., 2010; Liang et al., 2013; Zhu et al., 2004; Nguyen et al., 2011). Liang and colleages (2010) found that spinasaponin A methyl ester (**53)** from *P. stipuleanatus* exhibited significant cytotoxic activity against HL-60 (leukemia) and HCT-116 (colon cancer) cell lines with IC_{50} values of 4.44 and 0.63 μM,

respectively. Compound **53** activated intrinsic and extrinsic apoptosis pathways by upregulating DR-5 and Bax, downregulating Bcl-2, activating caspase-9, and cleaving poly-ADP-ribose polymerase (PARP) and activated ERK1/2 MAPK in the HCT-116 cells. The authors concluded that the oleanane-type saponin **53** might exhibit inhibitory effect on HCT-116 colon cancer cell growth by inducing both intrinsic and extrinsic apoptosis pathways through the activation of the ERK1/2 MAPK pathway (Liang et al., 2010). This findings support the use of these two *Panax* species in the cancer treatment of Vietnamese people.

4.2.3. Polysaccharides from Angelica acutiloba (Đương quy)

The plant *Angelica acutiloba* (Apiaceae; Figure 7) is native to Japan and Korea. The National Institute of Medicinal Materials (NIMM) of Vietnam domesticated and developed the cultivation of this plant in Vietnam in the early of 1990's (NIMM, 2004; Khoi and Thuong, 2012). The major compounds of *A. acutiloba* are essential oils (ligustilide), coumarins (scopoletin), and polysaccharide (Khoi and Thuong, 2012). It has been demonstrated that the polysaccharide from "Đương qui" exhibits the biological activity of enhancing physical strength, which could be applied for cancers and various serious diseases (Hatano et al., 2004; Lee et al., 2004). Therefore, NIMM developed the polysaccharide from this plant into a drug named Somanimm, which has indication of chemotherapeutic treatment of cancer and immunotherapy. Clinical studies have revealed that Somanimm reduced cancer development. The drug Somanimm has been approved by Drug Administration of Vietnam for human use since 2012 (Khoi and Thuong, 2012).

Figure 7. *Angelica acutiloba* and the anti-cancer drugs Somanimm.

4.2.4. Alkaloids and flavonoids from Crinum latifolium (Trinh nữ hoàng cung)

The aqueous extract of the leaves of the plant *Crinum latifolium* (Figure 8) has been reputed to reduce tumours in man for the treatment of prostate and uterine cancers (Loi, 2012). Vietnamese scientists have demonstrated that this plant exhibited antitumour and immunomodulatory activity. Using a sensitive marker reflecting the activation of cell-mediated immunity, neopterin production in human unstimulated peripheral mononuclear cells, Zvetkova and colleagues (2001) found that the extracts of *C. latifolium* (L.) enhanced immunomodulatory activity. The study also indicated that *C. latifolium* (L.) extracts seemed

to be more effective in reducing neopterin formation in stimulated cells than those of both green and black teas (Zvetkova et al., 2001). Recently, they reported that this aqueous extract of *C. latifolium* inhibited cell proliferation of highly metastatic human prostate carcinoma PC3 cells, androgen-sensitive prostate adenocarcinoma LNCaP cells, and benign prostate hyperplasia BPH-1 cells. Hence, they concluded that both inhibition of tumour cell growth and recovery of immune functions are important for the antitumour properties of *Crinum latifolium* (Jenny et al., 2011). The active constituents were recently characterized as alkaloids and flavonoids. In support of this, Nam N. H. isolated six compounds from the methanol extract of the leaves of *C. latifolium* by bioassay-guided separation (Nam et al., 2004). He found that the compound 4-senecioyloxymethyl-6,7-dimethoxycoumarin (**54**) strongly inhibited the *in vitro* tube-like formation of HUVECs, while manifesting no cytotoxicity in tumour cell lines (B16F10, HCT116), strong inhibitory activity was still observed at concentrations as low as 1 μg/mL (Nam et al., 2004). A new flavonoid compound 5,6,3'-trihydroxy-7,8,4'-trimethoxyflavone (**55)** showed a modest inhibitory effect on the tube-like formation of HUVECs.

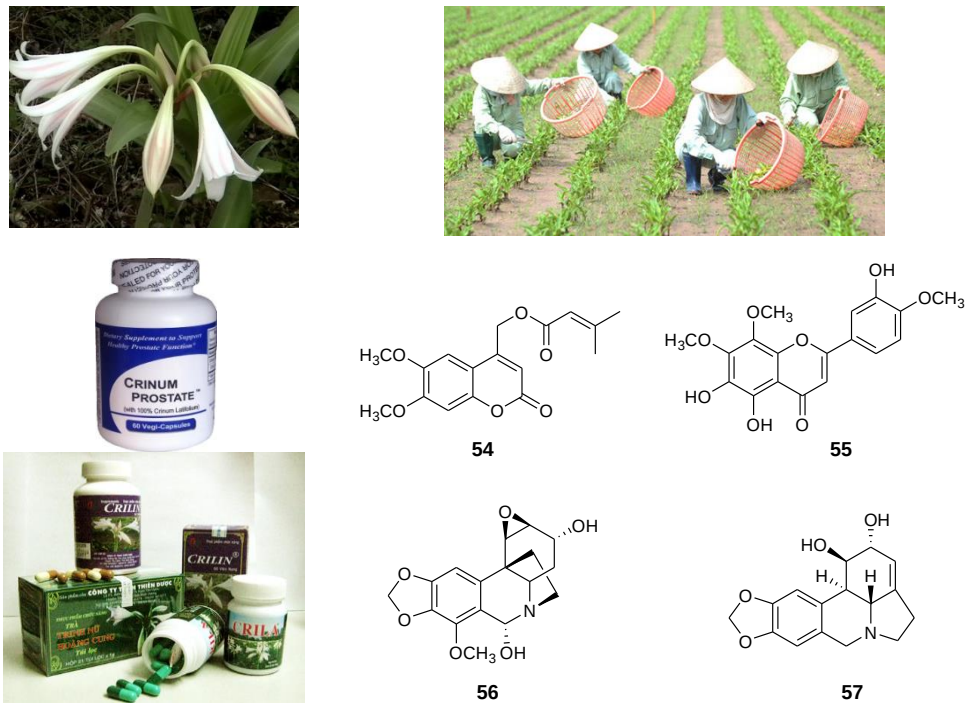


Figure 8. *Crinum latifolium* cultivated in Vietnam and drugs derived.

More recently, Nguyen et al (2013) showed that the total flavonoid extract of *Crinum latifolium* had high antioxidant activity, and showed an inhibitory action on cancer cells. Alkaloid extracts inhibited the proliferation of lymphoma cells either by directly acting on tumour cells or by activating the tumouricidal functions of syngeneic macrophages. The aqueous extract induced mRNA expression of tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin 6 (IL-6), indicating differentiation of macrophages into pro-

inflammatory M1 polarized macrophages. The total flavonoid, alkaloid extracts and an alkaloid fraction induced the expression of the formyl peptide receptor (FPR) on the surface of the polarized macrophages that could lead to the activation of macrophages towards the M1 phenotype. Aqueous and flavonoid extracts enhanced NADPH quinine oxido-reductase 1 (NQO1) mRNA expression in polarized macrophages, which could play an important role in cancer chemoprevention. All the samples studied were non-toxic to normal living cells, and the pure alkaloid tested, 6-hydroxycrinamidine (**56**), was not active in any of the models investigated. These findings indicate that *Crinum latifolium* extracts and alkaloid fraction (but not pure 6-hydroxycrinamidine) inhibit the proliferation of lymphoma cells in multiple pathways, in agreement with traditional usage (Nguyen et al., 2013). Our study (Thuong, 2014c) suggested that the active alkaloid compound of this plant might be lycorin (**57**). Taken together, further studies on this plant and its active principles should be pursued.

4.3. Medicinal Plants in Vietnam as Potential Agents for the Prevention and/or Treatment of Pancreatic Cancer

Vietnam clearly has a long and rich history of traditional medicines for numerous ailments, including cancer. However, paradoxically, there is very little information on plant-derived compounds and their application for pancreatic cancer. As described above, many compounds have been identified from native Vietnamese plants that confer quasi-ubiquitous anti-cancer activity. There remains great potential to tap into this unparalleled natural resource to develop novel therapeutic agents for cancers with limited therapeutic efficacy, such as pancreatic cancer. To address this, we have preliminarily investigated the anti-pancreatic cancer activity of compounds extracted from the native Vietnamese plants *Croton tonkinensis* and *Salacia cochinchinensis.* Both the *ent*-kaurane diterpenoid from the *C. tonkinensis* and pristimerin from *S. cochinchinensis* possessed potent growth inhibitory activity at low doses against pancreatic cancer cell lines while remaining essentially inactive against normal human pancreatic ductal epithelial (HPDE) cells (*Unpublished*). These findings, although preliminary, demonstrate great promise. Pristimerin has been shown to be a potent inhibitor of mTOR signalling in pancreatic cancer cells (Deeb et al, 2014), leading to an induction of apoptosis of pancreatic cancer cells. As described in the chapter of Jamieson et al. in this book, mTOR is a significant target for therapeutic intervention for pancreatic cancer, as recently highlighted by Morran et al (2014). Pristimerin has also been demonstrated to enhance sensitivity to gemcitabine (Wang et al. 2012). Further investigation into the mechanism of action and entry into pre-clinical trials are warranted. This is but one of the mounting examples of compounds isolated from Vietnamese native plants and being assess for their anti-pancreatic cancer activity. It is hoped, if not expected, that plant-derived bioactive compounds may show efficacy for pancreatic cancer phenotypes with as yet, untreatable mutations.

CONCLUSION

Medicinal plants have played an important role in the health management and development of civilization since ancient times. Plants have been the main source of crude drugs used to cure or alleviate human sickness. Today, drugs derived from natural products still make an enormous contribution to drug discovery and development. Many agents from natural sources have been used in clinical therapy. Different human civilizations transferred natural drugs knowledge to the followers, and this is recorded in their history. With the development of chemical and biological processes, scientists are able to prepare extracts and isolate active ingredients from plant materials and assess the pharmacological effects of single ingredients of plant materials, establishing the foundation of modern medicine. This system has grown since the last century, assisted by improved surgical procedures and the use of biotechnology. The progress of the system is fascinating and convincing, but the question of safety and nonexistence of side effects is not still answered. Due to this, the dependence on synthetics is over and people are returning to the natural products with the hope of safety and security. According to a recent survey conducted by WHO, about 80% of the world population uses medicinal herbs by one way or other. We hope that readers might be able to pass on information about the common medicinal plants available locally to those interested in their collection, practical uses, and development of priceless resources represented by medicinal plants in Viet Nam.

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 10

PLANTS IN THE SUBCONTINENT AS POTENTIAL ANTI-CANCER AGENTS

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ABSTRACT

Ayurveda, Siddha, Unani and Folk (tribal) medicines have a long history and are still used by many indigenous communities in the Subcontinent. Among these systems, Ayurveda is the most developed and widely practiced in countries like India, Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan. The Subcontinent has a rich source of medicinal plants. Numerous compounds have been isolated from plants and tested for their anti-cancer properties. This chapter briefly outlines the medicinal plants in the Subcontinent, their use as traditional medicines and their perspectives as anti-cancer agents. In addition, this chapter also discusses plant-derived agents in preclinical development, and their clinical use as anti-cancer agents. Further, we propose a future trend for using plants from the Subcontinent for the discovery and investigation of novel anti-cancer agents.

1. INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time minerals, plant and animal products were the main sources of drugs [1]. The Industrial Revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view

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regarding the concepts of health and disease existed within each culture. Obviously, this approach was against the new *modus vivendi* of the industrialized western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value. However, even if we only consider the impact of the discovery of penicillin obtained from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. Important drugs obtained from plants include digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin [2]. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds [3]. In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicines and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies [4].

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants [5-7]. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless. However, the use of these substances is not always authorized by legal authorities dealing with efficacy and safety procedures, and many investigations point to the lack of quality in the production, trade and prescription of phytomedicinal products. It is estimated that, in 1997, the world market for over the counter phytomedicinal products was 10 billion US dollars, with an annual growth of 6.5% [8]. The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs from plant origin in developing countries [9-10]. Eastern countries, such as China and India, have well-established herbal medicines industries and Latin American countries have been investing in research programs in medicinal plants and the standardisation and regulation of phytomedicinal products, following the example of European countries, such as France and Germany. In Germany, 50% of phytomedicinal products are sold via medical prescription, the cost being refunded by the individual's health insurance [11]. In North America, where phytomedicinal products are sold as "health foods" [12-13], consumers and professionals have struggled to change this by gathering information about the efficacy and safety of these products, and new guidelines for their registration are now part of FDA policy [14]. In 1997, the North American market for products of plant origin reached 2 billion US dollars [15]. Thus, the modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs [16-17] have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programmes worldwide. The NCI (National Cancer Institute, USA) has tested more than 50,000 plant

samples for anti-HIV activity and 33,000 samples for anti-tumour activity. In 1993, the International Program of Co-operation for Biodiversity (IPCB) was launched in order to promote natural products in Latin America and Africa, linking universities, industries and governments in a multidisciplinary programme for the sustained development and preservation of the environment [18]. Large pharmaceutical companies now have specific departments dedicated to the study of new drugs from natural sources [19]. However, the potential use of higher plants as a source of new drugs is still poorly explored. Of the estimated 250,000–500,000 plant species, only a small percentage have been investigated phytochemically, and even a smaller percentage has been properly studied in terms of their pharmacological properties; in most cases, only pharmacological screening or preliminary studies have been performed.

Each year, approximately 45,000 new patients are diagnosed with pancreatic cancer in the USA. Of note, approximately 40,000 patients in the US die from pancreatic cancer each year, making it the fourth leading cause of cancer-related death in the US. The incidence has been increasing since the 1930s, and the prognosis of patients with pancreatic cancer is extremely poor with a five-year overall survival rate for pancreatic cancer less than 5%. The poor prognosis had been attributed to the inability to diagnose while the tumour is resectable, its propensity toward early vascular dissemination and spread to regional lymph nodes, as well as ineffective therapeutic treatment agents [21-22].

2. MEDICINAL PLANTS IN THE SUBCONTINENT

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians [23]. According to the World Health Organization, ~80 % of people living in rural areas depend on medicinal herbs as their primary healthcare system.

Ocimum sanctum or Holy Basil is a sacred medicinal plant in India, where it is used as a carminative, antidysenteric, stimulant, demulcent for many different ailments in ethnomedicine [24]. *Cannabis sativa* is a dioecious, bushy plant, probably originating from central Asia [25]. *Caraway* is a medicinal plant in India and China, and have a large armamentarium of plants in their pharmacopoeias which are used throughout South Asia [26]. *Azadirachta indica*, called *Neem* or *Nim* in most parts of the world, is one of the very few trees known in the Indian subcontinent since antiquity [27]. *Urtica L.*, the stinging nettle (Urticaceae), is an annual and perennial herb, distinguished with stinging hairs. The leaves are opposite, and the flowers are green with yellow stamens. The male and female flowers are on separate plants. The fruits are achene. These are the characteristics of the *Urtica* genus, which belong to the family Urticaceae. The main varieties identified under the *Urtica* species are *U. dioica* L., *U. urens* L., *U. pilulifera* L., *U. cannabina* L., *U. membranacea* Poiret., *U. haussknechtii* Boiss., *U. atrovirens* Req., *U. rupestris* Guss., *U. chamaedryoides* Pursh., *U. ferox* Forst [28]. The *Magnoliaceae* is a family of about 220 species of deciduous or evergreen trees and shrubs native to Asia and America, with large showy flowers containing

both male and female parts. Its best-known representatives are the horticulturally important species of the genus *Magnolia*. Approximately 80% of the species are distributed in temperate and tropical Southeast Asia from the Himalayas [29]. The genus *Pueraria* has its species distributed over China and Japan, South and South-East Asia, and *Pueraria phaseoloides* var. *phaseoloides* in West Bengal. *P. tuberosa* is distributed widely in India, from the Western Himalaya to Sikkim, up to 4000 ft in Kumaon. It is found in the lower hills of the Punjab, Mount Abu, the hilly tracts of Bengal and in most of Southern India [30]. As a wild species, *Ginkgo biloba* is native to China and was probably a member of the mixed-mesophytic forest community that once covered the hill country bordering the Yangtze River valley [31]. *Vetiver* is a perennial graminaceous plant (Poaceae = Gramineae), originary from India, growing wild, half-wild or cultivated in many tropical and subtropical areas. Its fragrant roots contain essential oils used in the perfumery and cosmetic industry. The name derives from the Tamil "vetti" (khus-khus or cus-cus) and "ver" (root), alluding to aromatic roots and the oil obtained from its roots has been known to Indians from the time of Vedas. Vetiver grass, in particular the species *Vetiveria zizanioides* (L.) Nash, has been known to be a useful plant for thousands of years. It is mentioned in ancient Sanskrit writings and is also part of Hindu mythology [32]. The genera *Geranium* and *Pelargonium* are invariably confused by the general public and also plant sales personnel, health food shop workers and alternative medicine practitioners, especially aromatherapists [33].

3. TRADITIONAL USE OF PLANTS IN THE SUBCONTINENT FOR THE TREATMENT OF DISEASE

Derivatives of plant origin have long been known to possess biological activity [34]. Many traditional cultures remain mostly dependent on plants for their food and medicine, and often consider them both in the same context [35]. According to the World Health Organization, approximately 80% of the world's inhabitants currently rely on indigenous or traditional medicines for their primary health needs, and most of this therapy involves the use of plant extracts, often in aqueous solutions [36]. Of the plant-based foods used as medicines, none have received more attention as a group than herbal remedies [37]. The use of herbal preparations, typically prepared by steeping or heating the crude plant material, has prevailed for centuries and healthcare providers in Europe and Asia today often prescribe herbal teas. However, such practices are largely based on folklore and schools of traditional medicine rather than evidence-based research. In many cases, the bioactivity of these plants appears to be derived from secondary metabolites, such as the polyphenols [35]. Polyphenols, the most numerous and widely distributed class of phytochemicals, include classes of chromones, coumarins, lignans, stilbenes, xanthones and the ubiquitous flavonoids [38-39]. Within the past decade, many polyphenols, particularly the flavonoids, have been found to possess relatively potent antioxidant, anti-atherosclerotic, anti-inflammatory, anti-mutagenic, anti-tumour and anti-viral activities [40].

Observational studies have repeatedly shown that diets high in plant-based foods and beverages are associated with a lower risk of chronic diseases, such as cardiovascular disease and some forms of cancer [41-47], and suggests this correlation may be attributable to the phytochemical constituents as well as to the macro- and/or micronutrient content of these

foods. While further research is necessary to better understand and quantify the contributions of phytochemicals to health promotion and disease prevention, virtually all of the dietary guidelines created by regulatory agencies and healthcare organizations include recommendations for generous intakes of plant foods, including fruits, vegetables and whole grain cereals. Interestingly, recommendations for the consumption of plant-based beverages (except for fruit juices) such as tea (*Camellia sinensis*) and tisanes (herbal teas) are absent despite their being particularly rich sources of polyphenols. While there is an extensive literature suggesting health benefits associated with drinking black, green and oolong tea (i.e. *Camellia sinensis*) [48], evidence-based information regarding the effects of most herbal teas is quite limited.

O. gratissimum, O. viride and *O. suave* are native plants in different parts of Africa and are used in traditional medicine, mostly as expectorants. The essential oils of these species also exhibit large antimicrobial spectra. The composition of the fixed oil of *Ocimum* seeds has been studied lately and it was found to have anti-inflammatory activity [24].

Cannabis is used medicinally in a range of disorders. Traditional uses such as the relief of pain have been extended to include the reduction of intra-occular pressure in glaucoma, relief of spasticity in multiple sclerosis, treatment of chemotherapy-induced nausea and vomiting, and stimulation of appetite in patients with HIV/AIDS [25].

The therapeutic efficacy of Neem has been known since antiquity as a result of constant experimentation. Ancient man observed the unique features of this tree: a bitter taste, non-poisonous to man, but deleterious to lower forms of life. This might have resulted in its use as a medicine in various cultures, particularly in the Indian Subcontinent and later in other parts of the world. Whereas in folklore mainly the leaves and to some extent the oil was used in Ayurveda (the Indian system of medicine), Siddha (the system of medicine practiced in some parts of south India) and Unani Tibb (the Greco-Persian system of medicine), polyherbal preparations containing one, two or all five parts of the plant, i.e. leaves, bark, flower, fruit and root, called *panchang* in Ayurveda, were used. In the traditional systems of medicine, some of the preparations were for internal administration, while others such as nasal drops, medicated oils or fats were for external application [26].

U. dioica and *U. urens* have been known for a long time as medicinal plants. They are used as an expectorant, purgative, diuretic, haemostatic, vermifuge and for the treatment of eczema, rheumatism, haemorrhoids, hyperthyroidism, bronchitis and cancer. These plants have been consumed without any report of serious adverse effects [27].

Among *Magnolia* species, *M. obovata* and *M. officinalis* are very important in traditional Chino-Japanese herbal medicine [28]. Raw Ginkgo nuts, cleaned of their fleshy pulp, have long been used in traditional Chinese medicine to treat a variety of lung-related ailments such as asthma and bronchitis, as well as for the treatment of kidney and bladder disorders [29].

Pueraria is a plant widely used in traditional Indian Medicine. The tuber of *Pueraria* is sweet in taste and used in the indigenous system of Indian medicine as tonic, aphrodisiac, antirheumatic, diuretic and galactogogue. It is an important constituent of Ayurvedic medicines including Chywanprash, a popular tonic. In the traditional Indian medicine, the root of *P. tuberose* (*Radix puerariae*) has been mentioned for its antispasmodic activity. Certain Sadhus of Terai area of Uttar Pradesh (India) have been reported to consume these tubers for increasing general body resistance. Furthermore, it is active against angina pectoris, hypertension, deafness, optic nerve atrophy or retinitis. The root is also used as demulcent

and refrigerant in fevers. Peeled and bruised into a cataplasm, it is used to reduce swelling of the joints [30].

Vetiver oil is one of the most complex mixtures of sesquiterpene alcohols and hydrocarbons, and also one of the most viscous oils with an extremely slow rate of volatility. It is used extensively for blending in cosmetics and in the soap industry, and as a fixative in the perfumery industry prolonging the life of any composition to which it is added. In India it is used in herbal medicine as a carminative, stimulant and diaphoretic. Rural people have used Vetiver grass for centuries for the oil from its roots. Its center of origin appears to be in southern India and it has spread around the world through its byproduct value as a producer of aromatic oil for the perfume industry [30-31].

The numerous aromatherapeutic uses for Geranium oil are yet to be scientifically validated, although there is every reason to accept the scientific evidence that inhalation of the aroma and its action through the limbic system has a relaxing effect; theoretically, this could lead to the acceptance that many stress-related conditions like dermatitis, asthma, intestinal problems and headaches could be alleviated [32].

4. TRADITIONAL USE OF PLANTS AS FOLK MEDICINES IN THE SUBCONTINENT

Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practised in countries like India, Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan, while the traditional system of medicine in the other countries like Tibet, Mongolia and Thailand appear to be derived from Ayurveda.

Edible chrysanthemum is a bitter aromatic herb that has been experimentally shown to lower fever, soothe inflammation, dilate the coronary arteries thereby increasing blood flow to the heart, and inhibits the growth of pathogens. It is used in folk medicine for hypertension, coronary artery disease, angina, feverish colds, and liver-related disorders. Triterpenoids isolated from edible chrysanthemum flowers, and heliantriol C was found to be the major compound. The activity of heliantriol C was ten times greater than other pentacyclic triterpenes against TPA-induced tumour promotion in mouse skin [49].

Rosmarinus officinalis L., commonly called rosemary, is a woody perennial herb with fragrant evergreen needle-like leaves that are often used in cooking. It has been found to act both as a stimulant and as a mild analgesic, and has been in folk use to treat headaches and epilepsy. In European folk medicine, cabbage leaves are used to treat acute inflammation, and fresh cabbage juice has been shown to promote rapid healing of peptic ulcers [50-51].

Rakkyo (the bulbs of *Allium chinense* G. Don) is used in folk medicine as tonic to the intestines and a stomach. Chaga (the sclerotia of *Inonotus obliquus* (Pers. Fr.) Pil., Hymenochaetaceae) has been widely used as a folk medicine in the treatment of cancer, cardiovascular disease and diabetes in Russia, Poland, and several Baltic countries. In Japan and Korea, chaga is used as a supplement during cancer treatment. More recently, this herb has been assessed for its cancer-preventing activity. Several species and subspecies of *Taraxacum* are widely distributed in Japan, and the roots of these plants (*T. Platycarpum* Dahlst., *T. Japonicum* Koidz., etc.) have been used as bitter stomachic, diuretic, anti-

mastopathy and anti-inflammatory folk medicines in China and Japan. Interestingly, dandelion (*T. officinale* F.H. Wigg) leaves have been regarded as a vegetable in Europe [51].

Various essential oils have been used medicinally at different periods in history, while many common essential oils have medicinal properties that have been applied in folk medicine since ancient times and are still widely used still today in India. *Salvia libanotica* Boiss. & Gaill. (Labiatae) is a strongly aromatic perennial shrub. Its healing properties are well known and it is widely used by herbalists for the treatment of headache, stomachache and respiratory problems. The oil extract contains ketones such as camphor and α- and β-thujone, terpenes such as limonene and α- and β-pinene, and alcohols such as borneol and linalool. Moreover, oxides such as 1,8-cineol and esters such as linalyl acetate are also found in sage oil. Garlic oil from *Allium sativum* L. (Liliaceae) is rich in sulfur compounds, and a major component is diallyl disulphide, and is a suspected irritant. Onion oil from the seeds of *Allium cepa* L. (Liliaceae) may present a risk of skin irritation and/or sensitization similar to garlic oil. Sandalwood (*Santalum album* L., Santalaceae) comes from medium-sized fragrant trees and its oil has found wide use in the cosmetics industry [51].

5. PLANTS IN THE SUBCONTINENT AS POTENTIAL ANTI-CANCER AGENTS

Plants have been indispensable in treating diverse forms of diseases including cancer. Approximately 80% of the people living in rural areas depend on medicinal plants as their primary health care system. These practices are solely based on the knowledge of traditional uses of medicinal plants. Natural products are formulated to generate different types of effective drugs to enhance anti-cancer activities. Proper understanding of the complex synergistic interaction of various constituents of anti-cancer herbs would help in formulating drug design, and show efficacy against the cancerous cells without harming the normal cells within the body [52, 53].

Advances in the clinical research for anti-cancer agents have increased over the years and as a result, a number of drugs have been introduced into the clinic. Imperative organic compounds present in plants could exaggerate to diminish the toxicity caused due to chemotherapy. The task of modulating the adverse affect is feasible only through requisite perspective regarding the specificity of these molecules with combination therapy [54]. Only in the last decade there has been some success in developing "targeted" drugs and therapies with fewer side effects [55]. For example, the recent discovery and rapid licensing of Imatinib (Gleevec ^{TM}) for the treatment of chronic myelogenous leukemia is often heralded as the start of a new era in the development of non-cytotoxic agents targeted toward distinct biological pathways [55]. *Moringa oleifera*, Lam. (Moringaceae) is a tree that grows widely in the tropics and subtropics of Asia and Africa. Its leaves have been traditionally consumed by Asian village people, but it is a relatively novel food material in the western world [67]. *Moringa oleifera* contains several phytochemicals, some of which are of special interest because of their medicinal properties. Leaves of the *Moringa oleifera* contain flavonoid pigments, such as kaempferol, rhamnetin, isoquercitrin and kaempferitrin. In addition, these leaves are rich in a group of the glycoside compounds, glucosinolates and isothiocyanates [68] as well as beta-sitosterol, glycerol-1-(9-octadecanoate), 3-O-(6'-O-oleoyl-beta-D-

glucopyranosyl), beta-sitosterol and betasitosterol- O-beta-D-glucopyranoside, all of which have demonstrated anti-cancer properties in-vitro [69]. An in vitro study using human KB cells as a cancer model has shown that *Moringa oleifera* leaf extract exerts strong anti-tumour activity [70]. In addition, different leaf extracts of *Moringa oleifera* generate significant cytotoxic effects on human multiple myeloma cultured cell lines [70].

Clearly, plant derived anti-cancer agents have been shown to be valuable motifs for the treatment of various types of cancer. We know that pancreatic cancer is a rare disease, but with a survival rate of four to five percent, it is one of the worst cancers known to exist. The National Cancer Institute estimates that almost 44,000 people get pancreatic cancer every year and over 37,000 of them die. With a median survival of six months for metastatic disease, only 17 percent of patients live more a year and a half after their diagnosis [64]. The most common form of pancreatic cancer, pancreatic ductal adenocarcinomas, are very aggressive, spread rapidly, and are often found at a late stage. With current treatment options displaying limited efficacy, scientists continue searching for effective therapeutic strategies. Recent studies found that numerous plant-based alternatives have been effective in killing pancreatic tumours in murine models of pancreatic cancer [65], and warrant vigorous investigation in a pre-clinical context. For example, the recent investigations of Chugh and colleagues successfully synthesized a plant-based drug called Minnelide, which they derived from triptolide, a natural plant product which has been used for a long time in traditional Chinese medicine. *Tripterygium wilfordii*, known as thunder god vine (lei gong teng), has been shown to be efficient in alleviating symptoms of arthritis, fever, and other illnesses. According to their study, the plant also appears to effectively fight pancreatic tumours in mice. Pancreatic cancer cells possess a large amount of protective protein called HSP70, which makes tumours resistant to drugs. Triptolide appears to have the ability to inhibit that protein. However, as triptolide is not water-soluble, it has been hard to use for therapy in the human body. To circumvent this problem, Chugh et al. modified the agent, creating its water-soluble analog, Minnelide, which is now in early stage clinical trials [66]. The following section contains some of the plant derived anti-cancer agents in preclinical/clinical development and in clinical use.

5.1. Plant-Derived Anti-Cancer Agents in Preclinical Development

A number of naturally derived agents have been entered into clinical trials that have been terminated due to lack of efficacy or unacceptable toxicity. One of these, maytansine, has been revived through the application of targeting technology. Another example of an "old" drug in process of revival is bruceantin (I) (Figure 1), which was first isolated from *Brucea antidysenterica* J. F. Mill., a tree used in Ethiopia for the treatment of "cancer" [56].

Betulinic acid (II), another plant-derived compound with a long history, is a lupane-type triterpene that has been isolated from many taxonomically diverse plant genera [57]. The most important activities have been associated with inhibition of the replication of strains of the human immunodeficiency virus (HIV) [58].

The synthesis of new analogs having increased potencies have led to the synthesis of 2-cyano-3,12- dioxoolean-1,9-dien-28-oic acid (CDDO) (III) isolated from *Phytolacca*

americana and its methyl ester, which exhibit potent *in vitro* and *in vivo* anti-tumour activity against a wide range of cancers, including breast, pancreatic and leukemias [59].

For *pancreatic cancer*, a number of naturally derived agents have been entered into clinical trials but terminated due to lack of efficacy or unacceptable toxicity. One of these, irinotecan (CPT-11) (XIV) is a semisynthetic, watersoluble derivative of the plant alkaloid camptothecin. After conversion to its active metabolite, SN-38, irinotecan functions by inhibiting DNA topoisomerase I, thereby interfering with DNA replication and cell division [71, 72].

The chemotherapeutic effects of bioactive proanthocyanidins (XV) from grape seeds (GSPs) have been assessed using *in vitro* and *in vivo* models. Treatment of human pancreatic cancer cells with GSPs *in vitro* reduced cell viability and increased G2/M phase arrest of the cell cycle leading to induction of apoptosis in a dose- and time dependent manner. The GSPs-induced apoptosis of pancreatic cancer cells was associated with a decrease in the levels of Bcl-2 and Bcl-xl and an increase in the levels of Bax and activated caspase-3. Treatment of MiaPaCa-2 and PANC-1 cells with GSPs also decreased the levels of phosphatidylinositol-3-kinase (PI3K) and phosphorylation of Akt at ser473. SiRNA knockdown of PI3K from pancreatic cancer cells also reduced the phosphorylation of Akt [73].

Cyclopamine (XVI) is a steroidal alkaloid derived from the Lilly plant that inhibits Hedgehog (Hh) signaling via direct interaction with the protein Smoothened. In pancreatic cancer cell lines which over-express Hh pathway signaling, cyclopamine induced apoptosis, while other Hh non-expressing pancreas cell lines were resistant. In the same study, cyclopamine treatment inhibited growth of human pancreatic cancer xenografts. These data suggest targeting the hedgehog pathway is a promising approach for the treatment of pancreatic cancer. Although several studies have shown a cytotoxic effect of cyclopamine on various tumour cells that over express hedgehog pathway proteins, the potential use of cyclopamine as a single agent for treatment of pancreatic cancer is limited by heterogeneity of tumour populations, deferential tumour cell sensitivity, limited drug availability, and high production costs [71]. The expression of sonic hedgehog (SHH) and epidermal growth factor receptor (EGFR) signaling molecules in pancreatic cancer cells, and assessing the inhibitory effects through the blockade of the SHH and EGFR signaling pathways by cyclopamine and Iressa, respectively is also warranted [72].

Pterostilbene (XVII), an analog of resveratrol (trans-3, 5-dimethoxy-4-hydroxystilbene), is a phenylpropanoid-derived plant compound found in blueberries, several types of grapes, and tree wood. In nature, pterostilbene acts as a phylotaxin and is significantly increased during times of plant stress in response to microbial attack, ultraviolet light, and irradiation mainly as a protective mechanism. Additionally, pterostilbene has nearly three times the bioavailability of resveratrol [73]. Pterostilbene (3, 5- dimethoxy-4-hydroxystilbene), an antioxidant found in blueberries, reduces proliferation in pancreatic cancer *in vitro* through mitochondrial membrane depolarization, caspase 3/7 activation, and cell cycle arrest. To further elucidate the mechanism of pterostilbene and conducted a microarray genomic analysis of pterostilbene-treated pancreatic cancer cells to identify upregulated and downregulated genes. Manganese superoxide dismutase (MnSOD), an antioxidant located in the mitochondria, was found to be significantly upregulated by pterostilbene in the genomic microarray analysis. Hence, It was further investigated the effect of pterostilbene upon this enzyme. Interestingly, studies have shown that pancreatic cancer cells have decreased expression of MnSOD as compared to normal cells. MnSOD overexpression studies in

pancreatic cancer show that increased MnSOD activity correlates with decreased rates of tumour growth. It was hypothesized that pterostilbene would increase MnSOD enzymatic activity in a dose-dependent manner and decrease intracellular oxidative stress [73]. Pterostilbene alters gene expression in pancreatic cancer and increases the antiproliferative markers cytochrome C, Smac/DIABLO, and MnSOD/antioxidant activity. It was also shown to inhibit phosphorylated STAT3, a marker of accelerated tumourigenesis, and decrease pancreatic tumour growth *in vivo*. Further studies are warranted to elucidate the effects of pterostilbene in humans [73].

5.2. Plant-Derived Anti-Cancer Agents in Clinical Development

The structures of some plant-derived agents currently in clinical development are shown in Figure 2. Flavopiridol (IV) is totally synthetic, but the basis for its novel flavonoid structure is a natural product, rohitukine (V), isolated as the constituent responsible for anti-inflammatory and immunomodulatory activities from *Dysoxylum binectariferum Hook. f.* (Meliaceae), It is currently in phase I and phase II clinical trials, either alone or in combination with other anti-cancer agents, against a broad range of tumours, including leukemias, lymphomas, and solid tumours. Natural plant product (R) – Roscovitine, which shows inhibitions of CDK activity [97]. This will assist natural product chemists with their investigations into other natural an unnatural analogues from (R) –Roscovitine and to determine their effect towards other cancers and their pharmacokinetics properties [59].

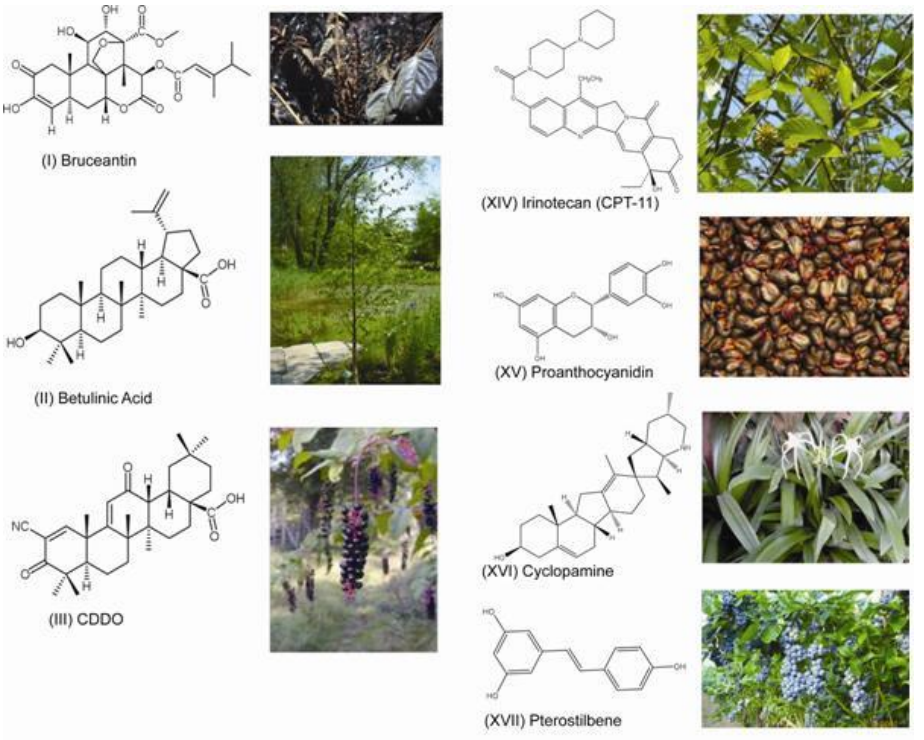

Figure 1. Plant-derived agents in preclinical development.

The Combretastatins (VI) were isolated from the South African "bush willow" *Combretum Caffrum* [60, 61]. A water-soluble analog, combretastatin phosphate (VII), has shown promise against thyroid cancer in early clinical trials [62].

Pancreatic cancer: The n-hexane extracts of the roots of three medicinally used Echinacea species exhibited cytotoxic activity on human cancer cell lines, with *Echinacea pallida* found to be the most cytotoxic. Acetylenes are present in E. pallid lipophilic extracts but essentially absent in extracts from the other two species. The cytotoxic effects of five compounds, two polyacetylenes (8-hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one (XVIII) and pentadeca-(9E)-ene- 11,13-diyne-2,8-dione (XX)) and three polyenes (8-hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one (XIX), pentadeca- (9E,13Z)-dien-11-yne-2,8-dione (XXI) and pentadeca-(8Z,13Z)-dien-11-yn-2-one (XXII)), isolated from the n-hexane extract of *E. pallida* roots by bioassay-guided fractionationed and investigated toward the potential bioavailability of these compounds in the extract. Cytotoxic effects were assessed on human pancreatic MiaPaCa-2 and colonic COLO320 cancer cell lines [74].

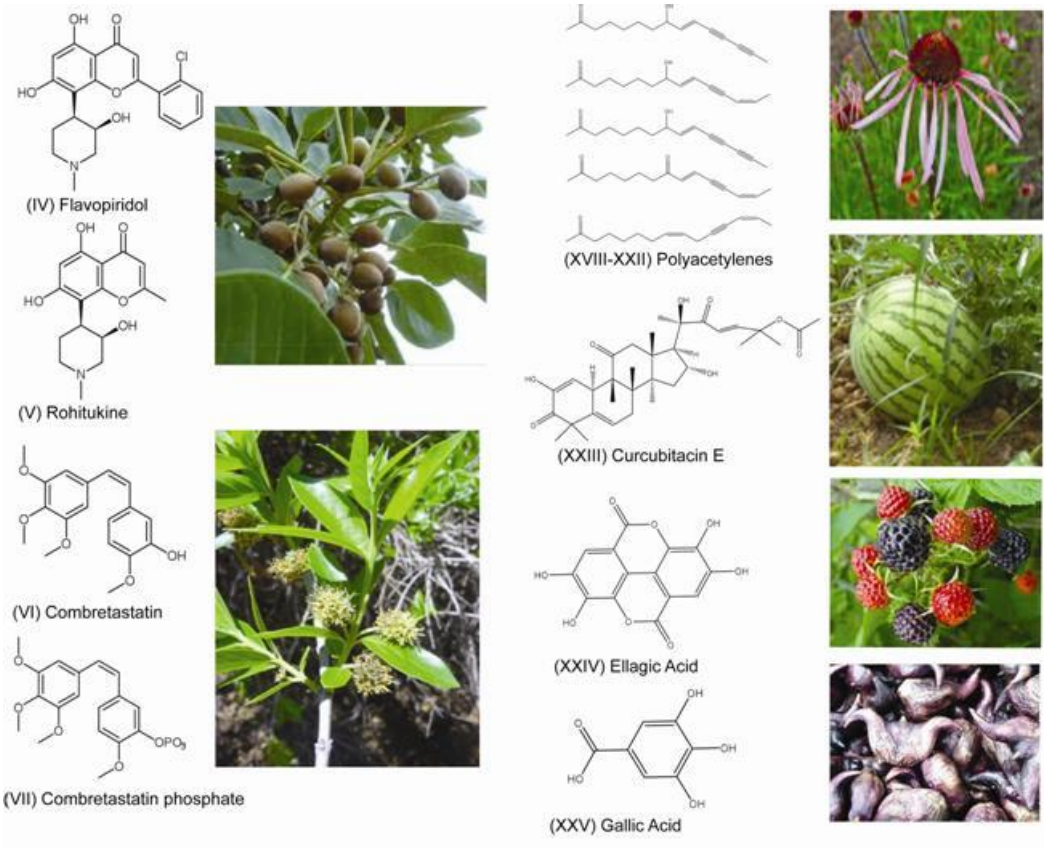

Figure 2. Plant-derived anti-cancer agents in clinical development.

Cucurbitacins are tetracyclic triterpenes isolated from plant in the Cucurbitaceae families that has been used in traditional medicine for centuries. Cucurbitacins from watermelons have potential to be used as a favorable phytochemical for cancer prevention. Several types of cucurbitacin compounds have been studied *in vitro* and *in vivo* for their anti-cancer effects. For example, Cucurbitacin E (XXIII) treatment can inhibit the viability of pancreatic cancer cells (PANC-1) and induce apoptosis via suppression of STAT3 phosphorylation and up-regulation of p53. Cucurbitacin E also inhibits the proliferation of prostate cancer cells and causes disruption of the cytoskeleton structure of actin and vimentin [74-76].

Ellagic acid (2,3,7,8-tetrahydroxy[1]benzopyrano[5,4,3-cde][1] benzopyran–5,10-dione) (XXIV) (Figure 2) is a plant-derived polyphenol found in a wide variety of fruits and nuts, for example raspberries, strawberries, walnuts, grapes, and black currants. Ellagic acid has a variety of biological activities including anti-oxidant, anti-inflammatory, anti-fibrosis and anti-cancer properties [77]. Ellagic acid protects against ischemia/reperfusion-induced gastric injury and carbon tetrachloride-induced liver fibrosis. The anti-cancer properties of ellagic acid include induction of cell cycle arrest and apoptosis, and inhibition of tumour formation and growth *in vivo*. The molecular mechanisms responsible for these effects remain largely unknown, but its potent scavenging action on both superoxide anion and hydroxy anion might be involved as oxidative stress plays an important role in the development of pancreatic fibrosis [77].

Gallic acid (GA) (XXV) is a plant phenol isolated from water caltrop, which is reported to have anti-inflammatory and anti-cancer effects. The antiproliferative effect of GA on human pancreatic cancer cell lines as well as hepatocytes as normal cells has been assessed and GA showed selective toxicity for cancer cells. GA can function as a cancer-selective agent by inducing apoptosis in pancreatic cancer cells via the mitochondria-mediated pathways and it should open up new opportunities for the therapy of pancreatic cancer [78, 79].

5.3. Plant-Derived Anti-Cancer Agents in Clinical Use

The structures of some plant-derived anti-cancer drugs currently in clinical use are shown in Figure 3. The most well known are the vinca alkaloids, vinblastine (VIII) and vincristine (IX), isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. More recent semi synthetic analogs of these agents are vinorelbine (X) and vindesine (XI) [63]. These agents act through the inhibition of tubulin polymerization and used for the treatment of a variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers.

Other plant-derived agents in clinical use are homoharringtonine (XII), isolated from the Chinese tree *Cephalotaxus harringtonia* var. drupacea (Sieb and Zucc.) [63] and elliptinium (XIII), a derivative of ellipticine isolated from species of several genera of the Apocynaceae family including *Bleekeria vitensis* A. C. Sm., a Fijian medicinal plant. A racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Elliptinium is marketed in France for the treatment of breast cancer.

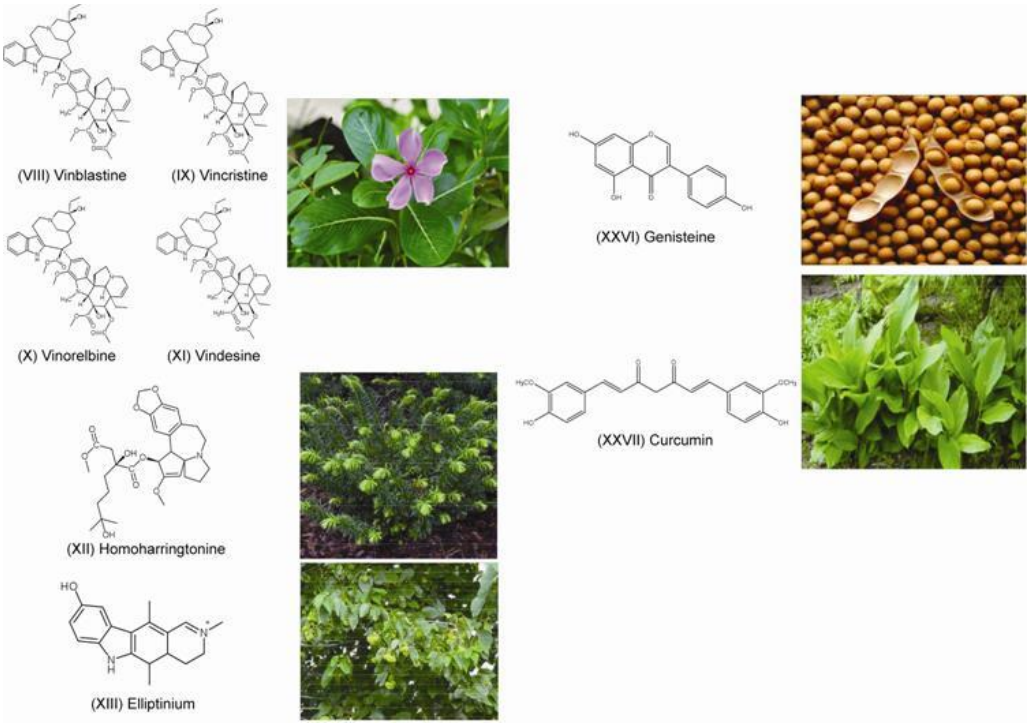

Figure 3. Plant-derived anti-cancer drugs in clinical use.

Pancreatic Cancer: In contrast, Asians who consume a diet high in soy products have a relatively low incidence of, and mortality due to pancreatic cancer, suggesting that a high intake of soy products may protect people against pancreatic cancer. Genistein (XXVI) (Figure 3), a natural isoflavonoid found in soybean products [80], has been believed to be a chemopreventive agent because it has been reported to be associated with lower incidence of pancreatic cancer. It has been found that genistein can inhibit the growth of various cancer cell lines both *in vitro* and *in vivo*. However, the molecular mechanisms by which genistein elicits its effects on pancreatic cancer cells has not been fully elucidated, and it is an emerging area of research. Genistein has been shown to infuce cell growth inhibition, as well as inducing apoptotic cell death in pancreatic cancer cell lines. Furthermore, genistein inhibited the expression of FoxM1 and its target genes. Therefore, genistein-mediated cell growth inhibition could be partly mediated via inactivation of FoxM1 activity. Indeed, it was found that down-regulation of FoxM1 by siRNA together with genistein treatment inhibited cell growth and induced apoptosis to a greater degree in pancreatic cancer cells compared to genistein treatment alone. Inactivation of FoxM1 by genistein results in the down-regulation of its target genes, which are believed to be linked mechanistically with genistein induced cell growth inhibition, induction of apoptosis and cell invasion [79].

Curcumin (XXVII) is derived from turmeric (*Curcuma longa*) and is a natural polyphenol. [79] Curcumin has long been used as a food, coloring agent, and in traditional medicine. More and more data support the idea that this chemical could be a promising anti-cancer drug for a variety of tumours. Preclinical studies demonstrated curcumin potentiates anti-tumour activity of gemcitabine against pancreatic cancer. Studies of curcumin have shown that this agent can be administered safely at doses of up to 12 g/day and some clinical

benefit in patients with pancreatic cancer. Thus, synergic effects of curcumin on gemcitabine in preclinical studies, and safety of curcumin monotherapy in clinical studies have been reported; however, at the time of planning this clinical trial in 2008, the safety and feasibility of curcumin in combination with gemcitabine-based chemotherapy had not been reported in a human study. More-recently introduced anti-metabolites in this family include gemcitabine, which is used intravenously with cisplatin for metastatic non-small cell lung, pancreatic, and bladder cancers [81].

6. FUTURE TRENDS FOR PLANTS IN THE SUBCONTINENT AS NOVEL ANTI-CANCER AGENTS

Natural products have been major molecular structural resources for drug discovery. Antibiotics, penicillin, the analgesic and antipyretic drug aspirin, the anti-cancer drug taxol, the anti-malarial drug artemisinin, and the anti-Alzheimer's disease drug huperzine A are typical, successful examples. The reservoir of natural products contains an abundance of chemical novelty and diversity: about 40% of the chemical scaffolds of the published natural products are unique and have not been made by synthetic chemistry. Another advantage is their chiral center. Most natural products are chiral and occur as single enantiomers; and many modern drugs are chiral and have their biological activity associated with only one of the enantiomers. Therefore, using natural products as enantiomerically pure starting materials is a good solution to this problem. Today, it becomes increasingly difficult to synthesize or discover new and interesting lead compounds. However, with the aid of techniques like high-throughput screening assays, 3-D protein–ligand models, virtual screening, computer assisted rational drug design, and the expanding knowledge of the molecular basis of tumourigenesis and metastasis, it seems feasible to harness the natural pool and discover novel compounds that rationally target the abnormal molecular and biochemical signals leading to cancer [82].

CONCLUSION

From the foregoing discussion, it is clear that plant products have made, and continue to make, an indispensable contribution to the discovery and development of effective drugs for the treatment of cancer. This observation applies equally as well for many other diseases afflicting humankind. A recent analysis of the new drugs marketed over the past 25 years during the period between 1981 and 2006 shows that some 50% owe their origin in one way or another to natural sources, and in some disease areas well over 60% are derived from natural products. In addition, plant products are an invaluable source of molecular probes in the study of pathways influencing cell cycle progression. While plant products are a proven source of novel bioactive molecules, the actual compound isolated from the parent source is often not suitable for development into an effective drug, but it may be regarded as a lead molecule which can form the basis for further chemical or biochemical modification. Currently, natural products are passing through a phase of reduced interest in drug discovery because of the enormous efforts, which are necessary to isolate the active principles and to elucidate their structures. However, if one considers the diversity of chemical structures found

in nature with the narrow spectrum of structural variation of even the largest combinatorial library it can be expected that natural products will become important again [26].

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In: Plant Bioactive Compounds … ISBN: 978-1-63463-324-6
Editors: Christopher J. Scarlett and Quan V. Vuong

Chapter 11

PLANTS IN AFRICA AS POTENTIAL ANTI-CANCER AGENTS

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ABSTRACT

Medicinal plants are important natural resources that serve as the most reliable source of drugs for rural populations in Africa. The knowledge of plant remedies is available through African traditions and folklore, thus the application of this popular knowledge on the use of medicinal plants can contribute to the development and production of products for the pharmaceutical market. In this chapter we review the development of anti-cancer drugs from African medicinal plants. A perspective on the methods of ethno-medical surveys of African folk remedies, concept and perceptions about cancers are discussed. The process of determination of the scientific basis for the continued use of specific medicinal plants in the treatment of cancers using appropriate pharmacological evaluation is also discussed. The isolation and purification methods of biologically active compounds from medicinal plants and a comprehensive discussion of isolated anti-cancer compounds from African medicinal plants that show reliable outcomes on etiology and pathogenesis of human cancers are given. The potential of isolated compounds with molecular targets and/or as inhibitors of cell signaling-enzyme pathways implicated in cancer are also discussed. Active anti-cancer products from African medicinal plants are derived from a wide variety of plant Genera and Species; herein we provide a review on the utilization of the plants from an ecological, sustainable development point of view.

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1. INTRODUCTION

Human ancestors had a large incentive for exploring the world around them for possible remedies against diseases and physical ailments. As far back as the paleolithic period, humans have being using plants, animals and minerals resources of the earth (Grifo et al.. 1997). The abundant productivity of nature was the only source of remedies for humans during these earlier times. Although it was not only plants that were used as remedies by man, plants are increasingly becoming an important source of drugs. This is mainly due to human observation of the environment and natural resources which led to traditional claims through folklore and scientific experimentation (Koehn and Carter, 2005). With these developments in human history, plants continue to play a central role in the healthcare system of large proportions of the world's population. Subsequently, the use of plants as remedies for diseases is referred to as herbal medicine (Rômulo and Lerêce, 2007).

It has been estimated that only about 5,000 plant species (1.5-2%) out a total of 250,000-300,000 world-wide have been studied for possible bioactive substances. Overwhelmingly, their use in traditional medicine was the main criterion for their selection (Iwu, 1995). This approach makes good sense, since it is a daunting task to construct large libraries of *de novo* synthetic compounds and test them for their medicinal effects. Ancient texts, historical records, ethnology, and pharmacological approaches provide important means to discover new drugs. Medicinal plant research is directed at verifying ethno-medical claims by herbalists and traditional medical healers with the ultimate aim of isolating active compounds and standardizing the crude extracts used in traditional medicines (Sofowora, 1986; Sofowora, 1993). There is a great diversity in the plants used in traditional medicine; the diversity varies with local cultures and tradition (Watt and Breyer-Brandwijk, 1962; Farnsworth and Morris, 1976).

The World Health Organization (WHO) estimates that 80% of the population of developing countries relies on traditional medicine for their primary care needs and the WHO has been in support for the development of traditional pharmacopeia to facilitate the well-being of African populations and other developing nations. Medicinal plants are the major resource of this folk medicine where several species are used for the treatment of diseases with an inflammatory and/or infectious component, as is the case of old wounds, skin diseases and malfunctions affecting internal organs such as liver, lung, prostate and kidney.

In recent times, medicinal plants occupy an important position for being the primary sources of drug discovery; and plants have been indispensable in treating diverse forms of diseases including cancer (Koehn and Carter, 2005 & Farnsworth, 1984, Farnsworth, 1988). According to WHO, 80% of the people living in the rural areas depend on medicinal plants as their primary health care system (WHO, 2003). There are at-least 250,000 plant species worldwide out of which more than one thousand plants have been found to possess significant anti-cancer properties (Fabricant and Farnsworth, 2001). In developing countries, herbal medicine has a long and uninterrupted history of use and accordingly the oldest component of the African health sector (Farnsworth, 1984).

The health care practitioners consist of traditional healers and birth attendants, who are the *de facto* providers of primary health care. Healers provide client-centered and personalized health care that is culturally appropriate, holistic and tailored to meet the needs and expectations of the patients (Iwu, 1994). There is also a general belief that the remedies

used in traditional medicine are safe and more readily acceptable by the population (Heinrich, 2000).

Recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of Western pharmaceuticals and healthcare (Farnsworth, 1988). Herbal medicines are more acceptable in these countries from their cultural and spiritual points of view. Among the human diseases treated with medicinal plants is cancer, which is probably the most important disease with high mortality rates. A large number of synthetic agents are used to cure various cancers, but they produce side effects that prevent their extensive usage. Many of these pathologies described by practitioners of traditional medicine have similarities with certain cancers, but the lack of training of many of these healers does not allow them to establish a link with cancer.

The potentials of plants in treatment of cancers is on the increase with several examples of plant extracts from plant parts including leaves, bark, wood, flowers, seeds, fruits and plant exudates (including resins, gums, latex, nectar). A large number of potential anti-cancer plants come from the rich African lora (Sawadogo 2012).

The Africa's flora is dictated by the local climate, and its natural vegetation is, therefore, very varied – from fertile rainforests where rain falls virtually every month of the year to arid desert landscapes where years can pass without rain (Burslem, 2001). While many molecules obtained from nature have shown wonders, there are a huge number of molecules that yet to be elucidated and harnessed by medicinal chemists. Research on natural anti-cancer molecules from African plants is still in its infancy because of very limited financial resources and the scarcity of adequate technical facilities. However, several plants were investigated for their anti-cancer properties through north-south or south-south partnerships. (Farnsworth, 1984 and Farnsworth, 1988).

2. AFRICAN TRADITIONAL MEDICAL SYSTEMS

Plants used in traditional medicine have stood up to the test of time and have contributed many novel compounds for preventive and curative medicine to modern science. Africa is sitting on a gold mine of a traditionally well practiced knowledge of herbal medicine. Madagascar has contributed the *Catharanthus roseus* (Madagascar periwinkle) and has the potential of contributing more in view of the diversity of the flora and fauna of the country. Famous African medicinal plants include *Acacia senegal* (Gum Arabic), *Aloe ferox* (Cape Aloes), *Aloe vera* (North African Origin), *Catha edulis* (Khat), *Commiphora myrrha* (Myrrh), *Harpagophytum procumbens* (Devil's Claw), *Hibiscus sabdariffa* (Hibiscus, Roselle), *Prunus africana* (African Cherry). All these plants are well described in official pharmacopoeias (United States pharmacopoeia, British pharmacopoeia, British Pharmaceutical Codex). The monographs in the pharmacopoeias provide the basic requirement for standardization of the crude drugs.

As the knowledge of African traditional medicine is poorly recorded, there is an urgent need for documentation of medicinal properties of plants and cultural perception of diseases and their remedies (Ameh et al. 2010). There is rapid loss of the natural habitats of some of

these plants because of anthropogenic activities (Heinrich, 2000). The exploitation of medicinal plants is introducing a new threat to the environment as large amount of plants are collected without replacement; with many of these plants being sold in open markets (Figure 1). Considering the rapid rate of deforestation and loss of biodiversity, there is a need for accurate documentation of traditional knowledge and experience through ethno-medical surveys (WHO, 2000).

The African continent is reported to have one of the highest rates of deforestation in the world. The paradox is that it is also a continent with a high rate of endemism with the Republic of Madagascar topping the list at 82% (Burslem, 2001 & Farnsworth, 1988).

2.1. Classical African Traditional Medicine

Africa is considered to be the cradle of Mankind with a rich biological and cultural diversity and marked regional difference in healing practices. African traditional medicine forms the mainstream coverage of the health needs of its population due to extreme poverty and shortage of health care professionals (Awodele et al., 2011). It is perhaps the oldest and the most diverse of all medicine systems. African traditional medicine is a holistic process involving both the body and the mind. Practitioners of traditional African medicine claim to be able to cure various conditions such as cancers, psychiatric disorders, high blood pressure, cholera, most venereal diseases, epilepsy, asthma, eczema, fever, anxiety, depression, benign prostatic hyperplasia, urinary tract infections, gout, and healing of wounds and burns (Etkin, 1998).

The healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines to treat the symptoms. Most of these prescriptions consist of plants that have contributed to the health and general well-being of local inhabitants and is handed down through generations.

2.2. Classical Arabic and North African Traditional Medicine

The oldest written information in the Arabic traditions comes from the Sumerians and Akkadians of Mesopotamia, thus originating from the same areas as the archeological records of Shanidar IV (Heinrich et al., 2004).

The Middle East is known as the cradle of civilisation and many plants grown nowadays have been domesticated in this region. The Babylonians, Assyrians and Sumerians recorded herbal remedies in cuneiform writing on numerous clay tablets; of special interest is the Code of Hammurabi (ca. 1700 BC), a comprehensive set of civil laws carved in stone and commissioned by the King of Babylon. It lists several medicinal herbs (Heinrich, 2004).

Figure 1. A significant number of medicinal plants are collected by local herbalists without replacement, and this may lead to scarcity or even extinction.

Figure 2. Herbalists displaying their wares Zaria city, Nigeria.

The Egyptians documented their knowledge (including medical and pharmaceutical) in wall-paintings of tombs dating from the Old Kingdom and on papyrus. The most important of these writings is the Papyrus Ebers, which originates from around 1500 BC and is reported to contain ancient medicinal knowledge from before 3000 BC covering all sorts of illnesses and includes empirical and symbolic forms of treatment. The Arabs developed a system of medicine by expanding their methods with Greco-Roman expertise and Chinese and Indian herbs. The Arabs were the first to establish privately owned drug stores in the 8th Century, and the Persian pharmacist, physician, philosopher and poet, Avicenna, contributed much to the sciences of pharmacy and medicine throughout the works such as Canon medicinae, regarded as the ‘‘final codification of all Greco-Roman medicine’’. Canon medicinae include elements of other healing cultures and forms the basis for a distinct Islamic healing system known today as Unani-Tibb (Heinrich, 2004). Among the famous medicinal plants of the Middle East and Egypt (Gurib-Fakim, 2006) are: *Allium cepa* (Onion), *Astracantha gummifera* (Tragacanth), *Carthamus tinctorius* (Safflower), *Carum carvi* (Caraway), *Ferula*

assafoetida (Asofoetida), *Lawsonia inermis* (Henna), *Papaver somniferum* (Opium poppy), *Peganum harmala* (Syrian rue), *Prunus dulcis* (Almond), *Punica granatum* (Pomegranate), *Rosa damascena* (Damask Rose), *Ricinus communis* (Castor Oil Plant), *Salvadora persica* (Toothbrush tree), *Senna alexandrina* (Senna), *Sesamum indicum* (Sesame), *Trachyspermum ammi (*Ajowan), *Trigonella foenum-graecum* (Fenugreek) and *Vitis vinifera* (Grape).

2.3. African Traditional Perceptions and Diagnosis of Disease

In African traditional medicine, the practitioner personally assesses patients in order to diagnose, treat, and prevent disease using their clinical judgment. The practitioner – patient relationship typically begins with interrogations through case-history taking and recourse to basic procedures such as divination to determine the cause of the patient's complaint (Etkin, 1998). Once the primary causes of the ailment are determined, the practitioner then prepares medicines, which may be derived from medicinal plants, animal parts or minerals. The practitioner's own experience, added to the accumulated knowledge handed down by their ancestors; allow the practitioner to offer cheap, but effective remedies for treating the populations of the African Region. Some healers may employ the use of charms, incantations, and the casting of spells in their treatments. The dualistic nature of traditional African medicine between the body and soul, matter and spirit and their interactions with one another are also seen as a form of magic. Using charms and amulets to cure diseases and illnesses is an uncertain and clouded practice that requires more scientific investigation (Cox, 2000; Neuwinger, 2000).

The main diseases treated by the practitioner include malaria, sexual dysfunction, mental problems, rheumatism, arthritis, anaemia, parasitic infections, bone fractures, conditions requiring midwifery services, stomach infections, respiratory problems, and more recently cancer, due to changing diets and social habits. When diagnosis is ascertained, the treatment is prescribed, usually consisting of an herbal remedy that has not only healing abilities, but also symbolic and spiritual significance. In African traditional medicine, illness is not derived from chance occurrences, but through spiritual or social imbalance. Many traditional medicinal practitioners are people without western education, who have received knowledge of medicinal plants and their effects on the human body from their fore fathers (Cox, 2000). They have a deep and personal involvement in the healing process and protect the therapeutic knowledge by keeping it a secret. The practitioners of traditional medicine specialize in particular areas of their profession. Some, such as the Inyangas of Swaziland and Magoris of Northern Nigeria are experts in herbalism, whilst others, such as the South African Sangomas and Babalawos (Yoruba, Nigeria) are experts in spiritual healing as diviners. Others practitioners specialize in a combination of both forms of practice. There are also traditional bone setters and birth attendants.

2.4. Role of African Traditional Medicine in Primary Health Care

The oldest component of the African primary health care component consist of tradtional medicine that provides a culturally appropriate, client-oriented and personalised health care delivery system (Awodele et al., 2011). Medicinal plants provide the bulk of raw material for

the African tradtional health system (Ameh et al. 2010). Historical records and folklores, as well as traditional practices of peoples around the world provide a rich source of potential natural products (animal, plant, and mineral) for discovering lead molecular structures for new drugs (Grifo et al. 1997). Ancient Meso-potamian, Egyptian, and Greek texts provided the impetus for the isolation and eventual modification of salicylic acid from willow tree bark (*Salix* spp.) in the 19th century; acetylsalicylic acid (aspirin) is today the most widely used analgesic and antipyretic drug.

The African concept of disease and medicine is the foundation of traditional medicine treatment. The traditional medical practitioner provides a link between a patient and the patient's own social, cultural and intellectual environmental background. In African traditional medicine, the curative, training, promotive and rehabilitative services are referred to as clinical practices (Etkin, 1998). These traditional health care services are provided through tradition and culture prescribed under a particular philosophy, norms, taboos, tradition and culture, which are the cornerstones of clinical practice of traditional medicine. The philosophical clinical care embedded in these traditions, culture and taboos have contributed to making traditional medicine practices acceptable and hence highly demanded by the population (WHO, 2003). Traditional healers, like any other profession, are rewarded for their services. In African societies, the payment for a treatment depends on its efficacy. Payments are not requested until the treatment is given. This is another reason many prefer traditional healers to western doctors who require payment before the patient has assessed the effectiveness of the treatment (Neuwinger, 2000). Due to the effectiveness, affordability and acceptability, traditional medicine is the main primary health care provider of African populations.

Medicinal plants are widely exploited through Africa and commerce in herbal resources is contributing to the income of the local populations (Figures 2 and 3). Herbs are now being sold on major market days in Africa, in fact there are some markets and market days that are dedicated solely to the sale of medicinal plants (Figure 2).

2.5. Traditional African Concept of Cancer and its Treatment

Cancer refers to a large group of diseases that can affect any part of the body and it is found found in several systems (circulatory, lymphatic, digestive, urinary, reproductive) as well as skin. Because of technological advances, the diagnosis of cancer is easier in modern, but not traditional medicine. The general African traditional concepts and etiology of cancers is complex and does not tally with modern medical practice (Table 1). Although some of these beliefs may have some scientific basis, others are bizarre and unfounded. Interestingly some practitioners of tradtional medicine have some basic knowledge of specific cancers.

The concepts and etiology of cancer in traditional medicine are complex and are not always compatible with modern medical practices (shown in Table 1). This can be justified by the fact that most of the healers of folk medicine are not educated enough to establish an adequate link between symptoms and type of pathology, which does not allow a proper clinical diagnosis; in addition, diagnostic tools are totally absent. The classical tradtional theory for etiology of of cancer is that these diseases are resulting from excessive production and deposition of fluids. Most traditional medical practitioners are of the opinion that production of excess body fluids is responsible for rheumatism, arthritis, abnormalities of the

gastrointestinal tract, and cancers of the male and female reproductive systems. Some localities also believe that the entry of some poisonous materials before birth is a contributory factor in the etiology of cancers. A bizarre belief about cancers is that they occur as a result of contact by victims with evil spirits in the forest or bush (Abubakar et al., 2007).

The method of preparation for the herbal prescriptions in African traditional medicine is diverse, remedies are prepared and used as concoctions, decoctions, infusions and powders dispensed with food or drinks. Underground plant organs such as roots, corms and bulbs were the most frequently used parts of plants for the treatment of cancer, these are prepared as decoctions and infusions. Also certain leaves and barks are used as herbal preparations for cancers. Useful plant parts are powdered and prepared as decoctions or infusions, these are filtered and are administered orally for internal cancer and applied topically on external cancers until signs of relief are obvious. The extraction solvent is water, although local spirits or gin is also used (wines, and other local brew). Latex and plant exudates may be applied topically on external cancers on a daily basis until the tumour heals.

Figure 3. Sales of medicinal plants is a contributory factor to the local economy, however there is need for standardization of the sale.

The treatment and concept of cancers are prone to superstitions but ethno-medical information can be used to pursue plants as a source of drugs (Trotter and Logan, 1986). There is a need to provide scientific basis for the use of some plants in the treatment of cancer especially by the extraction and isolation of the active principles (Grifo et al., 1997; Harvey, 2008). An active research program can be established by encouraging collaborations to develop drugs from these bio-resources (Etkin, 2001).

3. PLANTS WITH POTENTIAL FOR DEVELOPMENT INTO ANTI-CANCER DRUGS

There is a great diversity in ethno-medical claims of useful plants and perception of diseases. Current medicinal plant research is directed at verifying the ethno-medical claims of practitioners in treatment of diseases. The ethno-medical history is documented through

surveys of plants used in disease treatment and is carried out through interviews and interaction with local people and herbalists, mostly in their homes and/or clinics. Generally, the interview is conducted in the local languages spoken by the people. There is a need for the researcher to first adequately identify the perception of diseases by the people.

Information on the names of plants, parts used and methods of preparation are collected through questionnaires which are administered to herbalist traditional medical practitioners, traditional birth attendants, community dwellers, farmers, hunters who have the necessary knowledge of medicinal plants. In cases where the respondents cannot fill the forms, there is a need for the researcher to fill the forms while keeping audio and video records of such interviews. Table 2 show different surveys carried out for the identification of African plants with potential for the development of anti-cancer drugs.

Table 1. African perceptions about certain cancers

Location	Type of Cancer	Traditional Diagnosis
Skin	Malignant melanoma	Any skin condition which rapidly grows and has defied normal traditional and any conventional cure. Some practitioners are of the belief that furuncles, carbuncles and severe skin ulcers are a form of skin cancers
Digestive System	Stomach, pancreas, liver, spleen, colon, rectal and anal cancer	There is no clear-cut differentiation of digestive tract cancers in most traditional systems. However, any symptom associated with stomach distention and/or chronic stomach pains lasting several weeks is a stomach cancer (Hausa tribe Northern Nigeria). If localized on the right side that cannot be treated by the herbs for stomach ailment are considered as liver cancers while on the left side are considered pancreatic cancer.
Urinary System	Kidney, bladder, testicular, prostate	Not clearly diagnosed in traditional medicine. Most of these conditions are characterized as chronic sexually transmitted diseases
Cancer of Women	Ovarian cancer and other gynecological cancers	Not clearly diagnosed in traditional medicine. Most of these conditions are characterized as sexually transmitted and some practitioners believe that it has some spiritual origin
General cancer	All forms of cancers including lymphatic, brain and thyroid cancers	Most perception of cancers are unfounded and are perceived to be caused by contact with evil spirits

4. PLANT CONSTITUENTS WITH POTENTIAL ANTI-CANCER COMPOUNDS FROM AFRICAN PLANTS

Through ethno-medical surveys, several African plants have been found to contain biologicaly active compounds and a number are found to be useful in laboratory models of various cancers. The emergence of resistance to cancer chemotherapy has forced researchers to turn to natural products of plant and marine origin. More recently researchers are able to identify plants of interest for cancer treatment through ethno-botanical and ethno-pharmacological surveys (WHO, 2006).

Table 2. List of some African plants used in traditional treatment of cancers

Plant Family	Plants	Part Used	References	Type of Cancer
Acanthacea	*Dyscoriste perrotteti*	Aerial part	Abubakar, et al. 2007	Cancers (unspecified)/ Inflammations
Amaryllidaceae	*Crinum jagus*	Bulb	Soladoye et al. 2010	Cancers (unspecified)
Ampelidaceae	*Cissus ibuensis*	Leaves	Abubakar, et al. 2007	Skin Cancers
Anacardiaceae	*Lannea egregia* *Magnifera indica*	Leaves Bark	Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified)
Annonaceae	*Anona senegalensis* *Uvaria chamae* *Uvaria afzelii*	Leaves Bark Root	Abubakar, et al. 2007 Soladoye et al. 2010 Soladoye et al. 2010	Skin / leukaemia Cancers (unspecified) Cancers (unspecified)
Apocynaceae	*Alstonia congensis* *Alafia barteri*	Root bark Leaves	Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified)
Araceae	*Pistia stratiotes* *Culcasia scandens* *Anchomanas difformis*	Leaves Leaves Leaves	Soladoye et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Aristolochiaceae	*Aristolochia albida*	Rhizomes	Abubakar, et al. 2007	Many forms of cancers
Asclepediaceae	*Leptadenia hastata* *Calotropis procera* *Secamone afzelii* *Tylophora spp.*	Aerial parts Roots Leaves Leaves	Abubakar, et al. 2007 Abubakar, et al. 2007, Silva, et al. 2010 & Moustafa et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010	Many forms of cancers Tumours (unspecified) Cancers (unspecified) Cancers (unspecified)
Asteraceae	*Acanthospermum hispidum*	Flowering shoot	Ashidi et al. 2010	Cancers (unspecified)
Bignonaceae	*Kigelia africana* *Spathodea companulata*	Leaves & Bark Bark	Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified)
Bromeliadaceae	*Ananas comosus*	Juice	Soladoye et al. 2010	Cancers (unspecified)

Plant Family	Plants	Part Used	References	Type of Cancer
Burseraceae	*Boswellia dalzielli*	Stem bark	Abubakar, et al. 2007	Many forms of cancers and fibrosis
Caesalpiniaceae	*Erythrophleum guineense*	Leaves	Abubakar, et al. 2007	Skin cancer
	Berlinia grandiflora	Bark	Soladoye et al. 2010	Cancers (unspecified)
	Senna alata	Leaves	Soladoye et al. 2010	Cancers (unspecified)
	Senna fistula	Leaves	Soladoye et al. 2010	Cancers (unspecified)
	Cynometra mannii	Bark	Soladoye et al. 2010	Cancers (unspecified)
Celastraceae	*Celastrus indica*	Root	Soladoye et al. 2010	Cancers (unspecified)
Chenopodiaceae	*Chenopodium ambrosioides*	Aerial Part	Soladoye et al. 2010	Cancers (unspecified)
Combretaceae	*Terminalia avicennioides*	Bark	Soladoye et al. 2010	Cancers (unspecified)
Compositae	*Vernonia amygdalina*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Connaraceae	*Brysocarpus coccineus*	Root	Soladoye et al. 2010	Cancers (unspecified)
Crassulaceae	*Bryophyllum pinnatum*	Root	Soladoye et al. 2010	Cancers (unspecified)
Cucurbitaceae	*Luffa aegyptica*	Leaves	Abubakar, et al. 2007	Leukamia
Euphorbiaceae	*Bridelia ferrugineae*	Stem bark	Abubakar, et al. 2007	Skin cancer
	Croton lobatus	Leaves	Abubakar, et al. 2007	Skin cancer
	Acalypha wilkesiana	Whole plant	Lim et al., 2011	Breast Cancer
	Euphorbia unispina	Root	Soladoye et al. 2010	Cancers (unspecified)
Fabaceae	*Desmodium velutum*	Leaves	Abubakar, et al. 2007	Many cancers
	Indigofera pulchra	Aerial parts	Abubakar, et al. 2007	Cancers (unspecified)/Cellulitis
	Acacia macrostachya	Root bark	Ashidi et al. 2010	Cancers (unspecified)
	Cajanus cajan	Leaves	Sawadogo et al. 2012	Cancers (unspecified)
Guttiferae	*Garcinia kola*	Roots	Soladoye et al. 2010	Cancers (unspecified)
Hypericaceae	*Psorospermum senegalense*	Stem bark	Abubakar, et al. 2007	Skin Cancer
	Psorospermum febrifugum	Stem Bark	Soladoye et al. 2010	Cancers (unspecified)
	Vismia guineense	Leaves	Abubakar, et al. 2007	Cancer (unspecified)

Table 2. (Continued)

Plant Family	Plants	Part Used	References	Type of Cancer
Icacinaceae	*Pyrenacantha staudii*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Labiatae	*Ocimum basilicum*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Lecythidaceae	*Napoleona vogelii*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Liliaceae	*Aloe barteri* *Allium cepa* *Allium sativum* *Allium ascalonicum*	Leaves Leaves Bulb Bulb	Soladoye et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Loganiaceae	*Anthocleista djalonensis*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Meliaceae	*Khaya grandifoliola* *Pseudocedrela kotschyi* *Tricalysia macrophylla*	Bark Bark Bark	Soladoye et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Menispermaceae	*Jateorhiza palmata* *Sphenocentrum jollyanum*	Bark Seeds	Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified)
Mimosaceae	*Calliandra haematocephala* *Tetrapleura tetraptera*	Root Seeds	Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified)
Musaceae	*Musa sapientium*	Tubers	Soladoye et al. 2010	Cancers (unspecified)
Moraceae	*Antiaris Africana*		Soladoye et al. 2010	Cancers (unspecified)
Myristicaceae	*Pycnanthus angolensis*	Bark	Soladoye et al. 2010	Cancers (unspecified)
Nympheaceae	*Nymphaea lotus*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Olacaceae	*Olax mannii* *Olax subscorpioidea* *Ximenia americana*	Leaves Roots Fruits	Abubakar, et al. 2007 Soladoye et al. 2010 Abubakar, et al. 2007	Many cancers Cancers (unspecified) Cancers (unspecified)
Palmae	*Elaeis guineensis*	Bark	Soladoye et al. 2010	Cancers (unspecified)

Plant Family	Plants	Part Used	References	Type of Cancer
Periplocaceae	*Parquetina nigrescens*	Leaves	Soladoye et al. 2010	Cancers (unspecified)
Phytolacaceae	*Petiveria alliacea*	Root	Soladoye et al. 2010	Cancers (unspecified)
Piperaceae	*Piper guineense*	Seeds	Soladoye et al. 2010	Cancers (unspecified)
Plumbaginaceae	*Plumbago zeylanicca*	Root	Soladoye et al. 2010	Cancers (unspecified)
Poaceae	*Saccharum offinarum*	Juice	Soladoye et al. 2010	Cancers (unspecified)
Polygalaceae	*Securidaca longepedunculata*	Root	Soladoye et al. 2010	Cancers (unspecified)
Rubiaceae	*Nauclea latifolia* *Coffea bracteolate* *Morinda lucida*	Root Leaves Bark	Soladoye et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Rutaceae	*Clausena alata* *Citrus aurantifolia* *Citrus aurantium*	Bark Juice Root	Soladoye et al. 2010 Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Sapindaceae	*Paullina pinnata*	Fruit	Soladoye et al. 2010	Cancers (unspecified)
Solanaceae	*Solanum incanum* *Solanum Panduriforme* *Solanum acanthoideum* *Solanum tomentosum* *Capsicum frutescens* *Nicotina tabacum*	Fruits Fruits Wood Root Fruit Leaves	Abubakar, et al. 2007 Mabogo, 1990, SteenKamp and Gows, 2006 Mabogo, 1990, SteenKamp and Gows, 2006 Mabogo, 1990, SteenKamp and Gows, 2006 Soladoye et al. 2010 Soladoye et al. 2010	Stomach Cancer Cancers (unspecified) Cancers (unspecified) Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Verbanaceae	*Lantana ukambensis*	Whole plant	Sawadogo et al. 2012	Cancers (unspecified)
Zingiberaceae	*Zingiber officinale* *Curcuma domestica* *Aframomum melegueta*	Rhizomes Rhizomes Seeds	Kuete et al. 2011 Soladoye et al. 2010 Soladoye et al. 2010	Cancers (unspecified) Cancers (unspecified) Cancers (unspecified)
Zygophyllaceae	*Balanites aegyptiaca*	Seeds	Abubakar, et al. 2007	Cancers (unspecified)

The scientific investigations of these plants showed anti-inflammatory, antioxidant, anti-proliferative and cytotoxic activities against cancer cells. Despite the long history of cancer treatment using plants, the knowledge and experience of these local people is passed from one generation to the other through oral tradition. Several African plant constituents are of special interest in the development of drugs as they provide unique pharmacophores and medicinal properties (Ameh et al., 2011, Ameh et al., 2013). These include alkaloids, phenolic compounds (for example tannins and flavonoids) and terpenoids, which have been reported to possess antimutagenic and anti-cancer properties in many studies.

4.1. Alkaloids

Alkaloids are basic constituents, having nitrogen atoms within a heterocyclic structure. They are largely present in the stem, roots, barks or rhizomes of plants. They give a bitter taste to these plant organs. These compounds are well known to possess antiparasitic (antimalarial), antimicrobial and antibacterial properties. Several African plants are known to contain bioactive alkaloids some of which have exhibited significant anti-cancer activity.

1. ***Catharanthus roseas*** **(Madagascar periwinkle):** The Catharanthus (Vinca) alkaloids comprise a group of about 130 terpenoid indole alkaloids. Due to the pharmaceutical importance and the low content of vinblastine and the related alkaloid vincristine, *Catharanthus roseus* (Figure 4) became one of the best-studied medicinal plants. Figure 5 shows the chemical structures of some vinca alkaloids; vincristine (1), vinblastine (2), vindoline (3), and vinflunine (4). Vinblastine has been marketed for more than 40 years as an anti-cancer drug and became a true lead compound for drug development. Vinca alkaloids (Figure 5) are active against several cancers and there are significant clinical differences between the use of vincristine and vinblastine, the two major alkaloids of *Catharanthus*. Vincristine is used for the treatment of acute lymphatic leukaemia, Wilm's tumour, neuroblastoma, rhabdosarcoma, Erwing's sarcoma, lymphoma and cancers of breast, lung, bladder and cervix. Vinblastine is used for treatment of Hodgkin's disease and non-Hodgkin's lymphoma and cancers of testis and kidney. They are found to induce cell death through apoptosis leading to DNA fragmentation. This induction of apoptosis is mediated through activation of caspase-3 and/or caspase-7. In addition, the alkaloid exhibits its cytotoxic effect by stimulating c-Jun N-terminal kinase (JNK) triggered by cellular stress (Kruczynski et al., 1998).

2. ***Cryptolepis Sanguinolenta:*** Known as yellow-dye root, Ghanaian quinine, or gangamau (Hausa, Nigeria), *C. sanguinolenta* (Figure 6) is sold as a herbal tea bag preparation in Nigeria and Ghana for the treatment of Malaria and other ailments, and was found to contain the anti-cancer indoquinoline alkaloids cryptolepine (5) and Neocryptolepine (6) (Figure 7) (Dassonneville et al., 2000). These alkaloids have several advantages over mainstream anti-cancer agents. They are active against solid human tumours that include lung adenocarcinoma, lymphoma and breast tumours, with breast tumours being the most sensitive. Cryptolepine, however showed differences in activity in patient tumour samples in both solid tumours and

haematological malignancies (Dassonneville et al., 2000). These alkaloids' cytotoxic activity acts through an apoptotic pathway via caspase-3 activation. Cryptolepine (5) was found to be four times more toxic than its isomer proving that the positions of the indole and quinolone rings are crucial to the activity of these alkaloids.

Figure 4. *Catharanthus roseaus* (Madagascar periwinkle).

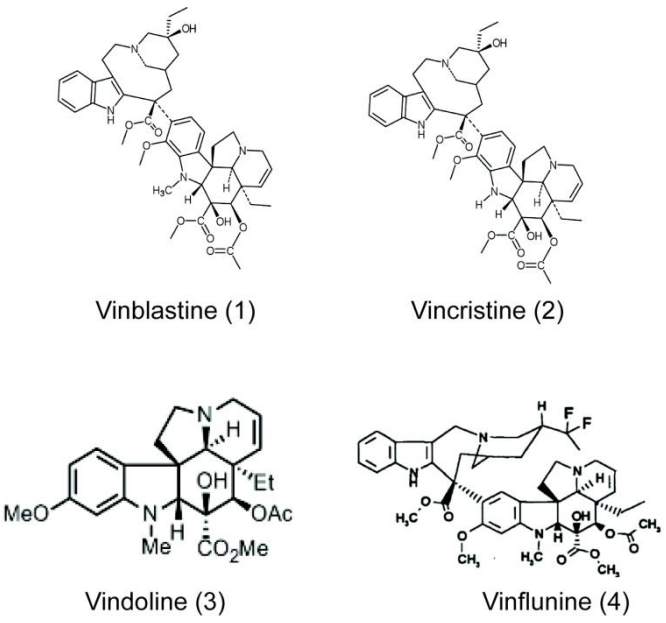


Figure 5. Structures of some *Catharanthus (vinca) alkaloids*.

Cyclopeptide Alkaloids

Cyclopeptides and cyclopepetide alkaloids are cyclic compounds formed mainly with the peptide bonds of protein or non-protein amino acids. More than 200 cyclopeptides have been obtained up to now and their structures have been determined. Cyclopeptides have mainly been found in plants of the following families: Annonaceae, Caryophyllaceae, Rhamnaceae, Rubiaceae; and also found in plants of Compositae, Hymenocardiaceae, Labiatae, Myrsinaceae, Pandaceae, Solanaceae, Sterculiaceae, Urticaceae, and Verbenaceae. All these families have representative genera and species in Africa and they may offer many active anti-cancer cyclopeptide alkaloids. Cyclopeptide alkaloids (Figure 8) are the principal anti-cancer compounds found in *Ziziphus* species like *Ziziphus mauritiana* (Figure 9) and *Z. spinacristi*; and *Anona* species like *Anona senegalense* (Figure 10) *A muricata* and *A. squamosa*.

The seeds, bark, leaves and roots of *Ziziphus* yield many cyclopeptide alkaloids (Figure 8) that includes mauritine series, e.g Mauritane A (7) and Mauritine B (8), and the *Ziziphine* series e.g Ziziphine G (9). Mishra and Bhatia (2011) recently described the anti-cancer potential of phytochemical constituents of the *Ziziphus mauritiana*. *In vitro*, the *Z. mauritiana* extracts demonstrated activity against numerous cancer cell lines (HL-60, Molt-4, HeLa, and the normal cell line HGF). *Z. mauritiana* was found to markedly inhibit the proliferation of HL-60 cells, as well as inducing apoptosis a dose-dependent manner. Anti-cancer activity was also observed using an *in vivo* model of Ehrich ascites carcinoma. The plant extract significantly reduced tumour volume and viable tumour cell count and improved haemoglobin content, RBC count, mean survival time and increased life span of the mice.

Figure 6. *Creptolepis sanguinolenta*.

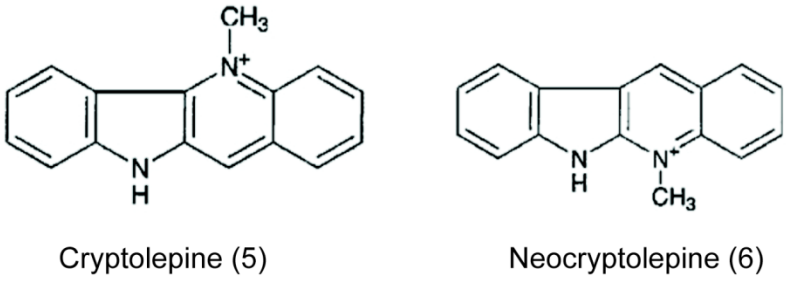
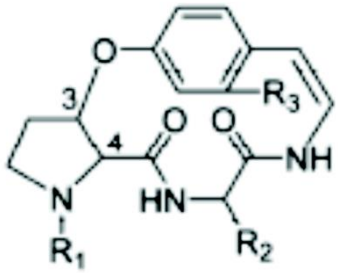


Figure 7. Indoquinoline alkaloid from *Cryptolepis sanguinolenta:* Cryptolepine (5); Neocryptolepine (6).



R_1	R_1	Name
R1=*l*-N,N- CH_3CH_3 Alanine-*l*-*Valine*	R2= *l*-Phenylalanine	Mauritine A (7)
N,N- CH_3CH_3 Alanine-Isoleucine-*Valine*	Phenylalanine	Mauritine B (8)
Isoleucine	Proline	Ziziphine G (9)

Figure 8. Some cyclopeptide alkaloids from *Ziziphus mauritiana* with anti-cancer properties.

Figure 9. *Ziziphus mauritiana*.

Figure 10. *Anona senegalensis*.

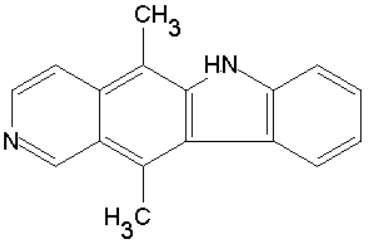


Figure 11. Structure of carbazole alkaloid Ellipticine (10).

Carbazole Alkaloids

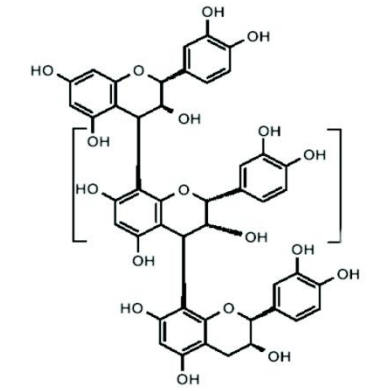
The tetracyclic natural product Carbozole alkaloid (Figure 11) was first isolated from the plant material of *Ochrosia elliptica.* In Africa, the plant is found only in the Seychelles. Investigations have centred on the biological activity of (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole); ellipticine (10). The ellipticine series of compounds uncovered potent *anti-cancer* properties and have been the subject of clinical trials. Ellipticine exhibited promising results in the treatment of osteolytic breast cancer metastases, kidney sarcoma, tumours of brain and myeloblastic leukemia (Stiborova et al. 2001). The ellipticine family of compounds exerts their biological activity **v***ia* several modes of action, the most well-established of which are intercalation with DNA and topoisomerase II inhibition. In recent times other modes of action have been revealed, including kinase inhibition, interaction with p53 transcription factor, bio-oxidation and adduct formation. Several analogues of ellipticine have been synthesised (Stiborova et al. 2011).

4.2. Plant Phenolic Compounds

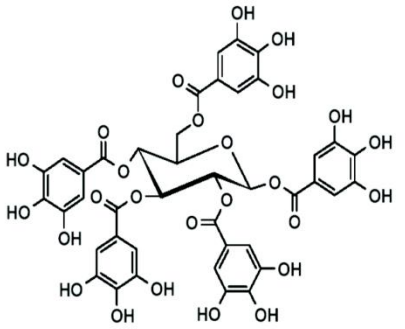
Plant phenolic compounds include; tannins (gallotannins, ellagitannins, pseudotannins), flavonoids, isoflavonoids, aurones chalcones, curcuminoids and stilbenes (Hadi, et al. 2007; Marin et al., 2002), and they possess a wide range of biological properties. Tannins are

complex phenolic compounds (Figure 12) derived from phloroglucinol, pyragallol and catechol and based on their structures they can be referred to as hydrolysable (12) or condensed (11). Flavonoids have diverse structures (Figure 12) and are principally benzopyrone derivatives (13, 14), while some tannins derived from flavonoids are referred to as pseudo-tannins.

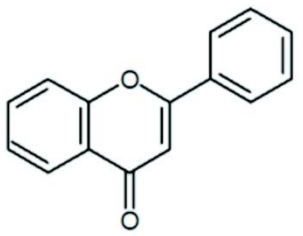
Phenolic compounds occur widely in the plant kingdom, however the pattern of distribution in different families is of taxonomic importance and thus tropical and subtropical plant phenolics may differ from those of temperate origin. As such plant phenolics are important compounds for the development of different drugs, for a range of disease types.



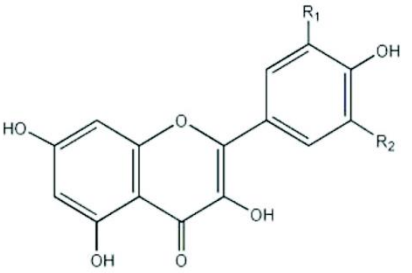
Condensed tannins monomer (11)



Hydrolysable tannins (12)

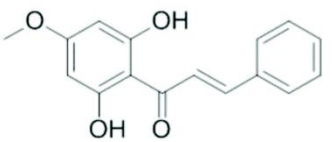


Flavone (13)



R_1 = H;	R_2 = H:	Kaempferol
R_1 = OH;	R_2 = H:	Quercetin
R_1 = OH;	R_2 OH:	Myricetin
R_1 = OCH_3;	R_2 = H:	Isorhamnetin

Flavonols (14)



Pinostrobin chalcone(15)

Figure 12. Structures of some polyphenolic compounds from plants.

Phenolic compounds owe their activity to their antioxidant properties, which is useful in cancer management. The ability of polyphenols (tannins, flavonoids, isoflavonoids) to

scavenge free radicals is considered to be responsible for their chemo-preventive effect. In addition polyphenols are able to interact with the cytochrome P450 CYP1 family enzymes that are promoters of the initiation stage of carcinogenesis. These interactions act by inhibiting the pro-carcinogen activation and by acting as substrate for the release of inhibitors of tumour cell growth. Several studies showed that plant polyphenols, such as flavonoids or tannins, (Azam, et al. 2004, Khan and Hadi, 1998) cause oxidative strand breakage in DNA in the presence or absence of metal ion such as copper. It is suggested that polyphenols can act as pro-oxidants catalyzing DNA degradation in the presence of transition metal ions such as copper. Recently, flavonoids were found to induce G2/M cell cycle arrest through regulation of proteins such as cyclin B1, cdc2, cdc25c and p21. Additionally, these compounds induce apoptosis by up-regulation of the ratio of Bax/Bcl-xL, caspase-3 activity and cleaved PARP, and by down-regulation of pro-caspase-3, -6, -8 and -9 (Khan and Hadi, 1998).

Figure 13. *Curcuma paradoxical*.

Chalcones (15) also present interesting anti-cancer potential by inhibiting NF-kB cell signaling pathways and by interfering with epigenetic regulation (Orlikova et al. 2011a; Orlikova et al. 2011b).

Curcuma longa (turmeric) and *C. paradoxica* (Figure 13) are cultivated along with *Zingiber officinallis* (ginger) in Northern Nigeria. Curcuma yields the anti-cancer constituent – curcumin (16) (Figure 14). Curcumin inhibits the growth of cancer by preventing the production of harmful eicosanoids, such as PGE-2. The effect has been demonstrated in all the steps of cancer development, i.e. initiation, promotion and progression of cancer. Also curcumin inhibits the genesis of cancer as well as promoting cancer regression (Nagabhushan and Bhide 1992). Curcumin suppresses the mutagenic effect of various mutagens including cigarette smoke condensates, 7, 12-dimethylbenz(a)anthracene (DMBA) and benzopyrene, and is also found to decrease levels of urinary mutagens. It also possesses anti-inflammatory and antioxidant properties, thus the cancer protective effects of *Curcuma longa* and its constituents are partially due to direct antioxidant effect. Studies have revealed that *Curcuma longa* inhibits production of nitrosamine that enhances natural antioxidant functions of the

body. *Curcuma longa* increases levels of glutathione and other non-protein sulphahydryl and acts directly on several enzymes. Curcumin has been used to treat squamous cell carcinoma of the skin and the ulcerating oral cancer. The plant also prevents malignant transformation of leukoplakia. The plant has been shown to suppress the development of stomach, breast, lung, and skin tumours (Kikuzaki and Nakatani, 1993). Polyphenol diversity, availability and multiple pharmacological properties justifies the enthusiasm of researchers to investigate this group of compounds.

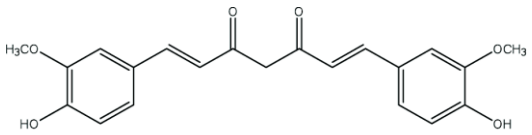
4.3. Plant Terpenoids

Terpenoids are extraordinarily diverse plant constituents (Figure 15) that originate through the condensation of the universal phosphorylated derivative of hemiterpene, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) giving geranyl pyrophosphate (GPP). In higher plants, IPP is derived from the classic mevalonic acid pathway in the cytosol but from the methylerythritol phosphate pathway in plastids. It is generally accepted that the cytosolic pool of IPP serves as a precursor of sesquiterpenes, diterpenes, triterpenes, sterols and polyterpenes whereas the plastid pool of IPP provides the precursors of terpenoids. Sesquiterpenes are a group of C-15 compounds resulting from the assembly of three isoprenoid units. They are found in essential oils of plants with a large structural variety having several aliphatic and cyclic types (mono, bi, tri and tetracyclic systems). They possess a variety of biological activity including antimalarial, antibacterial, antiviral, anti-inflammatory and anti-tumour activities. Mono- and sesquiterpenes are the chief constituents of the essential oils while the other higher terpenoids are constituents of balsams, resins, waxes, and rubber.

The main African plant families containing sesquiterpenes includes Asteracea, Apiaceae, Burseraceae, Compositae, Cyperacea, Graminae, Guttiferae, Labiatae, Piperaceae and Zingiberaceae. An excellent review of West African medicinal plants with anti-cancer properties was described by Sawadogo et al. (2012). Several papers were published on the anti-cancer properties of sesquiterpenes (Figure 15) that revealed their potential mechanisms of action (Robinson et al., 2008, Rodrigo et al. 2010 and Dall'Acqua et al. 2011). The investigation of isodihydrocostunolide (17), a sesquiterpene isolated from *Saussurea lappa* Clarke (Compositae), conducted by Robinson et al., (2008) showed the involvement of the exo-methylene group of the sesquiterpene chemical structure in cancer cell toxicity. Also, two sesquiterpenes from *Artmessia douglasiana* namely dehydroleucodine (18) and dehydroparashin-B were found to selectively inhibit migration and proliferation of melanoma cells. Another sesquiterpene, curcuphenol isolated from *Baccharis genistelloides*, demonstrated an inhibition of DNA replication and induction of apoptosis through the stimulation of caspase-3 activity (Robinson et al., 2008). Dall'Acqua et al. (2011) reported the antiproliferative and proapoptotic properties of sixteen isolated sesquiterpenes. All of them are cytotoxic at least against one of the tested cancer cell lines highlighting the interest of sesquiterpenes in cancer therapy.

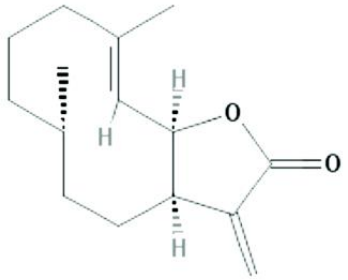
Diterpenes are C-20 phytochemicals derived from geranyl geranyl pyrophosphate. They are characterized by fascinating variations in their skeletons leading to several series of molecules with diverse biological properties. The chemical structures responsible for the pharmacological activity are often unsaturated α- and β-ketones, phenols groups and carbon–

carbon double bonds. They have chemopreventive and chemotherapeutic anti-cancer properties. Chemopreventive diterpenes may inhibit the initiation, promotion or progression stages of the carcinogenic process. Two diterpenes from coffee (cafestol and kahweol) were shown to prevent the DNA-binding of carcinogen agents such as benzo(a)pyrene and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine implicated in the initiation of breast, prostate, cancer of lymphatic system and colon cancers. This anticarcinogenic effect acts through several key mechanisms such as the induction of phase II enzymes (GST, UDP-GT, NAD(P)H) involved in carcinogen detoxification, reduction in the expression of phase I enzyme (P450CYP3A2, P450CYP2C11) of carcinogen activation or specific inhibition of P450 enzymatic activity and stimulation of intracellular antioxidant mechanisms by the increase of GSH intracellular concentration; as it is well known that GSH is a major antioxidant which plays a crucial role in the detoxification of activated xenobiotics (Cavin et al., 2001; Cacin et al., 2002; McMahon et al., 2001).

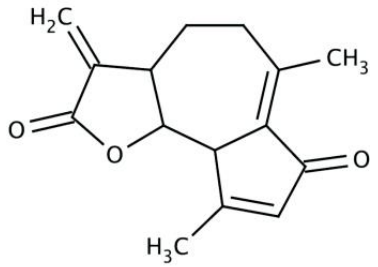


Curcumin (16)

Figure 14. Structure of curcumin from *Curcuma longa* and other *Curcuma* species.

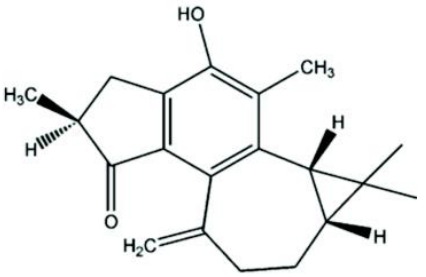


Isodihydrocostunolide (17)

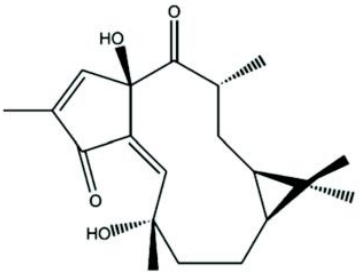


Dehydroleucodine (18)

Figure 15. Some sesquiterpenes with anti-cancer properties.



Jathropolone (19)



4-Z-Jatrogossidentadion (20)

Figure 16. Some diterpenes with anti-cancer properties.

The chemotherapeutic effect of diterpenes is due to their cytotoxicity enhanced by their protonophoric activity. Moreover their lipophilicity allows them to target biological membranes. Several investigations revealed the key mechanisms of diterpene cytotoxicity to be DNA damage by covalent bindings, topoisomerase I and II inhibitory activities and mitochondria dependent apoptosis (Fronza, et al. 2012). The non-specific targeting of these molecules is causing serious side effects and is a major hurdle for their development as anti-cancer drugs.

Two jatrophane diterpenes (A and B) isolated from *Euphorbia dendroides* were found to decrease vascular endothelial growth factor (VEGF) secretion, an anti-angiogenic effect. It is known that the up-regulation of VEGF expression in tumours is associated with poor prognosis in patients and potentially development of metastasis, the major cause of cancer-related death (Pesic et al., 2011; Weng and Yen, 2012).

Some diterpenes (Figure 16) showed a significant inhibitory effect against the function of ATP binding cassette (ABC) protein commonly called multidrug resistance (MDR), that is responsible for much cancer chemotherapy failure. The most known is the transporter P-glycoprotein (P-gp) that is overexpressed in many cancer cells enhancing the efflux mechanism of anti-cancer drugs with the consequence of cell survival. The investigation on anti- MDR effect of six diterpenes isolated from *Euphorbia lathyris* (chemical structure similar to that of diterpenes (structures 19 and 20; Figure 16) isolated from *Jathropa curcas;* (Figure 19) showed that the activity is dependant on the position of the carbon–carbon double bound in the different skeletons and the group containing nitrogen is benefit to the inhibition of P-gp (Jiao et al., 2009). The studies of Pesic et al. showed that the jatrophane diterpenes

(euphodendrophane A and B) from *E. dendroides* have an inhibitory effect against P-gp with a significant difference between the two diterpenes due to the presence of isobutyl group in the skeleton of euphodendrophane B that is favorable to the inhibitory ability of this molecule (Pesic et al., 2011).

Triterpenes (Figure 17) are C-30 compounds derived from squalene, a triterpene. Many triterpenes occur free, but others occur as glycosides, namely saponins. They have diverse structures with numerous biological effects, both the quassinoid and limonoids type tritepenes have reported anti-cancer properties in several studies. *Balanitis aegytica* (Figure 20) is found all over Africa and contains potent cytotoxic triterpenes known as balanitins (structures 21 and 22). Also Lin et al. (2003) reported the anti-cancer property of *Ganoderma lucidum* triterpenes, this is a polyporous mushroom found all over Africa and Asia. The tritepenes exhibited a significant inhibition of hepatoma cell growth through down regulation of protein kinase C (PKC) activity, activation of c-Jun N-terminal kinases (JNK) and p38 mitogen-activated protein kinases (p38 MAP kinases) and G2- phase cell cycle arrest.

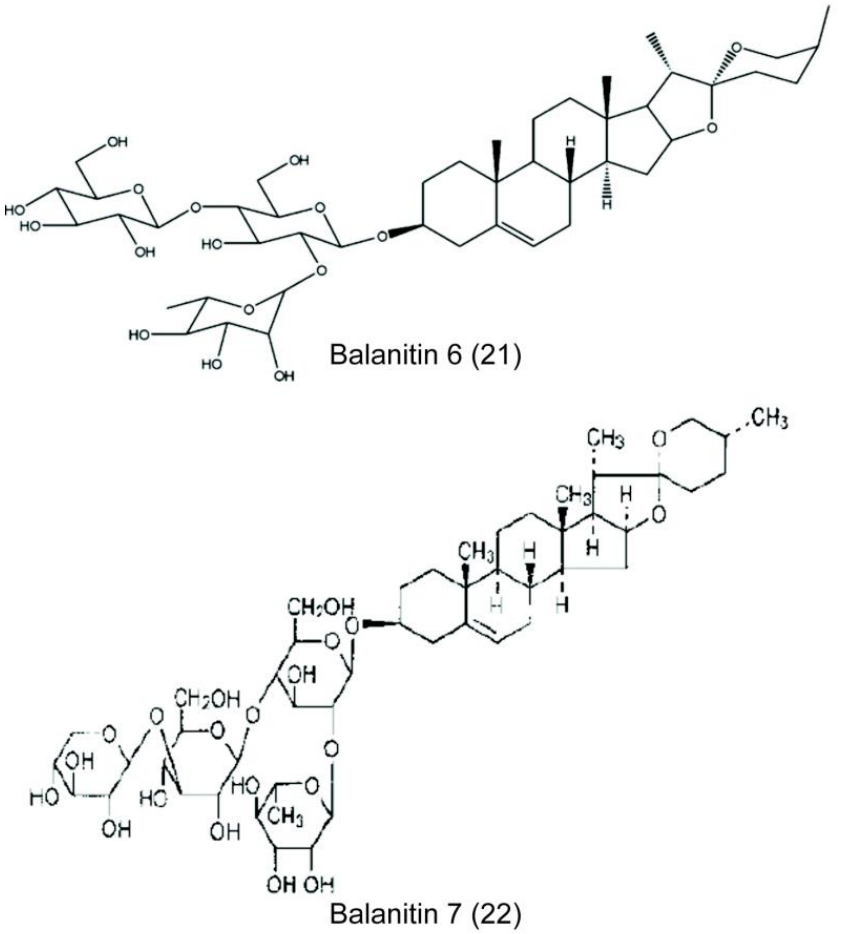
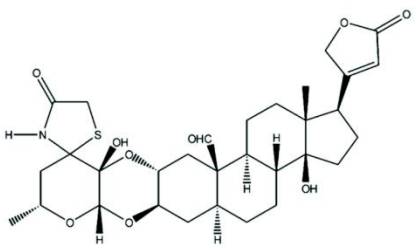
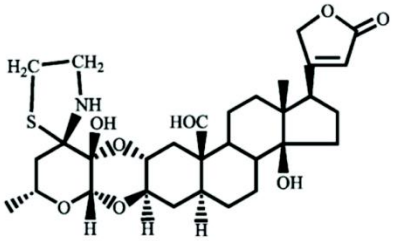


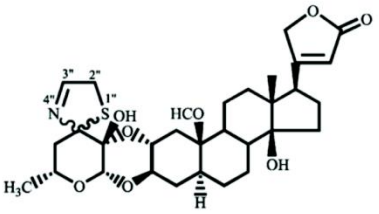
Figure 17. Some triterpenes with anti-cancer properties.



2”-oxovoruscharin (23)



Voruscharin (24)



Uscharin (25)

Figure 18. Some steroids with anti-cancer properties.

Figure 19. *Jathropa curcas*.

Steroids (Figure 18) are derivatives of triterpenes with 26 or more carbon atoms that have undergone a characteristic type of rearrangement. Steroids isolated from African plants are the poisonous cardenolides namely 2”-oxovoruscharin and uscharin both isolated from roots of *Calotropis procera* (Figures 18 & 21; structures 23, 24, 25). The anti-cancer activity of steroids is due to the induction of apoptosis through induction of variation in intracellular levels of Na+, K+, Ca^{2+} and H+, inhibition of NF-kB pathway and inhibition of glycolysis (Sreenivasan et al., 2003). In these cardenolides, the presence of free hydroxyl in the steroid

skeleton is necessary for the antiproliferative effect and inhibition of the sodium pump (Van Quaquebeke et al., 2005).

Figure 20. *Balanitis aegypitaca*.

Figure 21. A young *Calotropis procera* in its natural environment.

Plant Lignans

Lignans are naturally occurring compounds found in plants. A powerful anti-cancer lignan, Podophyllotoxin is used in the clinical treatment of small cell cancers. It was isolated from *Podophyllum* species (*Podophyllum hexandrum* and *P. peltatum*). The yield of the podophyllotoxin is low, synthesis is not feasible and the commercial source of the plant grows in an inhospitable region (the Himalayas). Thus alternative sources of lignans are being sourced (Arroo, et al., 2002).

Figure 22. *Boswellia dalzielli* growing in the Medicinal garden, Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

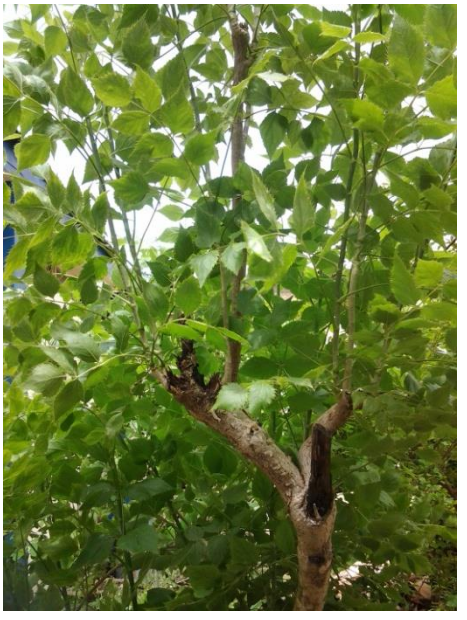


Figure 23. *Steganotaenia araliacea* growing in the Medicinal Garden Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

Several African plants families contain bioactive lignans these include Burseracea e.g. *Boswellia dalzielli* (Figure 22), *Commiphora Africana*; and Apiacea e.g. *Steganataenia araliaceae* (Figure 23), which yields anti-cancer steganacin lactones. Lignans acts in several ways as anti-cancer compounds. They act by blocking powerful growth factor receptors like

epidermal growth factor (*EGF*), Her2, Insulin like growth factor-1, and vascular endothelial growth factor (*VEGF*), the hormone responsible for stimulating blood vessels into tumours (Thompson et al. 2005). Lignans are incredibly effective at blocking estrogen II receptors at very low doses.

CONCLUSION

Natural product research has been developed specifically for the isolation of constituents with a particular biological activity. The identification and purification of bioactive constituents from plant extracts can be a tedious and expensive operation. In an attempt to cut down on the cost of drug discovery and to increase the probability of identifying useful compounds, research in natural products links drug development programs with traditional medicine/ethno-medicine. Traditional medicine has a long history and is still the major source of medicine in Africa and other developing countries.

Scientific evaluation of traditional medicine as a means to establish its efficiency and safety have led to the discovery of novel chemicals and novel drugs with interesting chemical structures that can serve as lead compounds/templates for the design of new drugs.

Many anti-cancer agents derived from plants have been developed through assessing cytotoxicity in cell lines and cell viability assays to provide important preliminary data to help select plant extracts with potential anti-cancer properties. The African tropical climate, a continent rich with traditional cultures, folklore and history has the potential of providing novel and potent drugs. Traditional treatments of diseases may be devoid of social and cultural barriers that affect patient's compliance (Holmstedt and Bruhn, 1983), which is an important component in the treatment of chronic diseases, particularly cancer.

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