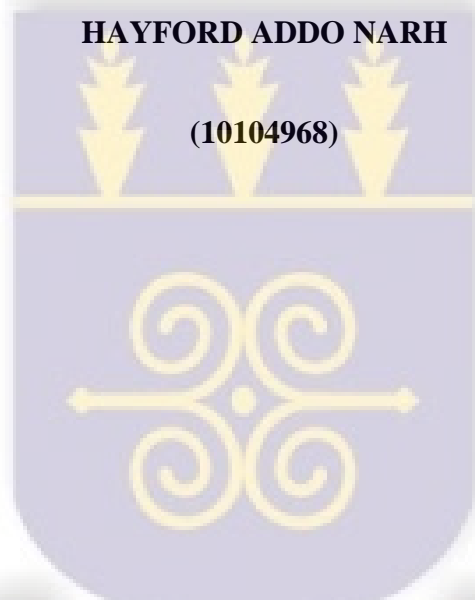


**ANTI-BREAST CANCER PROPERTIES OF SOME MEDICINAL PLANTS IN
GHANA**

BY



**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A
MASTER OF PHILOSOPHY DEGREE IN MEDICAL BIOCHEMISTRY**

JULY, 2015

DECLARATION

I, HAYFORD ADDO NARH, of the Department of Medical Biochemistry of the University of Ghana Medical School, do hereby declare that, with the exception of references duly acknowledged, this research work herein described was duly carried out by me and the results obtained are the true reflection of the work undertaken under the supervision of Dr. Bartholomew Dzudzor of the Department of Medical Biochemistry of the University of Ghana and Dr. (Mrs) Regina Appiah-Opong of the Department of Clinical Pathology of Noguchi Memorial Institute for Medical Research (NMIMR) also of the University of Ghana.

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ABSTRACT

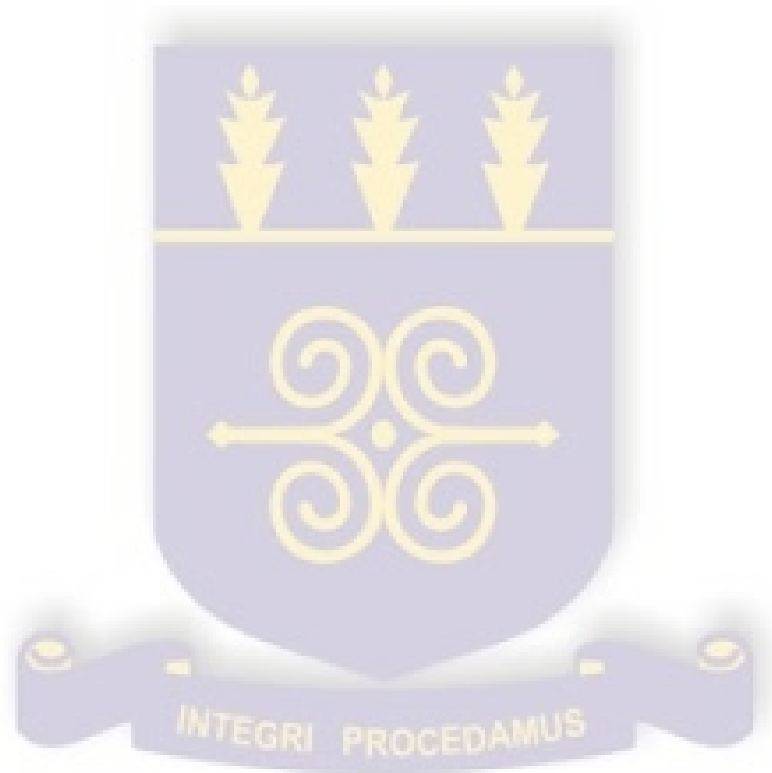
Cancer remains a killer disease worldwide and research into various anticancer agents must continue unabated to assuage the devastating effects of the disease. Medicinal plants have been utilised since antiquity to cure various ailments and plant species continue to serve as sources of prototypes for drug discovery. The study sought to screen Ghanaian traditional medicinal plants for their antiproliferative effect on MCF-7 breast cancer cell line and determine the mechanism of induction of cytotoxicity. A total of 40 fractions comprising 14 fractions of ethyl acetate, 14 fractions of dichloromethane and 12 fractions of petroleum ether from 16 plant species were examined for their cytotoxic activity on MCF-7 breast cancer cell line using the MTT assay. Aqueous and 50% hydroethanolic extracts of *M. oleifera* were also examined analogously to evaluate their antioxidant activities. The results showed a dose dependent activity of the fractions on the breast cancer cell line with varying IC₅₀ values. *F. exasperata*, *P. pinnata*, *H. indicum*, *P. macrocarpus*, *A. melegueta*, *M. charantia*, *A. conyzoides*, *S. rotundifolius* and *A. cordifolia*. were species that demonstrated the strongest cytotoxicity against the breast cancer cell line with IC₅₀ values ranging from 3.8 µg/mL to 33.7 µg/mL with selectivity indices for most of the bioactive fractions > 2. The selectivity indices for the most bioactive fractions ranged from 1.5 to 10.5. *O. gratissimum*, *F. aestuans*, and *A. zygia* demonstrated moderate cytotoxicity with IC₅₀ values of ranging from 41.9 µg/mL to 51.2 µg/mL. Aqueous and hydroethanolic extracts of *M. oleifera* showed no significant antiproliferative activities towards the MCF-7 breast cancer cells. The antioxidant activities of the aqueous and hydroethanolic extracts were 3.0 mg/mL and 3.1 mg/mL respectively indicating a good free radical scavenging property. The total phenolic content of the extracts were 177 mgGAE/g of extract and 168 mgGAE/g of extract for the aqueous and 50%

hydroethanolic extracts, respectively. The selectivity indices of some of the bioactive fractions identified them as excellent candidates for further research and possible utilisation as drug leads. Some of the results also give empirical evidence as to the anecdotal use of the plants in folk medicine. Examination of the petroleum ether fraction of *Aframomum melegueta* demonstrated that apoptosis is induced as a mechanism of cytotoxicity.



DEDICATION

This research work is foremost dedicated to the Almighty God for His grace that has been sufficient for me throughout my life. This thesis is also dedicated to my resourceful supervisors for their enormous contribution and mentoring in this research work. Finally I dedicate this work to my dear family, wife, Linda for their love, care, support and understanding over the years as well as my two lovely kids Henry and Mildred.



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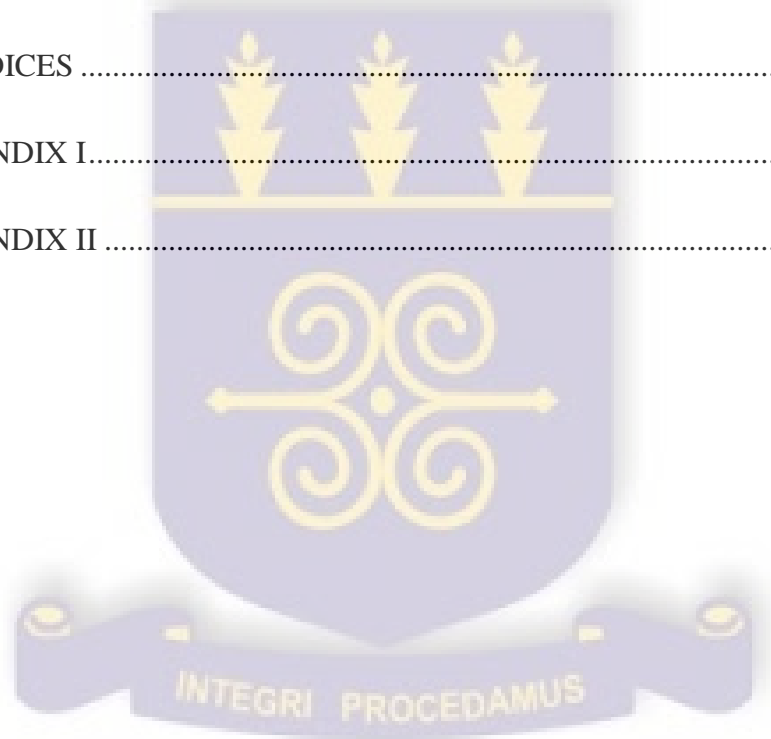
Last but not the least, I wish to thank my family and friends for their continuous love and support throughout all these years. To all individuals who were not mentioned by name, I would like to express my sincere gratitude for your support.

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LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
μ	Micro
±	Plus or minus
>	Greater than
<	Less than
H	Hour
min	Minute
³ H	Tritium
ABC	ATP Binding Cassette
AI	Aromatase Inhibitors
AO	Acridine orange
ATM	Ataxia Talangiectasia Mutated
ATP	Adenosine triphosphate
ATR	Ataxia Talangiectasia and Rad-3 related
BHT	Butylated hydroxytoluene
Cdk	Cyclin Dependent Kinase
Cip/Kip	Cyclin Dependent Kinase Inhibitors
CNS	Central Nervous System
CT	C-Terminal
DAPI	Diaminophenylindole
DMEM	Dulbeccos Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleid acid

DPPH	2,2 – diphenyl-1-picrylhydrazyl
EB	Ethidium bromide
EC ₅₀	Effective concentration at 50% activity
EGF	Epidermal Growth Factor
ER	Estrogen Receptor
ER+	Estrogen Receptor positive
FBS	Foetal Bovine Serum
FITC	Fluorescein isothiocyanate
G1, G2	Gap1, Gap2
GSH	Glutathione
GST	Glutathione transferase
HER2, ErbB2	Human epidermal growth factor receptor-2
IC ₅₀	Inhibitory coefficient at 50% activity
M	Mitosis
MAP	Mitogen Activated Protein
MCF-7	Michigan Cancer Foundation-7
mgGAE	Milligram gallic acid equivalent
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMIMR	Noguchi Memorial Institute for Medical Research
NT	N-Terminal
ORAC	Oxygen Radical Absorbance Capacity
ORID	Office for Research, Innovation and Development
PI	Propidium Iodide
pRB	Retinoblastoma protein
PS	Phosphatidylserine

PSG	Penicillin Streptomycin L-Glutamine
RTK	Receptor Tyrosine Kinase
S	Synthesis
SH2	Src homology 2
TGF α	Transforming Growth Factor alpha
TNFR1	Tumour Necrosis Factor Receptor 1
WHO	World Health Organization
WST	Water Soluble Tetrazolium

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Cancer is a killer disease worldwide. According to a 2012 World Health Organization report, it is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviours (Jemal *et al.*, 2011). It is estimated that 14.1 million new cases and 8.2 million cancer deaths occurred in 2012 worldwide with lung and breast cancers being the most frequently diagnosed cancers and the leading causes of death among both men and women (Torre *et al.*, 2015).

A growing body of evidence shows that breast cancer is the highest cause of death among women with cancer worldwide (Gonzalez-Angulo *et al.*, 2007). According to a WHO report (WHO, 2008) it is estimated that roughly 10% of new cases of breast cancers occur in Ghana each year. The disease is of great public health concern, and the needed actions must be taken to combat it. Chemotherapy is among several strategies such as mastectomy, radiotherapy, immunotherapy used to manage breast cancer. However, some challenges are being encountered in the use of current chemotherapeutic agents. These include cancer cells resistant to drugs due to various factors; the expression of energy – dependent transporters, that detect and eject anticancer drugs from cells, insensitivity of the cancer cells to drug-induced apoptosis and induction of drug detoxifying mechanisms that rapidly decreases drug levels in blood among others (Gonzalez-Angulo *et al.*, 2007; Ferlay *et al.*, 2002; Pal & Mittal, 2004). These may

either singly or in combination result in reduced drug levels in circulation and render the anti-breast cancer drug ineffective.

The cost of anti-breast cancer drugs also has direct bearing on the success of the chemotherapy treatment. Wiredu and Armah (2006) reported that drug accessibility and affordability is a major challenge to patients living in third world countries like Ghana and has a direct impact on the success or failure of chemotherapy. For example, patients' inability to purchase all their prescribed drugs due to financial challenges often leads to non-adherence to doctors instructions. Consumption of lower doses of the drugs renders the drug ineffective against the cancer cells and may lead to resistance. Even in some instances where patients can afford such drugs, they may not be accessible to patients due to transportation challenges (Guidry *et al.*, 1997).

1.2 Problem Statement

Breast cancer is a killer disease among women worldwide. Although much research has been conducted into various anticancer agents and many such as tamoxifen, vincristine, vinblastine, taxol and other anticancer agents derived from natural sources are being employed to treat various cancers (Balunas & Kinghorn, 2005), there have been reported cases of drug resistance with some of these drugs. The increasing rate of drug resistance among breast cancer patients on chemotherapy is a worrying trend that must be tackled head on. Available data indicates that approximately 30% of early stage breast cancer cases recur after treatment with chemotherapy (Gonzalez-Angulo *et al.*, 2007), with the rate of recurrence of early stage breast cancer in patients on treatment being equivalent to the rate of incidence of the disease worldwide (Anderson *et al.*, 2005).

Drug transporters such as ATP-binding cassette (ABC) amongst others have been implicated in the induction of multiple drug resistance in patients (Shukla *et al.*, 2011).

Additionally, Glutathione S-transferases (GSTs) have been implicated in the development of resistance toward chemotherapeutic agents (Motoyama *et al.*, 2002). They induce resistance via direct detoxification as well as acting as inhibitors of the mitogen activated protein (MAP) kinase pathway (Motoyama *et al.*, 2002).

Since cancers are becoming more resistant to current chemotherapeutic agents, the rate of survival of patients is reduced as well as prognosis for the patients (Vinogradov & Wei, 2013). As a result of the menace of drug resistance to the anticancer agents, researchers are now challenged to conduct new research into various drug leads and compounds to identify alternative therapies. Several Traditional Plant Medicines are used to treat cancers in Ghana. There is anecdotal evidence that these medicines are useful, however, empirical evidence is lacking. Therefore, in this research anticancer plant medicines were investigated for scientific evidence of their anti-breast cancer activities. The most bioactive plant medicines were also investigated for their apoptotic mechanism of action.

1.3 Rationale/Justification

Breast cancer is a major killer among women with cancer related cases and treatment is confronted with challenges such as drug resistance and cost. Thus there is the need to find alternative medicines which are more efficacious and cheaper. New compounds with anticancer activity can lead to the formulation of new anticancer drugs that will result in better treatment of cancer patients. Ghanaian medicinal plants that Traditional Medical Practitioners claim to use to cure/manage breast cancer were investigated to establish their anti-breast cancer activities. There was the need to carry out this project as Ghana has great biodiversity of plants and therefore the potential of unearthing lead compounds for development as anti-breast cancer agents exists. Research has shown that secondary plant metabolites and their derivatives form about 20% of drugs used in

various chemotherapeutic agents and they continue to provide the lead compounds in pharmacognosy (Balunas & Kinghorn, 2005; Kim *et al.*, 2014). It is worth noting that drugs such as vincristine, vinblastine, taxol and camptothecins used in modern day cancer chemotherapy of various cancers were derived from the plants *Catharanthus roseus*, *Taxus brevifolia* and *Camptotheca acuminata*, respectively (Balunas & Kinghorn, 2005).

1.4 Objectives of the Study

The aim of the study was to screen Ghanaian Traditional Medicinal plants for anti-breast cancer activities. The specific objectives pursued were:

1. To screen petroleum ether, dichloromethane and ethyl acetate fractions of Ghanaian traditional medicinal plants for cytotoxicity towards a breast cancer (MCF-7) cell line.
2. To prepare aqueous and 50% hydroethanolic leaf extracts of *Moringa oleifera* and test them for cytotoxicity towards MCF-7 cell line.
3. To estimate the antioxidant activity and total phenolic content of the *Moringa oleifera* extracts.
4. To investigate apoptosis as a molecular mechanism by which the most bioactive fraction induced anti-breast cancer activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Disease: Breast Cancer

Cancer is a complex disease in which cells in a specific tissue are no longer fully responsive to the signals within the tissue that regulate cellular differentiation, survival, proliferation and death. As a result, these cells accumulate within the tissue, causing local damage and inflammation (Tennant *et al.*, 2009). There are over 200 different types of cancer. Research has shown that breast cancer is the most common cause of cancer-related death among women worldwide, with case fatality rates highest in low-resource countries (Anderson *et al.*, 2005). Each year, breast cancer is newly diagnosed in more than 1.1 million women, and these cases represent more than 10% of all new cancer cases. The disease accounts for more than 1.6% of all female deaths worldwide (Anderson *et al.*, 2005; Ferlay *et al.*, 2002) with more than 410,000 deaths. The disease is an urgent public health problem in high-resource regions and is becoming an increasingly urgent problem in low resource regions, where incidence rates have been increasing by up to 5% per year (Stewart & Kleihues, 2003; Ferlay *et al.*, 2002). Despite significant scientific advances in its management, most of the world faces resource constraints that limit the capacity to improve early detection, diagnosis, and treatment of the disease (Anderson *et al.*, 2005). In Ghana although there is a dearth of a national published data on the disease, a study on 447 women in 2005 at Accra indicated a risk of breast cancer among women presenting symptoms of pain in the breast as 16% (Clegg-Lampety *et al.*, 2007). This rate is quite alarming and much attention is needed to combat the disease in its entirety. The age group at risk is 40 – 49 years with majority of the patients reporting late to hospital with advanced stages of the disease (Clegg-Lampety & Hodasi, 2007). In a recent study conducted by Brakohiapa and others (2013)

on mammography reports of patients reporting with breast lesions, the age group that have been found to be susceptible to breast cancer is 40 – 50 years. The breast cancer type that is most prevalent in Ghana is triple negative breast cancer followed by estrogen receptor-positive and progesterone receptor positive with human epidermal growth factor receptor-2 being the least expressed (Amankwaa-Frimpong *et al.*, 2014).

Low-resource countries have generally not identified cancer as a priority health care issue because infectious diseases are the predominant public health threat (Stewart & Kleihues, 2003). Nonetheless, resources are spent on breast cancer treatment when patients seek medical care for what is typically advanced-stage disease (Pal & Mittal, 2004). Breast cancer is becoming an increasing public health problem as the control of communicable diseases improves and life expectancy increases (Ferlay *et al.*, 2002). However, obstacles to improving breast cancer care arise from, including deficits in public knowledge and awareness, social and cultural barriers, challenges in organizing health care, and insufficient resources (Anderson *et al.*, 2005). In addition to advanced age, a few dozens of other breast cancer predisposing factors have been identified (Singletary, 2003); however, all these diverse risks can be assigned to either of two major categories: excessive exposure to estrogens and deficiency in maintenance of genomic integrity.

2.1.1 Mechanism of Breast Cancer Development and Drug Targets

Research shows a high endogenous level of estrogens associated with breast cancer and administration of estrogen antagonist, tamoxifen proved efficacious against 50% of breast cancers (Leopold *et al.*, 2002), which express estrogen receptor (ER). According to Wiredu and Armah (2006), during the malignant transformation process, breast epithelial cells accumulate a high number of somatic genetic events (mainly gross chromosomal alterations and methylation abnormalities). These DNA alterations

activate oncogenes and inactivate tumour suppressor genes, which eventually results in the manifestation of 'The hallmarks of cancer'. "Molecular determinants of the most essential tumour properties might serve as potential therapeutic targets" (Singletary, 2003).

Several therapeutic agents ranging from endocrine, chemotherapy, mastectomy, radiation, immunotherapy, gene therapy and many other therapies have been utilised in the management of breast cancers and cancers in general. The type of therapy used depends on the breast cancer type and the stage of progression of the disease as well as the severity (Evgeny & Kaido, 2004). For example, aromatase inhibitors (AIs) are considered standard first-line endocrine therapy for estrogen receptor-positive (ER+) metastatic breast cancer in postmenopausal women (Fan *et al.*, 2014). Although treatment with the AIs leads to a significant decline in mortality from breast cancer, disease resistance to AIs commonly develop during therapy (Gluck, 2014). Several chemotherapy agents have also been developed to manage breast cancer that are due to overexpression and amplification of human epidermal growth factor receptor-2 (HER2, ErbB2) (Pauletti *et al.*, 2000). In Ghana, similar findings regarding the breast cancer type and the therapy have been made by Amankwaa-Frimpong and others (2014) where triple negative breast cancer patients showed good prognosis with second line standard treatment but poorer response to the first line treatment. However, patients with ER+ breast cancer who do not show good response to first line treatment are also resistant to second line treatment.

Although drugs ranging from chemotherapeutics, immunotherapeutics as well as conjugates of the two therapies have been discovered over the years to manage metastatic breast cancer (Barok *et al.*, 2014), the problem of resistance still persists. The mechanisms employed by the resistant metastatic cells include masking of binding

epitopes of HER2 (Nagy *et al.*, 2005; Palyi-Krekk *et al.*, 2007), expression of p95HER2 (Arribas *et al.*, 2011), activation of insulin-like growth factor I receptor pathway (Lu *et al.*, 2001), defects in the phosphatase and tensin homology-phosphatidylinositol 3-kinase/protein kinase B pathway (Nagata *et al.*, 2004), overexpression of cyclin E (Scaltriti *et al.*, 2011) and autocrine production of epidermal growth factor-related ligands (Motoyama *et al.*, 2002) amongst others. In addition, glutathione transferase (formerly glutathione S-transferases) has been implicated as responsible for drug induced resistance by cancerous cells. GSTs serve two distinct roles in the development of drug resistance via direct detoxification as well as acting as an inhibitor of the MAP kinase pathway. The link between GSTs and the MAP kinase pathway provides a rationale as to why in many cases the drugs used to select for resistance are neither subject to conjugation with GSH, nor substrates for GSTs. GSTs have emerged as a promising therapeutic target because specific isozymes are overexpressed in a wide variety of tumors and may play a role in the etiology of other diseases, including neurodegenerative diseases, multiple sclerosis, and asthma (Motoyama *et al.*, 2002).

2.2 Cell Cycle Regulation as Targets for Anticancer Therapeutics

Advancement in knowledge of the cell cycle regulatory proteins and molecular breaks has provided new opportunities to understand the mechanisms of action of various anticancer agents. This has led to the development of management strategies for specific alterations in any tumour. The cell cycle consists of initiation after a resting stage or restriction point known as gap 1 (G1) and gap 2 (G2) alternating between DNA synthesis (S) and mitosis (M) respectively (Hunter, 2000). Certain cyclin dependent kinases, cyclin D-Cdk4/6 and cyclin E/A-Cdk2 are needed for the cells to progress through the G1–S transition (Hunter, 2000). The synthesis (S) phase is activated by the

action of the receptors Cdk4/6 and Cdk2 on the cell at the G1-S transition. The activity of Cdk2 is maintained through the S phase until a decline in its level signals exit from the S phase. Finally, Cdk1 becomes active in G2 and its activity persists through mitosis (Hunter, 2000; Malumbres & Barbacid, 2005) as shown in Figure 2-1.

Cyclin dependent kinase inhibitors- Cip/Kip and INK4 are involved in the regulation of cyclin-Cdk kinases (Kohn, 1999). The three members of the Cip/Kip family: p21^{Cip1}, p27^{Kip1}, and p57^{Kip2} upon associating with cyclin E/A-Cdk2 and cyclin B-Cdk1 complexes, inactivate them and prevent the cells from entering into the S phase and binding of p27^{KIP} to cyclin A-Cdk2 inhibits passage through S phase. The protein p21^{Kip1} can bind and inhibit both cyclin E/A-Cdk2 and cyclin B-Cdk1. Conversely, interaction of Cip/Kip family with cyclin D-Cdk4 or D-Cdk6 complexes appears to have a stimulatory effect (Blain *et al.*, 1997; Cheng *et al.*, 1999). In addition, binding of Cip/Kip proteins to cyclin D-Cdk4/6 kinases prevents their association with cyclin E/A-Cdk2 resulting in facilitation of the kinases in completing the G1 phase of the cell cycle and subsequent initiation of DNA synthesis (Sherr & Roberts, 1999). It has been shown that the INK4 family of proteins inhibit catalytic activity of Cdk4/6 kinases by preventing binding to their regulatory cyclin D subunits (Sherr & Roberts, 1999).

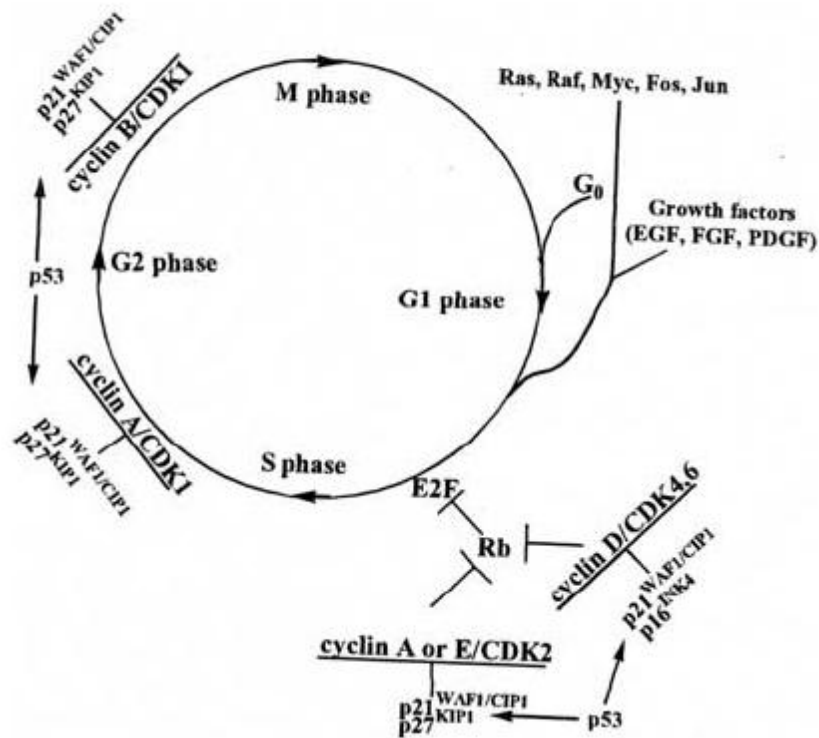


Figure 2.1 Key players in the cell cycle regulation. The G1, S, G2, and M phases of the cell cycle are regulated by various proteins and growth factors.

(Source: Xu *et al.*, 1999)

Research has demonstrated that the retinoblastoma tumour suppressor protein (pRb) acts as the substrate for cyclin D-dependent kinase and is involved in regulating G1 progression as well as being a key component of the molecular network controlling the restriction point. The pRb suppresses the transcriptional activity of various E2F family of proteins (Chen *et al.*, 1999; Flemington *et al.*, 1993). Cyclin D dependent Cdk's phosphorylate pRb in late G1 which disrupts the association of pRb, and E2F activation allows the coordinated expression of many genes that encode proteins necessary for S phase entry and progression (Kato *et al.*, 1993). Cyclins E and A are regulated by E2F which associate with Cdk2 to effect transition from the G1 to S phase (Girard *et al.*, 1991).

Apart from the regulators of cell cycle progression, there are molecular checkpoints or brakes that monitor the cell regulatory pathways and DNA structure before the cells enter the next phase of the cell cycle (Malumbres & Barbacid, 2005). A tumour suppressor gene, p53 is frequently mutated in various cancers. The p53 gene product initiates cell cycle arrest at the G1 phase of the cycle when there is DNA damage (Levine, 1997). It has been shown that non-lethal DNA damage/cellular damage; hypoxia, aberrant oncogenic signalling elevate the levels of p53 due to enhanced stabilization (Shieh *et al.*, 1997). It causes apoptosis by inducing the transcription of pro-apoptotic genes such as *BAX*. Levels of p53 are negatively regulated by mouse double minute 2 homolog (MDM2) through a feedback loop (Hu *et al.*, 2012). The p53 gene is required for the G1 to S checkpoint and is a main component of the G2 to M checkpoint (Harper *et al.*, 1993). The Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad-3 related (ATR) are activated by mechanisms that sense double stranded DNA breaks and transmits signals to arrest the cell cycle after DNA damage. It acts through p53 in the G1 to S checkpoint. At the G2 to M checkpoint, it acts both through p53-dependent mechanisms and through the inactivation of CDC25 phosphatase, which disrupts the cyclin B-Cdk1 complex (Nilsson and Hoffmann, 2000). They are components of a network of genes that include *BRCA1* and *BRCA2*, which link DNA damage with cell-cycle arrest and apoptosis (Yoshida & Miki, 2004).

2.3 Growth Factor and Signal Transduction as Targets for Cancer Therapy

The erbB family of receptor tyrosine kinases (RTKs) comprises four distinct membrane glycoproteins consisting of epidermal growth factor (EGF) receptors erbB1, erbB2, erbB3 and erbB4 commonly referred to as HER1, HER2, HER3 and HER4 respectively. They are widely expressed in epithelial, mesenchymal and neuronal tissues (Leopold *et al.*, 2002). Structurally, they have an extracellular ligand-binding

domain, a single transmembrane region, an intracellular domain possessing protein tyrosine kinase activity, and a C-terminal tail that contains specific tyrosine containing sequences which upon phosphorylation, become binding sites for src homology region 2 (SH2) – containing signalling proteins (Khazaie *et al.*, 1993). Several ligands have been identified that interact with these receptors. The first group of ligands include EGF, transforming growth factor α (TGF- α), and amphiregulin (Goldman *et al.*, 1990; Wada *et al.*, 1999; Johnson *et al.*, 1993).

2.4 Other Targets for Cancer Therapeutics

The Src kinases included in the non-receptor tyrosine kinases subfamily is a protein tyrosine kinase pp60Src and represents a prototype of the well established superfamily of protein tyrosine kinases. The Src have significant structural homology to roughly ten proteins known as the Src family kinases namely Lck, Fyn, Tes, Trk, Blk, Fgr, Hck, Lyn and Frk. They are widely expressed in mammalian cells and mostly associated with breast cancers and multiple myeloma (Nam & Parang, 2003). Src mutations/over expression is reportedly correlated with many transformed phenotypes in cancer (Thomas & Brugge, 1997), hence can be used as a target to anticancer drug development. There has been research into the Src inhibitors as therapy using “combinatorial chemistry”, structure-based design and high throughput screening (Nam & Parang, 2003). Structural elucidations have shown Src kinases containing five components/domains: N-terminal (NT), Src homology 2 (SH2), Src homology 3 (SH3), kinase (catalytic, including NT and C-terminal (CT) lobes), and C-terminal non catalytic domain (Figure 2.2). They have conserved Ser/Thr and Tyr kinases that are responsible for the catalytic activity. The NT lobe is an important target for Src kinase inhibitors through ATP –competitive inhibitors. Additionally, the CT lobe, SH2, SH3

domains have been employed as targets for Src kinase inhibition (Dalgarno *et al*, 1997; Sawyer, 1998)

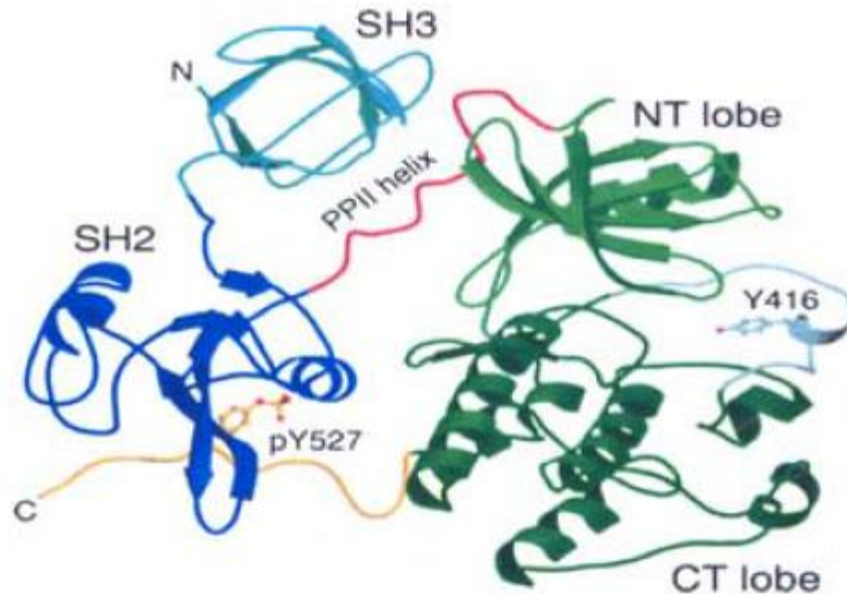


Figure 2.2 Structural representation of the c-Src protein tyrosine kinase in its inactive state (adapted from Xu *et al.*, 1999).

The Ras proteins function in normal and transformed cell growth (Nam & Parang, 2003). Studies on the protein structure, function and regulation of Ras proteins revealed that they act as intermediates in signal transduction pathways that mediate proliferative and other types of signals emanating from receptor tyrosine kinase (Boguski & McCormick, 1993). According to Nam and Parang (2003), mutated Ras proteins have been implicated in 30% of cancers and greater proportion of pancreatic and colon adenocarcinomas. Ras transduces extracellular signals provided by growth factors and cytokines by associating with the inner surface of the plasma membrane. The attachment is facilitated by several postranslational chemical modifications which involves farnesylation of cysteine residues, proteolytic cleavage of the AAX (A is any aliphatic amino acid, X is any amino acid) peptide by proteases and carboxymethyl

transferases (Casey, 1989; McCormick, 1993). Farnesyltransferases (FTases) catalyse the farnesylation step by recognizing the CAAX motif of Ras C –terminus and transferring a 15 carbon farnesyl isoprenoid from farnesyl diphosphate (FDP) to form thioether bond with the Ras cysteine (Omer *et al.*, 1997). FTase inhibitors are being utilized to target inactivation of Ras.

Telomerases have been implicated in cellular senescence (mortality stage 1, or M1), characterized by irreversible cell cycle arrest (Nam & Parang, 2003). They are specialized DNA–protein structures that cap the ends of linear chromosomes. Studies demonstrated that the telomere length decreased in normal diploid cells as they are grown in culture (Hiyama & Hiyama, 2003). In mammalian cells, it has been shown to decrease with the age of the cell (Nam & Parang, 2003). Additionally, it has been shown to progressively shorten with each cell division until a critical length is reached beyond which the cells cannot divide anymore. This implies that in order to prevent replicative cells from entering senescence, there's the need to prevent DNA telomere loss otherwise the cells will succumb to senescence eventually. DNA telomerase enzyme is activated in order to prevent telomere length shortening. Telomerase is a reverse transcriptase and maintains telomeric DNA at the end of chromosomes (Morin, 1989). Research has demonstrated an elevated level of telomerase in about 80%-85% of all cancers studied (Bears *et al.*, 2000; Neidle & Parkinson, 2002) except normal cells suggesting it might both serve as a diagnostic marker as well as target for the design of broad spectrum anticancer drugs.

2.5 Importance of Medicinal Plants in Drug Discovery

Due to the cumbersome nature of the drug discovery process, many pharmaceutical companies have scaled down or eliminated drug research into natural products (Butler, 2004). Various sources have been explored to acquire compounds for drug discovery

such as isolation from plants, invertebrates and other natural sources, synthetic chemistry, combinatorial chemistry and molecular modelling (Kell, 2013). Although pharmaceutical companies have recently developed interest in molecular modelling, combinatorial chemistry and other synthetic chemistry techniques, natural products of plant origin and invertebrates continue to serve as important sources of prototypes for new drugs and chemical entities (Newman & Cragg, 2007; Gullett *et al.*, 2010; Usami *et al.*, 2010). For example, the compound artemether (trade name artemotil) is a potent antimalarial drug derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* L. (Asteraceae) a plant used in traditional Chinese medicine (Ferreira *et al.*, 2010). The discovery of the anticancer drugs, vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae), introduced a new era of the use of plant material as a medication for treatment (Hostettmann *et al.*, 2000). They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine are used in combination with other cancer drugs, for the treatment of various kinds of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers (Hostettmann *et al.*, 2000). Vinflunine is a modification of vinblastine (Balunas & Kinghorn, 2005). Exatecan is an analog of camptothecin isolated from the Chinese ornamental tree, *Camptotheca acuminata* Decne (Nyssaceae) and is in the process of development toward as an anticancer agent (Balunas & Kinghorn, 2005). Earlier clinical trials involving camptothecin was dropped due to severe bladder toxicity. However, other derivatives of camptothecin including topotecan and irinotecan have been developed and employed for the treatment of ovarian and small cell lung cancers, and colon cancers, respectively (Balunas & Kinghorn, 2005).

Another important step in the drug discovery process has been the isolation of paclitaxel (Taxol) from the bark of the Pacific Yew, *Taxus brevifolia* Nutt. (*Taxaceae*) (Rao, 1993). Various parts of *Taxus brevifolia* and other *Taxus* species (e.g., *Taxus Canadensis* Marshall, *Taxus baccata* L.) have been utilised by several Native American tribes for the treatment of various diseases. *Taxus baccata* was however reported to be used in India as a medicine for the treatment of cancer (Newman & Cragg, 2007). Paclitaxel was initially introduced into the American market in the early 1990s and is known to be active against various cancer types, namely: ovarian cancer, advanced breast cancer, small and non-small cell lung cancer (Miele *et al.*, 2012; Rao, 1993).

2.5.1 Various Uses of Ghanaian Traditional Plants

Paullinia pinnata (Sapindaceae) is commonly called “sweet gum” and in the Akan dialect “toa-ntini”. The leaves of the plant have a strong free radical scavenging activity and the methanolic extract has been shown to demonstrate moderate reducing power *in vitro* (Jimoh *et al.*, 2007). The leaf juice is used in the treatment of sore throat and the infusions are used to treat fever (Abbiw, 1990). It has been reported to correct erectile dysfunction in men (Zamble *et al.*, 2006). The leaf extracts of the plant is highly antioxidative and it also induces a slight transcriptional activity of peroxisome proliferator activated receptor-alpha (Zamble *et al.*, 2006). *P. pinnata* is a rich source of polyphenols (Zamble *et al.*, 2006) and also contains the ceramide, paullinomide A, β -amyryn, steroids, the coumarinolignoid 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one, 5 α -poriferastane-3 β ,6 α -diol and l-quebrachitol (Dongo *et al.*, 2009). The triterpenoids lepeyl steryl ether, 3-oxo-11 α -hydroxyl-20(29) lupen and the lupeol-3-isovanilloyl ester paullinoyl (3-O-isovanilloyl-3R,5R,8R,9R,10R,13R,14S,17S,18R,19R-lup-20 (29)-en) are present in the plant (Lasisi *et al.*, 2015). Flavone glycosides such as diosmetin-7-O-(2'-O- β -d-

apiofuranosyl-6'-acetyl- β -d-glucopyranoside) and tricetin-4'-O-methyl-7-O-(2'-O- β -d-apiofuranosyl-6'-acetyl- β -d-glucopyranoside) have been isolated from the plant (Abourashed *et al.*, 1999). Fred-Jaiyesimi and Anthony (2011) reported larvicidal activities of leaf extracts and fractions of the plant.



Figure 2.3 Fruit and leaves of *Paullinia pinnata*

Moringa oleifera (Moringaceae) is commonly known as horse radish tree or drumstick tree (Ramachandran *et al.*, 1980) and in Hausa as “zogale” (Burkill, 1985). The leaves possess good nutritional values due to the high content of minerals, vitamins and amino acids. *M. oleifera* is native to the sub Himalayan regions of North West India and indigenous to many African countries, Arabia, South East Asia, South America and other countries (Anwar *et al.*, 2007). All the components of the plant have been found useful: the root, bark, gum from the bark, leaf, fruit (pods), flowers, seeds and seed oil (Morimitsu *et al.*, 2000; Anwar *et al.*, 2007). *M. oleifera* is used in ethnomedicine remedies for inflammation, infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatorenal disorders (Morimitsu *et al.*, 2000).

The flowers and roots are employed in folk medicine for treatment of tumors and the seeds for abdominal tumors as well as a purgative (Anwar *et al.*, 2007). Poultices of the leaves are applied on temples for headache and on sores (Anwar *et al.*, 2007). Antiproliferative activity has been reported against gram negative and gram positive pathogenic bacteria *in vitro* (Carceres *et al.*, 1992) and also shown to exert a “chemomodulatory” (Bharali *et al.*, 2003) effect against “skin papillomagenesis” in mice. Additionally, cytotoxic activity has been reported on human Hela cancer cell lines by leaf extracts of the plant (Jafarian *et al.*, 2014). Phytochemicals present include glucosinolates and isothiocyanates, (Morimitsu *et al.*, 2000) and alkaloids like Moringinine and Moringine from the bark. The flowers contain alkaloids like kaempferol, rhamnetin, isoquercetin and kaempferitin (Faizi *et al.*, 1994)



Figure 2.4 Matured leaves of *Moringa oleifera*.

Fluerya aestuans has the common name: “nettle weed” or “West Indian wood nettle” and “bhonho” in Akan (Abbiw, 1990). It belongs to the family Urticaceae and is native

to tropical Africa although widespread due to its introduction into western and eastern hemispheres. The medicinal uses of the plant in Africa include treatment for eye infections, swelling, wounds, burns, and gonorrhoea (Dokosi, 1998). In Ghana, the plant is used in preparing soup for women after childbirth (Glew *et al.*, 2009). Medicinal applications of *Fluerya aestuans* in Ghana include using the leaves of the plant to make a poultice to treat scorpion stings (Glew *et al.*, 2009). Also, the leaves are used to make soup with palm nuts that is consumed to treat stomach ailments and irregular menstruation (Dokosi, 1998). The chemical components in the leaves include mostly fat, fiber, carbohydrates, tannins and oxalate and low amount of proteins (Akpan & Udoh, 2004). In addition, the leaf was found to be rich in sodium, calcium, magnesium, iron, zinc, and copper. Cadmium, cobalt, manganese and lead were found in trace amounts.

Ocimum canum Sim (Lamiaceae) is of tropical African origin. In Ghana the leaves are used to enhance flavour in sauces. Research has shown that fasting blood glucose levels and body weight decreased significantly in diabetic and non-diabetic C57BL/KsJ mice, which were administered aqueous extract of *Ocimum canum* (Nyarko *et al.*, 2002). *In vitro*, the *O. canum* extract significantly enhanced insulin release from isolated rat pancreatic β -islet cells (Nyarko *et al.*, 2002). The dried leaves of *O. canum* contain the essential oil linalool (Weaver *et al.*, 1991; Ekundayo *et al.*, 1989) and gram positive bacteria are sensitive to the essential oil (Bassole *et al.*, 2005). The leaves consist mainly of 1, 8-cineole (60.1%) (Bassole *et al.*, 2005). The essential *O. canum* leaf oil of type I is characterized by a high percentage of monoterpene alcohols (total 91.9%), represented especially by linalool (44.9%) and geraniol (38.2%) (Ngassoum *et al.*, 2004). The plant is also rich in polyphenols (Oboh, 2008).

Solanum nigrum (Solanaceae) has the common name of “black nightshade” and known in Akan as “bachinia” (Abbiw, 1990). In Africa and Asia, it is used in the treatment of the following conditions: itching, burns, cuts, skin diseases, heart disease, fever and eye disease (Jansen, 2008). In the Volta Region of Ghana, a poultice made from the leaves is applied externally to treat Guinea worm infection (Glew *et al.*, 2009). The leaves are boiled in soup and may be used to treat jaundice, in addition to being used as a purgative and diuretic (Dokosi, 1998). In Nigerian folk medicine, it’s been used for the treatment of epilepsy (Sei-Jung *et al.*, 2006). The plant has been reported to have antipyretic and anticancer activity (Sei-Jung *et al.*, 2006). Extracts of the plant have been reported to show CNS depressant activity as well as a promising agent for the control of schistosomiasis (Wannang *et al.*, 2004; Ahmad *et al.*, 2005). *S. nigrum* contains several antioxidants, such as gallic acid, pyroglutamic acid (PCA), catechin, caffeic acid, epicatechin, rutin and narigenin, and possesses strong antioxidative activity *in vitro* (Chou *et al.*, 2008). Other researchers reported that a glycoprotein isolated from *S. nigrum* has a strong scavenging effect against reactive oxygen radicals, and growth inhibition effects against JA221 and XL1-Blue *Escherichia coli* cells. Moreover, it has been reported that glycoprotein has a cytotoxic effect against breast MCF-7 and human colorectal adenocarcinoma HT-29 cancer cells, even at low concentrations (Heo, 2004). Their protein content and mineral elements (magnesium being prominent) are considerable and *Solanum nigrum* oil is an important source of linoleic acid (Dhellit *et al.*, 2006). The treatment of rats both prior to or after stress with crude extract of *S. nigrum* leaves and its active constituents resulted in a significant increase in the antioxidant enzymes activities and GSH level along with a decrease in lipid peroxidation (Zaidi *et al.*, 2014). The leaves contain the highest concentration of

gentisic acid, luteolin, apigenin, kaempferol, and *m*-coumaric acid in the plant (Huang *et al.*, 2010).

Heliotropium indicum (Boraginaceae) is locally known as “Akonfem atiko” – Cock’s comb in Akan (Burkill, 1985). It is also known as “Indian Heliotrope” and is used for treating vomiting during amenorrhoea, baby thinness, ocular infections and high blood pressure (Togola *et al.*, 2005). According to literature, a decoction of the leaves is used in Sierra Leone for washing new-born babies (Kerharo & Adams, 1974). In Nigeria and Ghana, the sap is applied to gum boils or swellings, to clean ulcers and to heal eye infections. In Guinea the decoction of the whole plant is taken as a febrifuge (Kerharo & Adams, 1974). In Senegal the leaf powder is applied to dermatitis and especially to suppurating eczema and impetigo in children (Kerharo & Adams, 1974). The leaf decoction is used in Indonesia for thrush and in poultices for treatment of herpes and rheumatism. In Ivory Coast the dried leaf powder is taken up by the nose as decongestant in colds and sinusitis (Burkill, 1985). N-oxide of the alkaloid, indicine was isolated from *H. indicum* (Kugelman *et al.*, 1976). The compounds n-hexacosanol, sitosterol, stigmasterol, chalinasterol and campesterol are found in the plant (Andhiwal *et al.*, 1985). It has been reported that petroleum ether fraction of the leaf extract repels the oriental fruit fly (Areekul *et al.*, 1988).



Figure 2.5 Leaves and flowers of *Heliotropium indicum*

Alchonea cordifolia (Euphorbiaceae) is called “gyama” in Akan and employed in the treatment of gonorrhoea and toothaches and as fodder to feed ruminants (Abbiw, 1990). Other uses include treatment of cuts, burns, bruises, ulcers and piles. The leaf infusion or decoction is used to treat colds, bronchial problems, stomachache, dysmenorrhoea, fever and eye problems (George *et al.*, 2010). Ethno-botanical bioactive substances such as tannin, saponin, flavonoid, cardiac glycoside and anthraquinone have been isolated from the plant. The leaf extract isolate, isopentenyl guanidine is reported to exhibit antibacterial activity on *Staphylococcus aureus* and *Escherichia coli* (George *et al.*, 2010). The leaves also contain a range of hydroxybenzoic acids, gallic acids and its ethyl ester, gentisic acid, anthranilic acid, protocatechuric acid and elagic acid (George *et al.*, 2010). In Senegal a leaf decoction is used to treat tachycardia (Burkill, 1985).



Figure 2.6 *Alchornea cordifolia* plant with ripe fruits

Ficus exasperata P. Beauv (Moraceae) known in Akan as “nyankerenee” is widely distributed in tropical Africa and also occurs in Yemen, India and Sri Lanka. It is used in folk medicine for treating various pathological states including inflammatory disorders. Abotsi *et al.*, (2010) have reported anti-arthritic and antioxidant properties of *F. exasperata*. It has been employed in the treatment of hypertension (Buniyamin *et al.*, 2007). Other uses include haemostatic agent, ophthalmia, coughs and haemorrhoids, eczema, dewormer and venereal diseases treatment (Odunbaku *et al.*, 2008). In Nigeria, the young leaves are employed in the treatment of ulcer (Gamaniel *et al.*, 1997). Some phytochemicals isolated from the plant include tannins, flavonoid, saponins, phlobatannins, glycosides and steroids (Adebayo & Ishola, 2009).



Figure 2.7 Leaves of *Ficus exasperata*

Momordica charantia (Cucurbitaceae) is of tropical origin and widely occurs in Africa, India and the Carribean. In Ghana it is called “nyanya” in Akan. It is used in the treatment of various cancers (lymphoid leukemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumor, prostatic cancer, squamous carcinoma of tongue and larynx, human bladder carcinomas and Hodgkin’s disease). Other uses include treatment of diabetes, as a carminative and in the treatment of colics (Yeşilada *et al.*, 1999). According to Satyavati *et al.* (1987) it is employed in anti-ulcerogenic folk remedy for its anti-*Helicobacter pyloric* activity in Turkey. Other folk medicinal uses of the plant include treatment of wounds, internally as well as externally for management of worms and parasites, as emmenagogue, antiviral for measles and hepatitis, as contraceptive, antimalarial and laxative and treatment of dysmenorrhea, eczema, galactagogue, gout, jaundice, kidney stone, leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism and scabies. Biologically active chemicals in the plant include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and

steroids (Raman & Lau, 1996). The immature fruits are a good source of Vitamin C and also provide Vitamin A, phosphorus, and iron. Several phytochemicals such as momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, multiflorenol, have been isolated from the plant (Hussain *et al.*, 1994; Xie *et al.*, 1998; 1999; Prakash *et al.*, 2002; Murakami *et al.*, 2001).



Figure 2.8 *Momordica charantia* leaves, flower and unripe fruit

Ocimum gratissimum (Lamiaceae) is commonly known as “Clone Basil”, “African Basil” and in Hawaii as “Wild Basil”. In Ghana it is known as “nunum” in Akan. It has been reported to show antioxidant properties (Odukoya *et al.*, 2005; Akinmoladun *et al.*, 2007; Aprioku & Obianime, 2008) and used as an antidiarrhoeal (Adebolu &

Oladimeji, 2005), antihelminthic (Fakae *et al.*, 2000; Pessoa *et al.*, 2002). *O. gratissimum* is also used as an antiseptic agent (Agnaniet *et al.*, 2005) particularly by the local folks and for the treatment of several disease conditions including infections, oncogenic and neurological disorders. Ash, vitamins A, B2, and D, calcium, phosphorus, selenium, iron, zinc and magnesium (Oboh *et al.*, 2009) are some micronutrients that have been isolated from the leaves. Additionally, thymol, eugenol, terpenes, xanthenes and lactones α -pinene, β -pinene 1, 8-cineole, β -caryophyllene, a murolene and sehirene have been isolated from the plant (Sainsbury & Sofowora, 1971; Oboh, 2008). Other constituents include germacrene, x-copaene, humutene, β -elemene, β -bourbonenem and serinerel (Pande & Pathak, 2009). The essential oil eugenol had shown some antibacterial activity and a leaf extract of the plant together with other plant extracts had shown analgesic properties Pande & Pathak, 2009).

Albizia zygia is known as “okuro“ in Ghana and used as ornamental shade tree (Abbiw, 1990). The young leaves can be consumed whole as vegetables or added to soups and sauces. Some medicinal uses include the use of the bark sap for ophthalmia (Abbiw, 1990). Additionally, gum from the bark is used as a stabilizer in the food industry (ice cream preparation), cosmetic industry and pharmaceutical industry (drug coating). Some medicinal uses of the plant include a bark decoction for the treatment of bronchial disease and fever (Abbiw, 1990). Pounded or rasped bark are used to treat sores, wounds and toothache. Research has shown anti-inflammatory activity of *Albizia sp.* an ethno medical plant, in acute and chronic animal models of inflammation (Babu *et al.*, 2009). It has been shown to contain substantial amounts of flavanoids and anti-malarial potency (Abdalla & Laastsch, 2012). Saponins have been isolated from *Albizia sp.* (Kokila *et al.*, 2013).

Petersianthus macrocarpus belongs to the family Lecythidaceae and is known as ‘asia’ in Akan. Some ethno medicinal uses include analgesic; anti-arthritic agent, anti-rheumatic agent and anti-pulmonary agent (Burkill, 1985). Additionally, *P. macrocarpus* is used for curing menstrual disorders, as an abortifacient, ecboic and other medical disorders. *In vitro* activities of the plant extracts on human Loa loa (filarial worm) isolates and cytotoxicity for eukaryotic cells have been reported (Mengome *et al.*, 2010). Some phytochemicals isolated from the stem bark of the plant include two new saponins petersaponins III and IV as well as glycosides and steroids (Olugbade *et al.*, 2000).

Aframomum melegueta is a species belonging to the ginger family Zingiberaceae. It is commonly known as grains of paradise, melegueta pepper, alligator pepper, guinea grains. In Ghana it is known as “fom wisa” (Abbiw, 1990). It imparts a pungent peppery flavor with hints of citrus. The plant is native to swampy habitats along the West African coast. Some folk medicinal uses of the plant include treatment of abscess, pile, cough, measles, hernia as well as serving as a purgative (Igwe *et al.*, 1999). Some phytochemicals isolated from the plant are (S)-2-heptanol, (S)-2-heptyl acetate and (R)-linalool. These have been shown to be responsible for the repellent activity of extracts of the plant against the maize weevil (Ukeh *et al.*, 2009). Studies have shown antioxidant and anti-inflammatory properties of the *A. melegueta* extracts (Umukoro & Ashorobi, 2007).



Figure 2.9 Unripe fruits of *Aframomum melegueta*



Figure 2.10 Plant of *Aframomum melegueta*

Secamone afzelii Rhoem. (Asclepiadaceae) is a climber and scrambling plant found in the secondary forest in Ghana (Irvine, 1961). It is known as “kwantemaa” in Akan. According to Burkill, (1985) some folk medicinal uses include wound healing. Research has demonstrated that the mechanism of wound healing may be due to the antimicrobial and antioxidant properties of the leaf extracts (Mensah *et al.*, 2006)

Ageratum conyzoides L. (Asteraceae) is commonly known as billygoat weed, chick weed, goatweed, whiteweed and native to tropical America especially Brazil and tropical Africa. In Ghana it is called “guakro” in Akan. It is an annual herbaceous plant with a long history of traditional medicinal uses in several countries. It is employed as an insecticide due to its toxicity as well as a nematocide. Okunade (2002) in a review reports of several phytochemicals isolated from the plant namely, pyrrolizidine alkaloids, lycopsamine, echinatine, friedelin, β -sitosterol, stigmasterol, flavonoids, benzofuran, chromene, chromone, coumarin and others.

Mallotus oppositifolius (Euphorbiaceae) is found in Africa and Madagascar and have several medicinal properties. In Ghana it is known as “nyanyafurowa (pimpim)” in Akan. Some folk medicinal uses include the treatment of psychiatric disorders, for treating anemia and many others (Abbiw, 1990). According to Kukuia *et al.* (2014), the plant has antidepressant activity. Additionally, it has shown some anti-proliferative effect against human ovarian cancer cell lines and has anti-fungal activity (Adekunle & Ikumapayi, 2006). Some of the phytochemicals isolated from the leaf include dimeric phloroglucinol mallotojaponins with antimalarial activity as well as phenolic compounds (Adekunle & Ikumapayi, 2006).

2.6 Apoptosis as a Mechanism of Cell Death

Apoptosis is a term coined by Kerr (1972) and colleagues to describe the unique morphology associated with cell death that differs from necrosis (Kerr *et al.*, 1972). It is termed “programmed cell death” as it involves a tightly regulated process. The unique characteristics of apoptosis include a change in refractive index of cells, (Hengartner, 1997) followed by cytoplasmic shrinkage and nuclear condensation, blebbing of the cell membrane and eventually the blebs separate from the dying cell and form “apoptotic bodies” (Hengartner, 1997). The phospholipid membrane asymmetry is lost as phosphatidylserine (PS) appears on the outer leaflet of the membrane (Williamson, 2000). There is change in membrane potential of mitochondrial outer membrane (MOM) due to formation of pores in the MOM, resulting in leakage of cytochrome c from the MOM into the cytoplasm (Waterhouse *et al.*, 2001). Finally, adjacent cells or macrophages phagocytose apoptotic bodies and the cell dies.

Caspases have been implicated in the activation of apoptosis in higher organisms (Earnshaw *et al.*, 1999; Xing *et al.*, 2011). They constitute a large protein family that is highly conserved among multicellular organisms. There are two subfamilies based on function namely; caspases involved in inflammation and have homology to caspase-1 (Interleukin 1 β -Converting Enzyme), and those caspases involved in apoptosis (Launay *et al.*, 2005). Caspases are secreted as inactive zymogens by most cell types and processed by proteolytic cleavage to gain full activity (Earnshaw *et al.*, 1999). Human caspase-8 and caspase-9 are involved in initiating apoptosis through different signalling mechanisms and are known as “initiator caspases” (Launay *et al.*, 2005). They can activate the effector caspases including caspase-3, by proteolytic cleavage processing.

Caspase-3 transduces the signal downstream by targeting and cleaving other zymogens and irreversibly commits the cell to apoptosis (Brentnall *et al.*, 2013).

Many external stimuli including activation of cell surface receptors such as Fas, tumour necrosis factor 1 (TNFR1), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1), TRAIL-R2 and p75-nerve growth factor receptor (p75-NGFR) (Wajant *et al.*, 2003; Nowsheen & Yang, 2012) are involved in the activation of apoptosis through the extrinsic pathway. They have two distinct signalling motifs: death domains and death effector domains that enable them interact with other proteins down the apoptosis signalling cascade. The extrinsic pathway involves activating caspase-8, which in turn either activates caspase-3 or cleaves the Bcl-2 family member, Bid, leading to the formation of the apoptosome and activation of caspase-9 (Hengartner, 2000). The intrinsic pathway can be activated by DNA damage and other events (Rich *et al.*, 1999; Nowsheen & Yang, 2012). In a study involving mice caspase-12 a third pathway for activating apoptosis through the endoplasmic reticulum has been proposed (Nakagawa *et al.*, 2000).

The presence of phosphatidylserine, caspases and nuclear condensation in apoptotic cells has been utilized in the design of several assays to study apoptosis in dying cells. Flow cytometry involves the binding of calcium dependent Annexin V to PS (Koopman *et al.*, 1994). This reaction is specific as Annexin has high affinity for PS. Annexin V binding is assessed using bivariate flow cytometry and cell staining is evaluated with fluorescein isothiocyanate (FITC)-labelled Annexin V (green fluorescence), simultaneously with dye exclusion of propidium iodide (PI) (negative for red fluorescence) (Koopman *et al.*, 1994). The test discriminates intact cells (FITC⁻/PI⁻), apoptotic cells (FITC⁺/PI⁻) and necrotic cells (FITC⁺/PI⁺) (Vermes *et al.*, 1995). In comparison with existing traditional tests the Annexin V assay is sensitive and easy to

perform (Koopman *et al.*, 1994). The Annexin V assay offers the possibility of detecting early phases of apoptosis. Several assays including colorimetric, luminescence, fluorescence have been developed in the estimation of caspase activity in apoptosis (Vermes *et al.*, 1995). Fluorescent dyes with aromatic amino or guanidine groups, such as propidium iodide (PI), ethidium bromide (EB), diaminophenylindole (DAPI), acridine orange (AO), and Hoechst dyes, interact with nucleotides to emit fluorescence (Vermes *et al.*, 1995). EB and PI molecules intercalate inside the DNA double helix. DAPI and Hoechst dye molecules attach at the minor groove of the DNA double helix. On the other hand, AO can form complexes with either double-stranded DNA or single-stranded DNA and RNA. One molecule of AO can intercalate with three base pairs of double-stranded DNA to emit green fluorescence with the maximum wavelength at 526 nm.

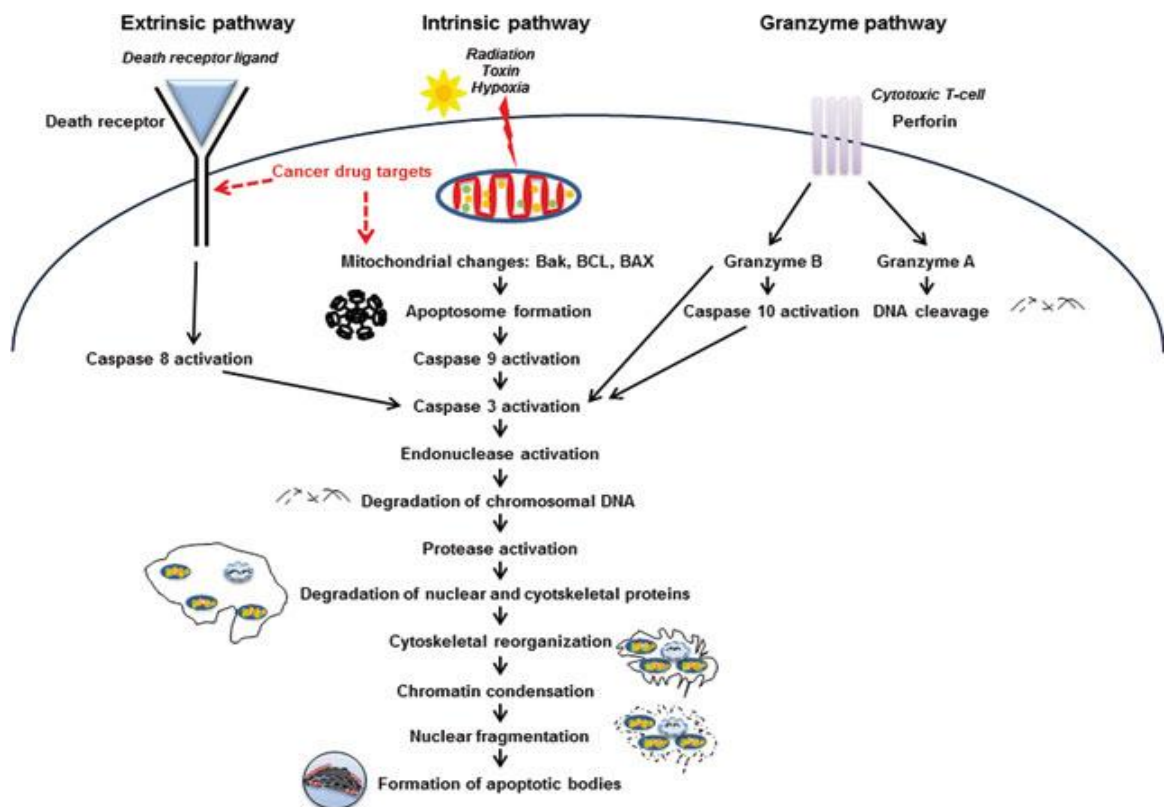


Figure 2.11 Illustration of pathways and key proteins involved in apoptosis

(Source: Nowsheen & Yang, 2012)

2.7 Estimation of Cell Viability and Cytotoxicity

There have been several methods employed in the estimation of cell viability and cytotoxicity of drugs. The assay used depends on the cell function that is being estimated. Cell functions such as enzyme activity, cell adherence, ATP production, coenzyme production, and nucleotide uptake amongst others. Many assays such as colony formation method, crystal violet method, tritium-labeled thymidine (^3H) uptake method, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) (WST) methods have been used to estimate live numbers of cells as well as cytotoxic effect of drugs on cells (Vega-Avila & Pugsley, 2011).

Trypan blue is extensively employed for staining dead cells for counting (Riss, 2005). Thus cell viability is determined by counting the unstained cells with a microscope or other instruments. A setback of this assay is that it cannot distinguish between healthy cells and cells that are alive, but have lost certain cellular functions (Tennant, 1964). In the case of the colony formation method, the numbers of colonies are counted using a microscope as a cell viability indicator. With regard to tritium-labeled thymidine uptake method, [^3H]-thymidine is involved in the cell nucleus due to the cell growth and the amount of ^3H in the nucleus is then measured using a scintillator counter (Riss, 2005). This assay is sensitive. However, a drawback to its use, is the use of radioisotope which raises various concerns.

Cellular enzymes like lactate dehydrogenase, adenylate kinase, and glucose-6-phosphate dehydrogenase are also used as cell markers (Stoddart, 2011). However, in vitro activities of adenylate kinase and glucose-6-phosphate dehydrogenase are unstable and only lactate dehydrogenase does not lose its activity during cell death

assays. Here cell death assays involving lactate dehydrogenase are more reliable than the other enzyme based cell death assays. The (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) and water soluble tetrazolium (WST) assays are enzyme based methods that rely on a reducing agent with coloring reagent and dehydrogenase in a viable cell to determine cell viability with a colorimetric measurement (Yin *et al.*, 2014). The advantages of these over the previously mentioned methods include easy to use, safe, high reproducibility and are widely used in viability and cytotoxicity assays. The MTT assay determines mitochondrial dehydrogenase activities in the living cells. In the method, MTT is reduced to a purple formazan by a dehydrogenase enzyme. However, MTT formazan is insoluble in water but soluble in organic solvents and it forms purple needle shaped crystals in the cells. Therefore prior to measuring the absorbance, an acidified organic solvent is required to solubilise the crystals.

2.8 Antioxidant and Phenolic Assays

Reactive oxygen species resulting from free radicals may cause cancer through two possible mechanisms: genetic mutations and the effects on signal transduction and transcription factors (Valko *et al.*, 2006; Ziech *et al.*, 2010). Oxidative stress affects the stability of DNA, phospholipids, proteins and carbohydrates on the cell membrane. Oxidation and injury to DNA induce genetic mutation (Ziech *et al.*, 2010). Naturally occurring antioxidants have been shown to mitigate the effect of oxidative stress in living tissues. Glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase are known catalytic antioxidants in cells (Silici *et al.*, 2010). The non catalytic antioxidants namely vitamin A, C, E, glutathione, lipoic acid, mixed carotenoids, several bioflavanoids, antioxidant minerals (copper, zinc, manganese and selenium) and many others are acquired from dietary sources (Silici *et al.*, 2010; Dai & Mumper,

2010). Vitamin E for example inhibits lipid peroxidation. Plant phenols are known to be potent antioxidants (Dai & Mumper, 2010) hence an estimation of the phenolic content will give an indication of the antioxidant properties of the plant. Several methods are used to estimate the antioxidant potential of phytochemicals namely: inhibition of 2,2-diphenyl picrylhydrazyl (DPPH) free radical scavenging, oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant potential (Aljadi & Kamaruddin, 2004) assays.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Materials and Reagents

Human breast cancer cell line (MCF-7) and normal prostate cells (PNT-2) were obtained from Dr. Takuhiro Uto (Nagasaki International University, Japan). DMEM (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), Trypsin (Wako Pure Chem. Ind., Tokyo, Japan), FBS (Hyclone Lab. Inc., South Logan, Utah, U.S.A.), PSG (Wako Pure Chem. Ind., Tokyo, Japan), MTT (Wako Pure Chem. Ind., Tokyo, Japan), Trypan Blue (Wako Pure Chem. Ind., Tokyo, Japan), Guava Nexin (California, U.S.A.), Hoechst 33258 Reagent (Dojindo, Japan), DPPH (Hamburg, Germany), Gallic Acid (Hamburg, Germany), BHT (Hamburg, Germany). Chemicals and reagents used for the project were all obtained from standard suppliers.

3.2 Plant Samples Used for Assay

Freeze dried plant fractions of the various plant species already prepared according to the method described by Hostettman *et al.* (2000) and Sarker *et al.* (2005) were obtained through the University of Ghana Research Fund/Office of Research, Innovation and Development (UGRF/ORID) funded project (grant number URF/5/LMG-001/2-11-2012) titled “Screening of Ghanaian Traditional Medicinal Plants for bioactive anticancer agents”. Support for my work was obtained from this project as well as the Department of Clinical Pathology, NMIMR. Aqueous and ethanolic extracts of *Moringa oleifera* were also prepared and used for the assays.

3.3 Aqueous Extraction of *Moringa oleifera* Leaves

Extraction procedures were done as described by Sarker *et al.* (2005) and Harbone (1973). Matured and young leaves of *M. oleifera* were harvested in December from one plant early in the morning at East Legon, Greater Accra. The harvested leaves of *M. oleifera* were air dried at ambient temperature (25°C) till the leaves were brittle and then pulverized. A 10X suspension of the sample was prepared with distilled water by weighing out 50 g of the pulverized leaves and adding 500 mL of distilled water to it in a reagent bottle. The mixture was heated for 1 hr at 80°C. After cooling, the mixture was then centrifuged at 4500 rpm for 15 min with an Eppendorf centrifuge 5810R to obtain the supernatant. The extraction process was repeated to obtain another supernatant with the residue after which the supernatants were pooled together, frozen at -20°C and freeze-dried. The percentage yield was then calculated.

3.4 Sequential Extraction of Aqueous Freeze Dried *Moringa oleifera* Extracts with Ethanol

A 10X dilution of the aqueous freeze dried extract with 50% ethanol was prepared by weighing 10.1 g of aqueous extract and adding 100 mL of 50% ethanol in a conical flask. The flask was then placed on a shaker for 24 hr and the mixture was centrifuged at 4500 rpm for 15 min (Eppendorf centrifuge 5810R, Germany) to obtain the supernatant. The procedure was then repeated for the residue to obtain another supernatant. The supernatants were then pooled together, concentrated by rotary evaporation, frozen at -20 °C and freeze-dried (Harbone, 1973). The percentage yield was then calculated.

3.5 Cytotoxicity Assays

3.5.1 Cell Culturing

3.5.1.1 Cell Recovery

The stored MCF-7 breast cancer cells were removed from -80°C refrigerator and thawed for 5 min without the ice melting completely and suspended in 5 mL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Foetal Bovine Serum (FBS) and 0.1% Penicillin-Streptomycin-L-Glutamine (PSG). The cells were spun at 1000 rpm (Tomy LC200 centrifuge, Japan) for 2 min with a centrifuge to get rid of the DMSO. The supernatant was discarded and the pellets resuspended in 1 mL of complete DMEM. The resuspended cells were then pipetted into 25 cm³ culture flask containing 5 mL of complete DMEM and incubated in a humidified chamber at 37°C in the presence of 5% CO₂.

3.5.1.2 Passing of Cells

The flask containing the MCF-7 cells was removed from the incubator and inspected for confluence at 80% - 100%. The spent medium was pipetted off and the cells washed with 4.5 mL of PBS for the 25 cm³ flask (9 mL for 75 cm³ flask) to get rid of dead cells and to stabilize the pH of the cells. The MCF-7 breast cancer cells were again washed with PBS and treated with 1.5 mL of trypsin (3 mL for 75 cm³ culture flask) and incubated for 4 min to detach the cells. After detaching the cells completely, they were washed with 8.5 mL (17 mL for 75 cm³ flask) of the medium and pipetted into eppendorf tubes. The MCF-7 breast cancer cells were then spun at 1000 rpm (Tomy LC200 centrifuge, Japan) for 5 min. The supernatant was discarded and the pellet resuspended in fresh complete DMEM (1 mL). A volume of 9 mL complete DMEM (18 mL for a 75 cm³ flask) was added to the initial suspension to obtain a homogenous

mixture. A volume of 30 μL of trypan blue was pipetted and 10 μL of the MCF-7 cells added into a micro tube to get a 4X dilution factor. The mixture was mixed thoroughly and 10 μL loaded onto a clean hemacytometer for counting. After counting the cells were passaged at a given concentration into 25 cm^3 or 75 cm^3 culture flasks.

3.5.2 MTT- Tetrazolium Based Colorimetric (MTT) Assay

Breast cancer cells, MCF-7 cell lines that were maintained in DMEM supplemented with 10% FBS and 0.1% PSG and incubated in a humidified chamber at 37°C in the presence of 5% CO_2 were used for the assay. Cytotoxic effect of the extracts and fractions were assessed using tetrazolium based colorimetric (MTT) assay (Ayisi *et al.*, 2011). For the MTT assay, MCF-7 breast cancer cells (100 μL) were seeded at 1×10^5 cells/mL concentration into 96-well plates and incubated overnight in a humidified chamber at 37°C in the presence of 5% CO_2 for 24 hr. The MCF-7 cells were then treated with concentrations of 0 - 50 mg/mL serial dilutions of the extracts and fractions (0 $\mu\text{g}/\text{mL}$, 62.5 $\mu\text{g}/\text{mL}$, 125 $\mu\text{g}/\text{mL}$, 250 $\mu\text{g}/\text{mL}$, 500 $\mu\text{g}/\text{mL}$, 1000 $\mu\text{g}/\text{mL}$) in triplicates and incubated for 72 hr. After incubation the cells were treated with 20 μL of 2.5 mg/mL MTT solution (refer to appendix II for preparation) and incubated for 4 hr as indicated above. The samples were subsequently treated with 150 μL of acidified isopropanol (refer to appendix II for preparation) and the plates were incubated in the dark overnight at room temperature (26°C) to dissolve formazan crystals formed from MTT. The optical density was read using a spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria) at 570 nm. Curcumin was used as a positive control compound. The negative control consisted of 1% of DMSO which was the dissolution solvent for the fractions. Percent cell survival was evaluated using the formula,

$$\% \text{ Cell survival} = [(A_1 - A_0) / A_2] \times 100,$$

Where A_1 is the absorbance of the test experiment, A_0 is the absorbance of the test control and A_2 is the absorbance of the negative control. A plot of % cell survival versus concentration of the fractions were used to determine the inhibition concentration at 50% cell survival (IC_{50}) values. To test the safety and efficacy of the fractions, they were tested on non-cancerous (normal) prostate cells (PNT-2) using the method described above. The IC_{50} values obtained were used to determine the selectivity indices (SI) of the fractions using the formula,

$$SI = (IC_{50} \text{ of normal cell}) / (IC_{50} \text{ of cancer cell line})$$

A significant SI value must be ≥ 2 .

The samples were coded to avoid any bias in the running of the assays. A total of forty fractions of the medicinal plants were assessed for their activity against the MCF-7 breast cancer cell line.

3.6 Molecular Mechanistic Studies

Studies on molecular mechanism by which the most bioactive fraction (petroleum ether fraction of *Aframomum melegueta*) induced cytotoxicity was examined using test for apoptosis via nuclear morphology and flow cytometry.

3.6.1 Tests for Apoptosis

3.6.1.1 Nuclear Morphology

The method employed was that described by Xing and others (2011) with slight modifications. The MCF-7 cells (6 mL at $1 \times 10^6 \text{ cells/mL}$) were seeded into sterile petri dishes and incubated for 24 hr at 37°C and 5% CO_2 . A concentration based experiment was performed by treating the cells at concentrations of $0 \mu\text{g/mL}$, $2 \mu\text{g/mL}$, and $4 \mu\text{g/mL}$.

of the petroleum ether fraction of *Aframomum melegueta* and re-incubated for 24 hr. The treated MCF-7 cells were transferred from the petri dishes into 15 mL centrifuge tubes and spun at 1000 rpm for 5 min. The supernatant was then discarded and the pellet resuspended in 1 mL of phosphate buffered saline (PBS). The cells were then transferred to 1.5 mL eppendorf tubes and centrifuged at 1000 rpm for 5 min. The supernatant was discarded and the cells were treated with 200 μ L of 1% glutaraldehyde and incubated at room temperature (26°C) for 30 min. Urosolic acid was used as a positive control and 1% DMSO as a negative control. The supernatant was removed and 50 μ L of PBS and 8 μ L of Hoechst solution added and mixed thoroughly. The samples were viewed under a fluorescent microscope (Olympus BX53, Tokyo, Japan) for nuclear morphological changes.

3.6.2 Flow Cytometric Analysis

The method described by Xing and others (2011) with slight modifications was used for this assay. The MCF-7 cells (3 mL at 2×10^5 cells/mL) were seeded into sterile petri dishes and incubated for 24 hr at 37°C and 5% CO₂. The cells were treated with the petroleum ether fraction of *Aframomum melegueta* at concentrations of 0 μ g/mL, 2 μ g/mL and 4 μ g/mL and re-incubated for 24 hr. Curcumin was used as a positive control. The cells were harvested by gently scraping them with a spatula to detach them and 100 μ L of the cell suspension was aliquoted into wells in a 96-well plate. An equal volume (100 μ L) of Guava Nexin Reagent was added and incubated for 20 min in the dark at 25°C after which a flow cytometric analysis was done using a Guava easyCyte HT flow cytometer, U. S. A.

3.7 Antioxidant: Inhibition of Free Radical Scavenging Activity – DPPH assay

The free radical scavenging activities of the *M. oleifera* extracts and were evaluated using the 2,2 – diphenyl-1-picrylhydrazyl (DPPH) assay with slight modification (Blois, 1958; Coruh *et al.*, 2007). The extract solutions were prepared by weighing 20 mg of the dry *M. oleifera* extracts and dissolved in 1 mL of methanol (20 mg/mL) separately. The mixtures were then vortexed for complete dissolution. A volume of 100 μ L diluted extract solution (1 - 20 mg/mL) in serial concentrations of 0.027 mg/mL, 0.082 mg/mL, 0.247 mg/mL, 0.741 mg/mL, 2.22 mg/mL, 6.67 mg/mL were pipetted into 96 well-plates in triplicates and 100 μ L of DPPH (0.5 mM) was added to the samples. After incubation of the samples in the dark at room temperature (25°C) for 20 min, the absorbance was recorded at 517 nm using Tecan Infinite M200 Pro plate reader. Butylated hydroxytoluene (BHT) of 10 mM concentration was used as the positive control in serial dilutions 0.014 mM, 0.041 mM, 0.12 mM, 0.37 mM, 1.11 mM, 3.33 mM, and 10 mM. Methanol was used as the negative control. The concentrations were converted to mg/mL for comparison. Radical scavenging capacity of each extract was calculated as the percent DPPH radical scavenging effect.

$$\% \text{ DPPH Scavenging effect} = [(A_0 - A_1) / A_0] \times 100,$$

Where A_0 is the absorbance of the negative control and A_1 is the absorbance of the test experiment. The IC_{50} values were determined from a plot of % DPPH scavenging effect versus concentration of test sample.

3.8 Determination of Total Phenolic Content of *M. oleifera* Extracts

A gallic acid standard curve was prepared using (0 mg/mL - 5 mg/mL) stock of gallic acid to estimate the total phenolic content of the extracts. A volume of 10 μ L (5 mg/mL, 2.5 mg/mL and 1 mg/mL) of each of the extract concentration was aliquoted into an

eppendorf tube and 0.79 mL of distilled water added and thoroughly mixed after addition of 50 μL of Folin-Ciocalteu reagent and incubated at 25°C for 8 min. A volume of 150 μL of Na_2CO_3 (0.25 g/mL) was then added and the mixture was incubated for 2 hr at a room temperature of 25°C. The resultant solution was aliquoted into 24 well-plates in triplicates and absorbance was read at 750 nm. (Waterhouse, 2002).

CHAPTER FOUR

4.0 RESULTS

The cell viability graphs showed a concentration dependent activity of the fractions on the MCF-7 breast cancer cell line. Refer to Figures 4.1 to 4.40 for graphs of cytotoxicity assays of the dichloromethane, petroleum ether and ethyl acetate fractions of the medicinal plants. The cell viability graphs were used to estimate the IC₅₀ values of the various fractions as well as the SI values (Tables 4.1, 4.2 and 4.3). Tables 4.1 to 4.3 shows the IC₅₀ values and SI values of the ethyl acetate, dichloromethane and petroleum ether fractions of the medicinal plants, respectively. In Table 4.1 *A. cordifolia* and *P. macrocarpus* were the species with IC₅₀ values close to 30 µg/mL (41.6 µg/mL and 40.9 µg/mL) with the rest of the species having IC₅₀ > 100. The ethyl acetate fraction of the medicinal plant species demonstrated insignificant antiproliferative potential towards the MCF-7 breast cancer cell line.

The IC₅₀ values of *A. cordifolia*, *M. charantia* and *S. rotundifolius* in Table 4.2 are less than 30 µg/mL suggesting that they can serve as promising candidates for further research. The SI values of some of the medicinal plants also make them promising candidates for further research. In Table 4.3 the petroleum ether fractions of *F. exasperata*, *P. pinnata*, *O. gratissimum*, *P. macrocarpus*, *H. indicum*, and *A. melegueta* showed potent cytotoxicity against the MCF-7 breast cancer cell line with IC₅₀ < 30 µg/mL. The SI values ranged from 1.2 to 10 suggesting weak to strong efficacy on normal cells. The remaining plant species demonstrated moderate cytotoxicity towards the MCF-7 breast cancer cell line.

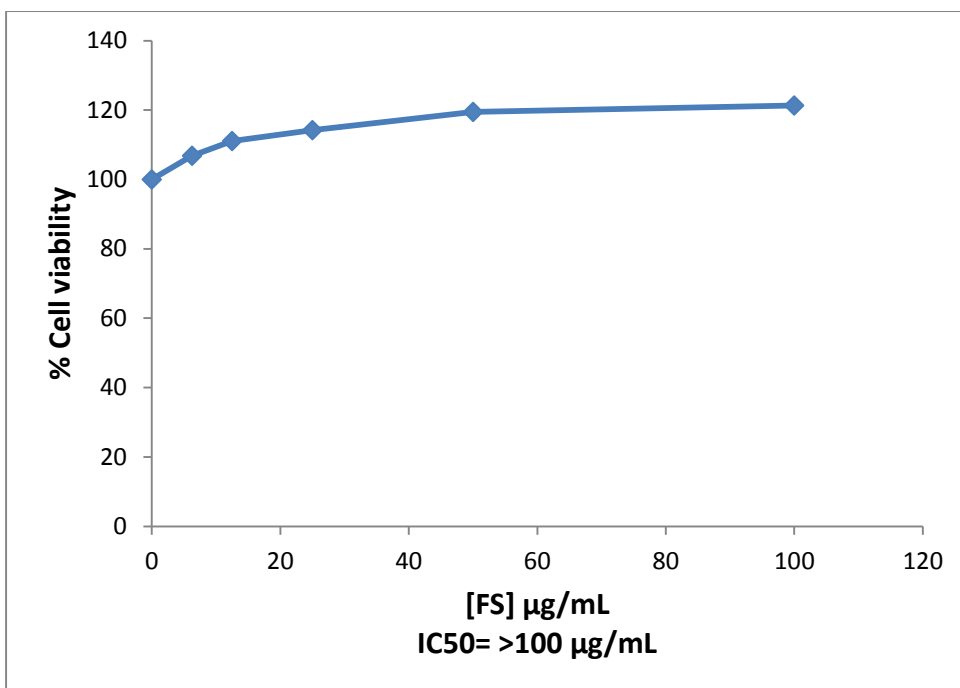


Figure 4.1 Cell viability curve of ethyl acetate fraction of *Ficus exasperata*.

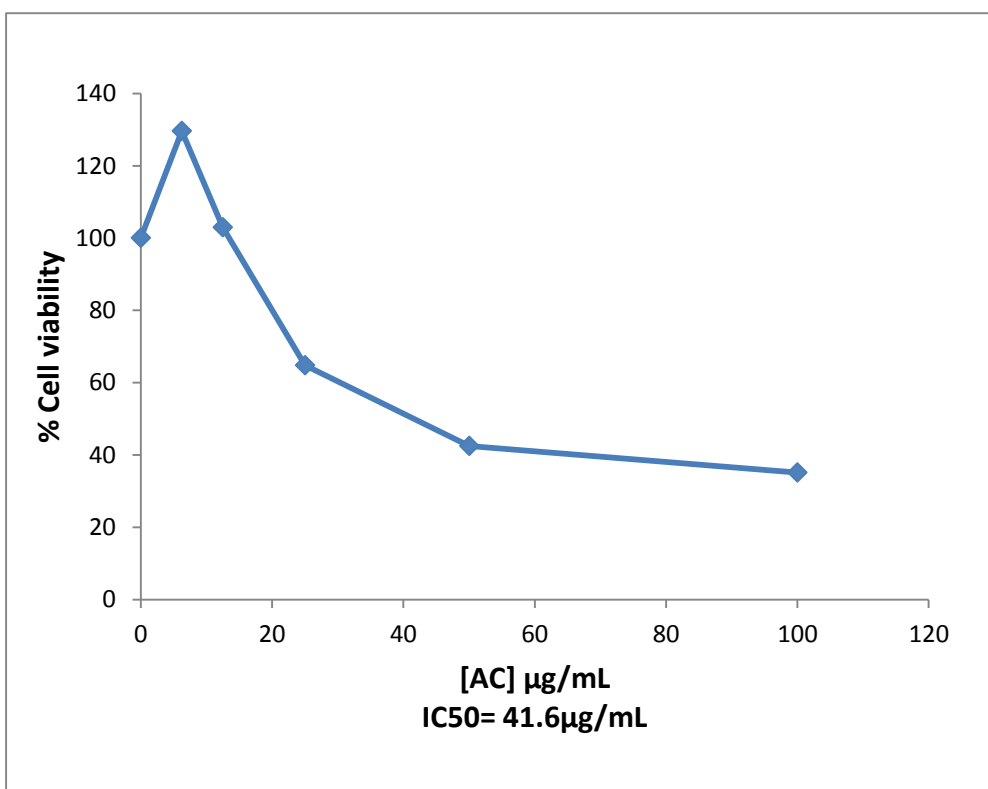


Figure 4.2 Cell viability curve of ethyl acetate fraction of *Alchornea cordifolia*.

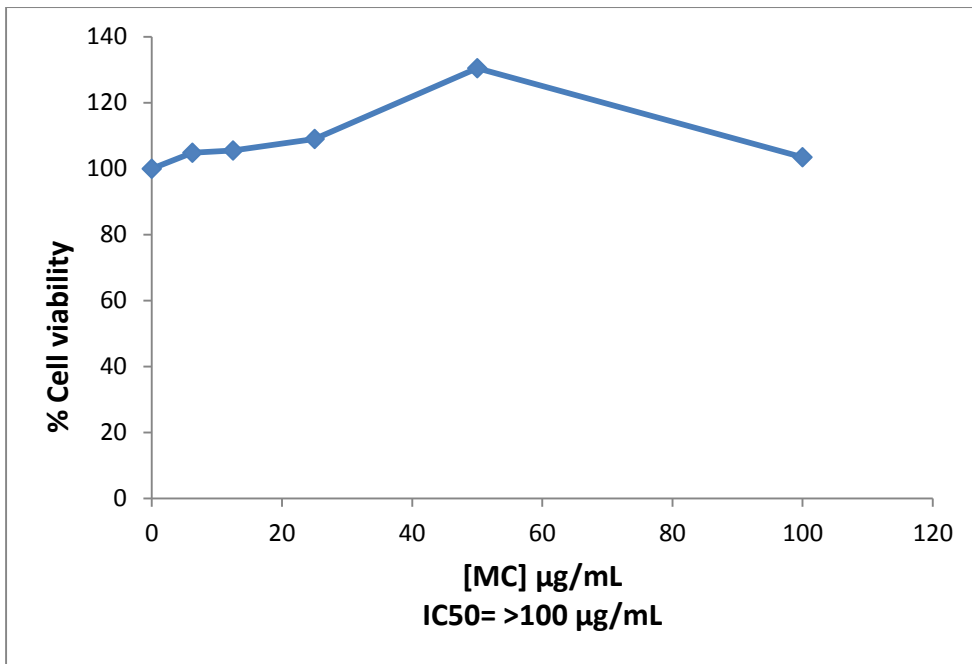


Figure 4.3 Cell viability curve of ethyl acetate fraction of *Momordica charantia*.

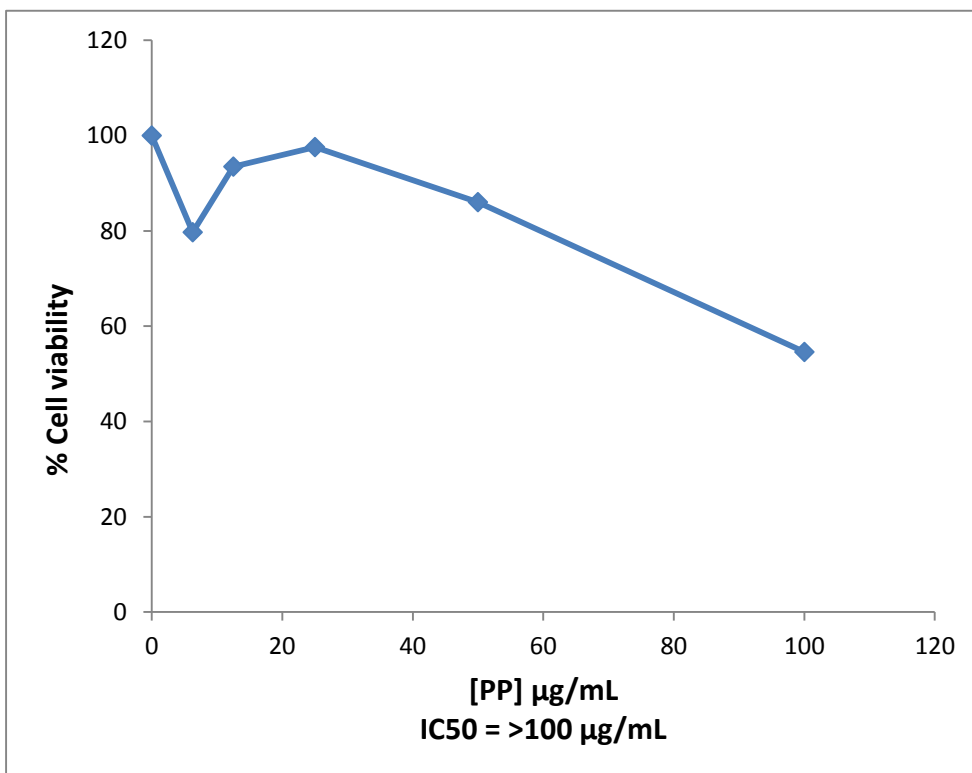


Figure 4.4 Cell viability curve of ethyl acetate fraction of *Paullinia pinnata*

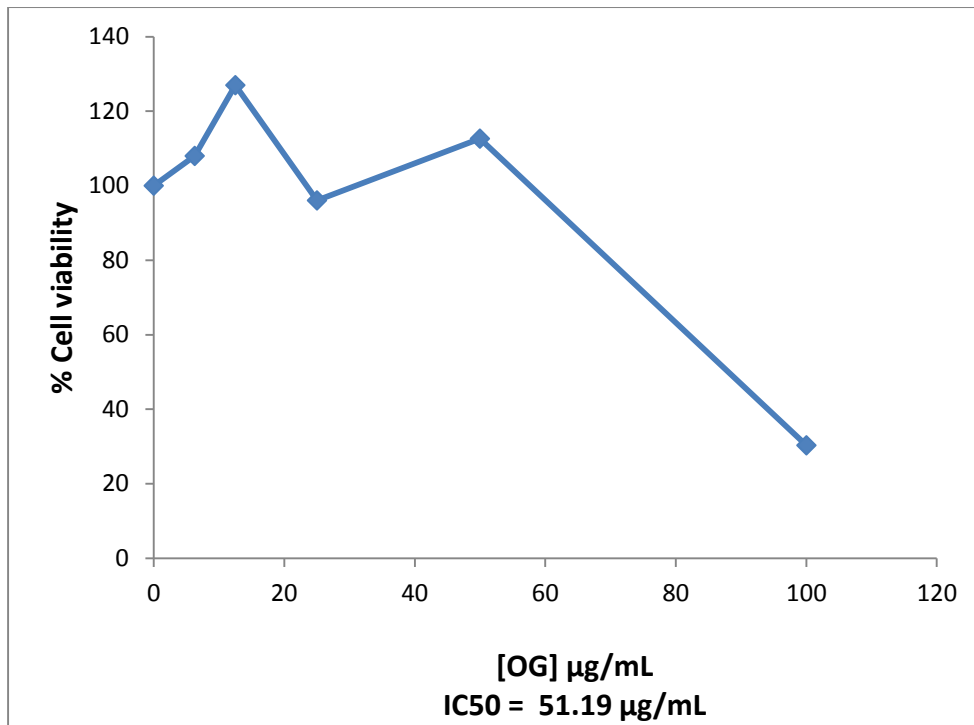


Figure 4.5 Cell viability curve of ethyl acetate fraction of *Ocimum gratissimum*.

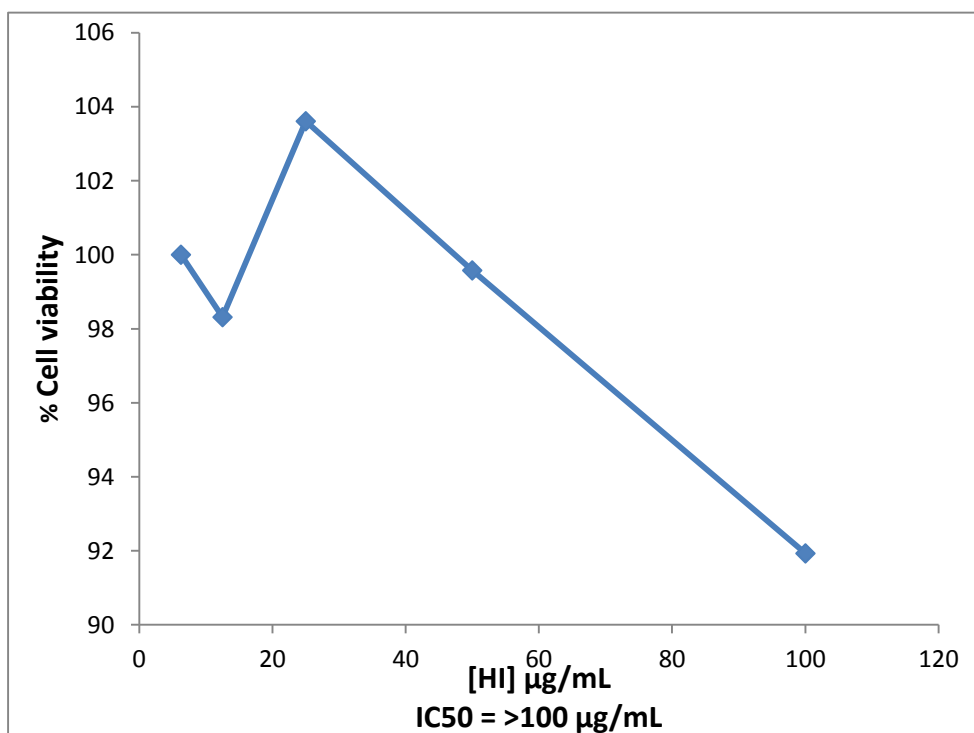


Figure 4.6 Cell viability curve of ethyl acetate fraction of *Heliotropium indicum*.

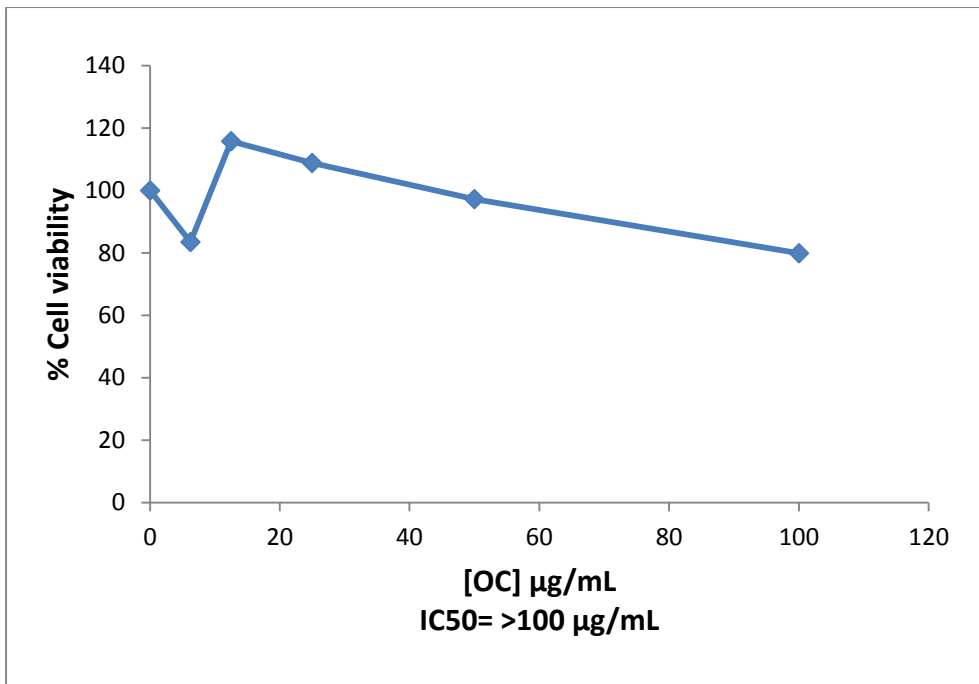


Figure 4.7 Cell viability curve of ethyl acetate fraction of *Ocimum canum*.

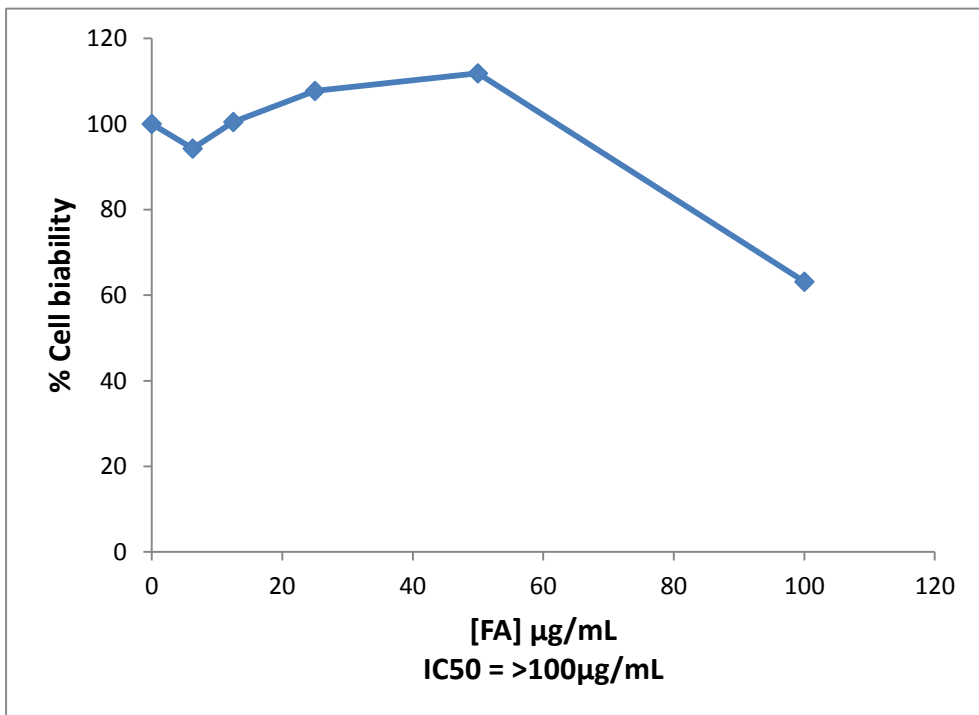


Figure 4.8 Cell viability curve of ethyl acetate fraction of *Fleurya asteuans*.

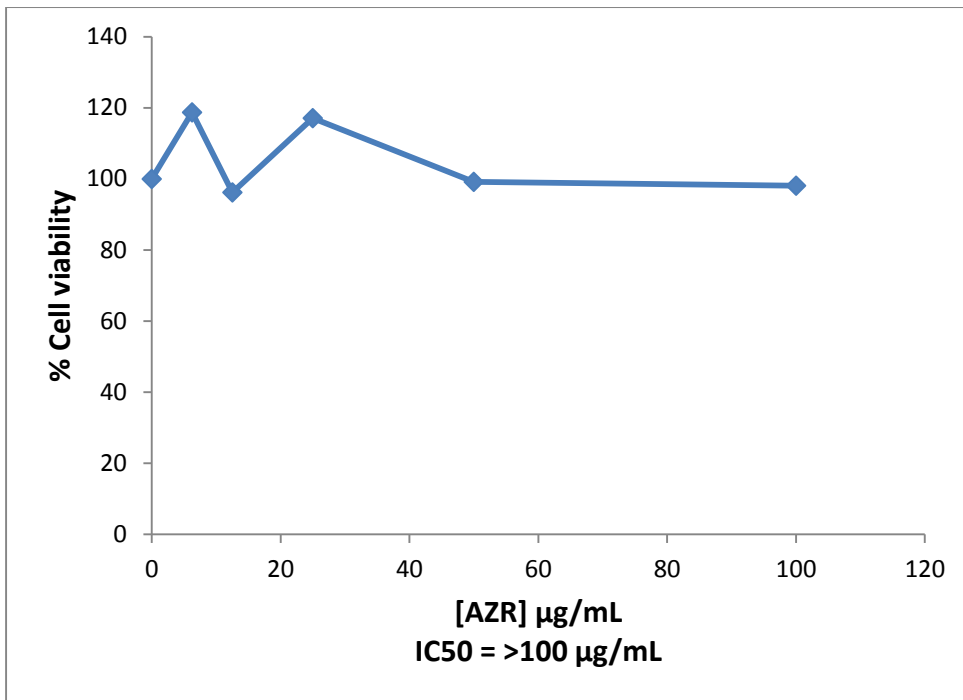


Figure 4.9 Cell viability curve of ethyl acetate fraction of *Albizia zygia*.

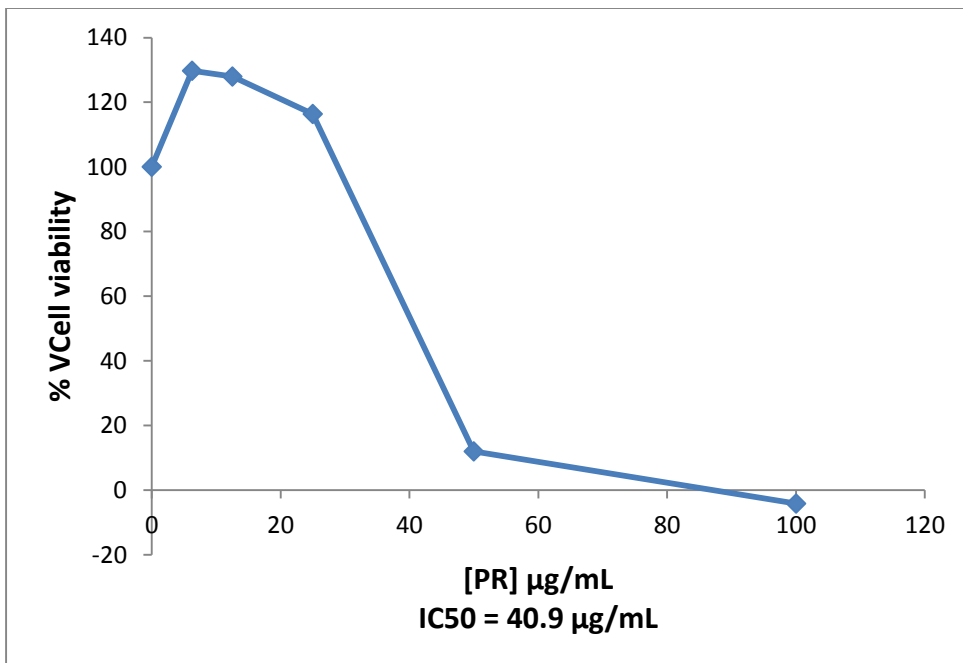


Figure 4.10 Cell viability curve of ethyl acetate fraction of *Petersianthus macrocarpus*.

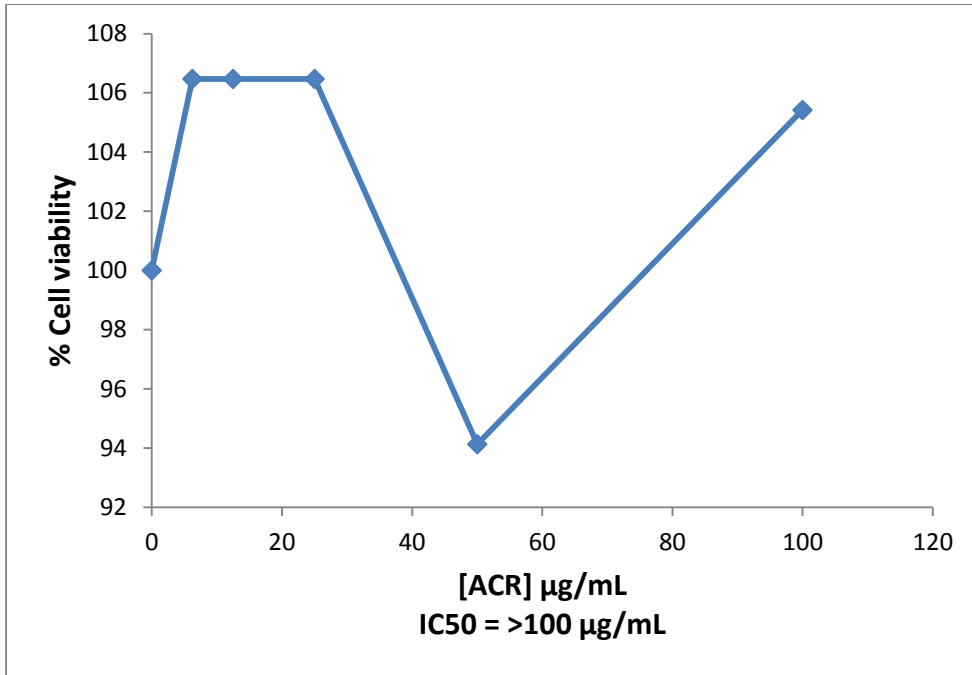


Figure 4.11 Cell viability curve of ethyl acetate fraction of *Alchornea cordifolia*.

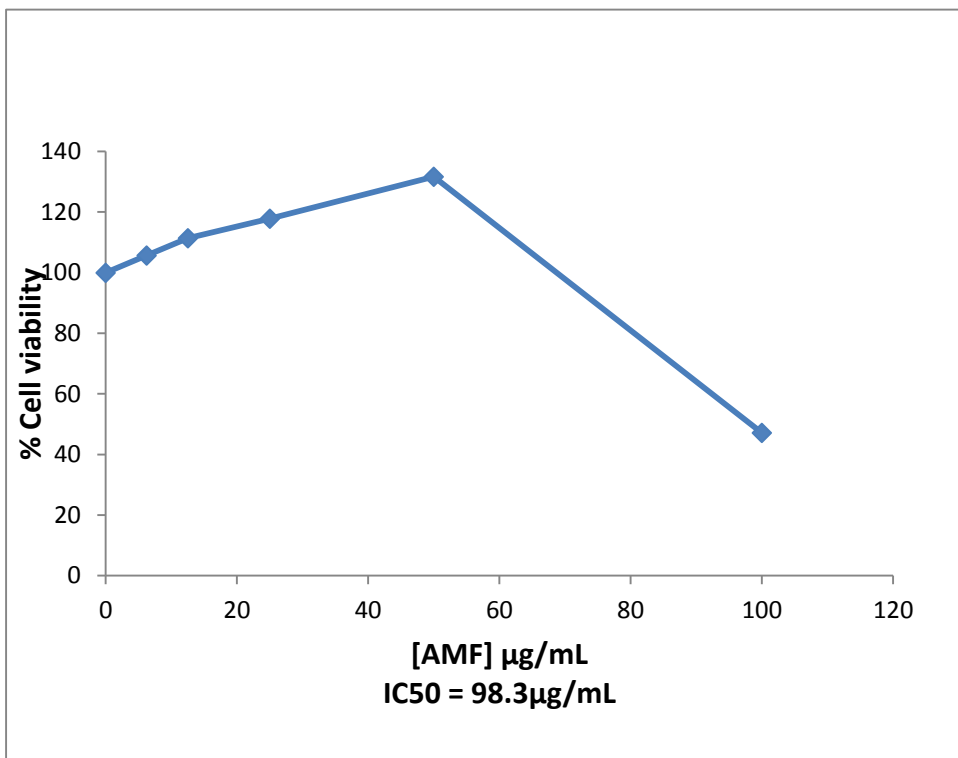


Figure 4.12 Cell viability curve of ethyl acetate fraction of *Aframomum melegueta*.

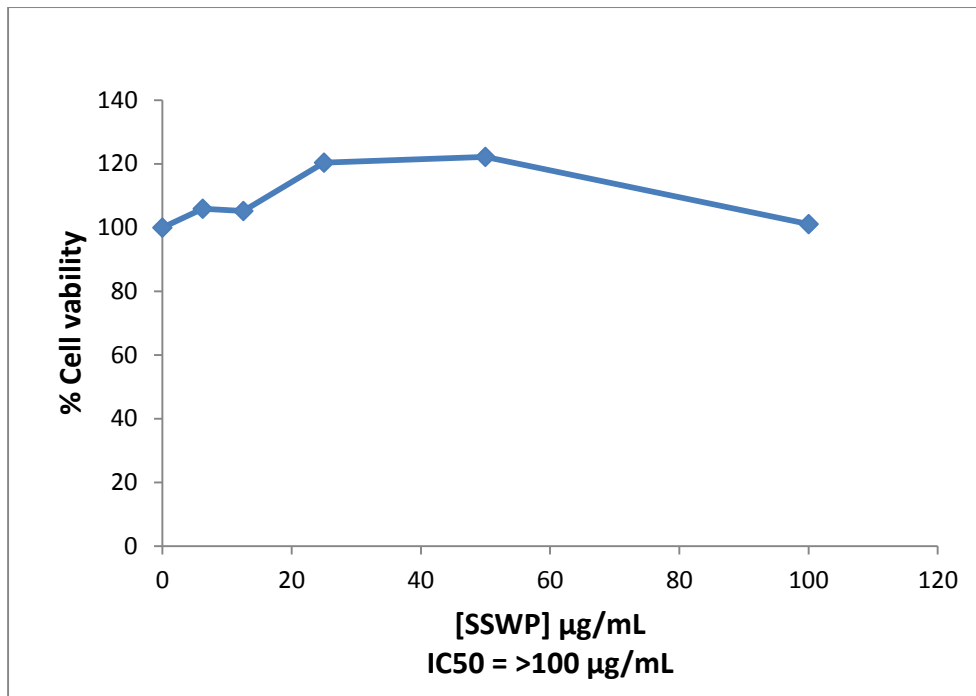


Figure 4.13 Cell viability curve of ethyl acetate fraction of *S. rotundifolius*.

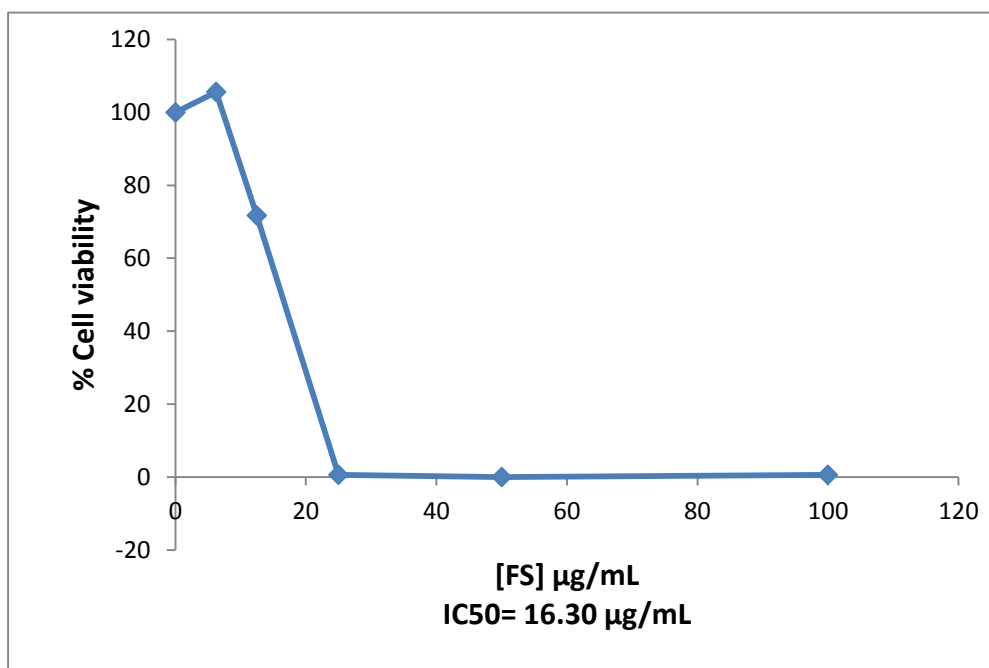


Figure 4.14 Cell viability curve of petroleum ether fraction of *Ficus exasperate*.

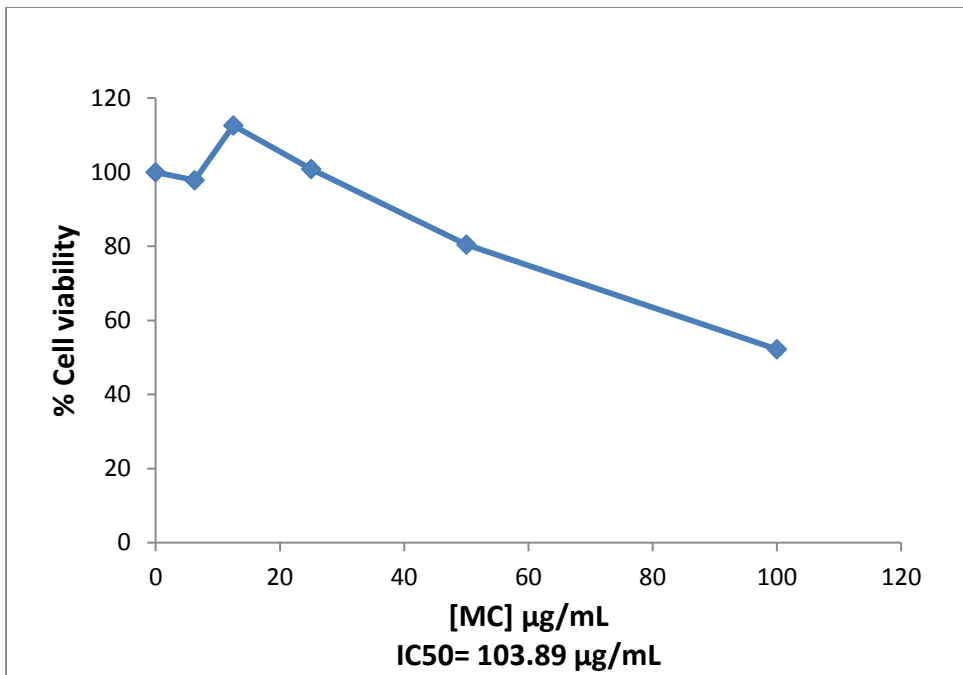


Figure 4.15 Cell viability curve of petroleum ether fraction of *Momordica charantia*.

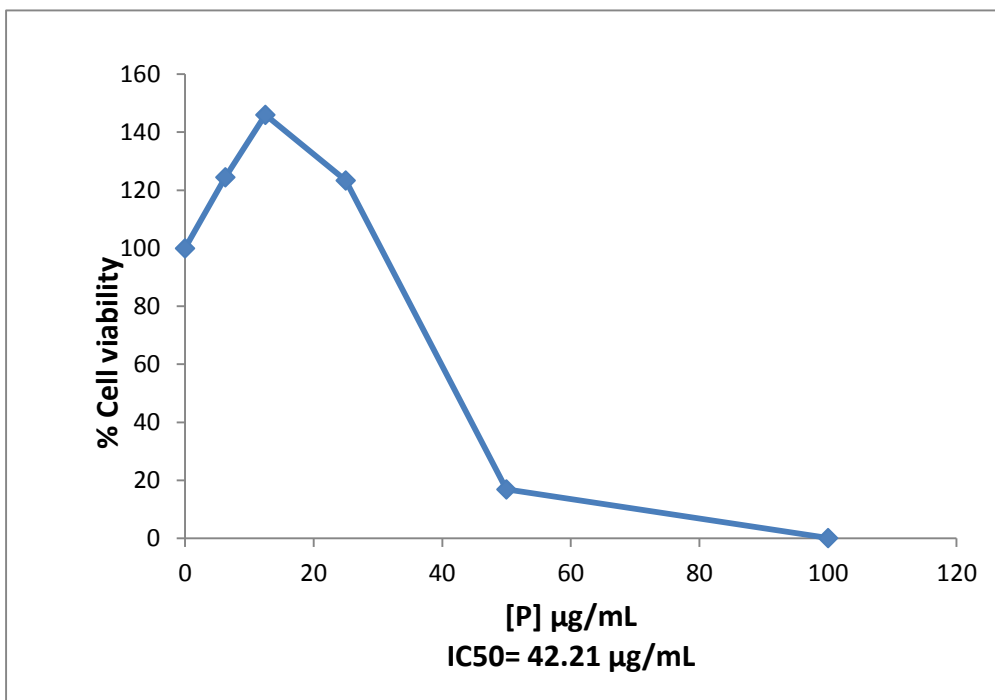


Figure 4.16 Cell viability curve of petroleum ether fraction of *P. macrocarpus*.

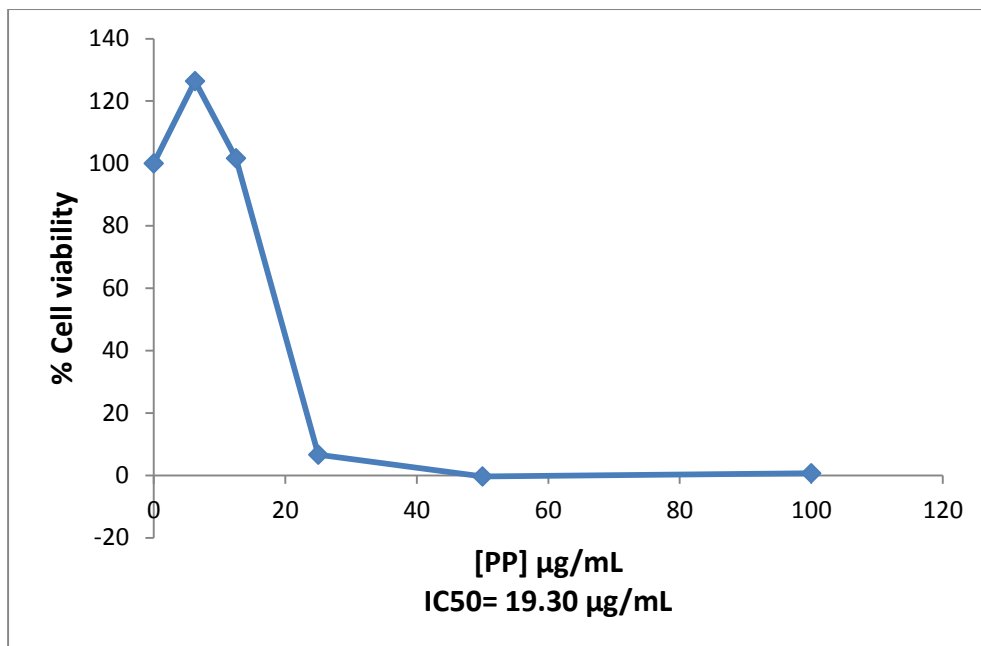


Figure 4.17 Cell viability curve of petroleum ether fraction of *Paullinia pinnata*.

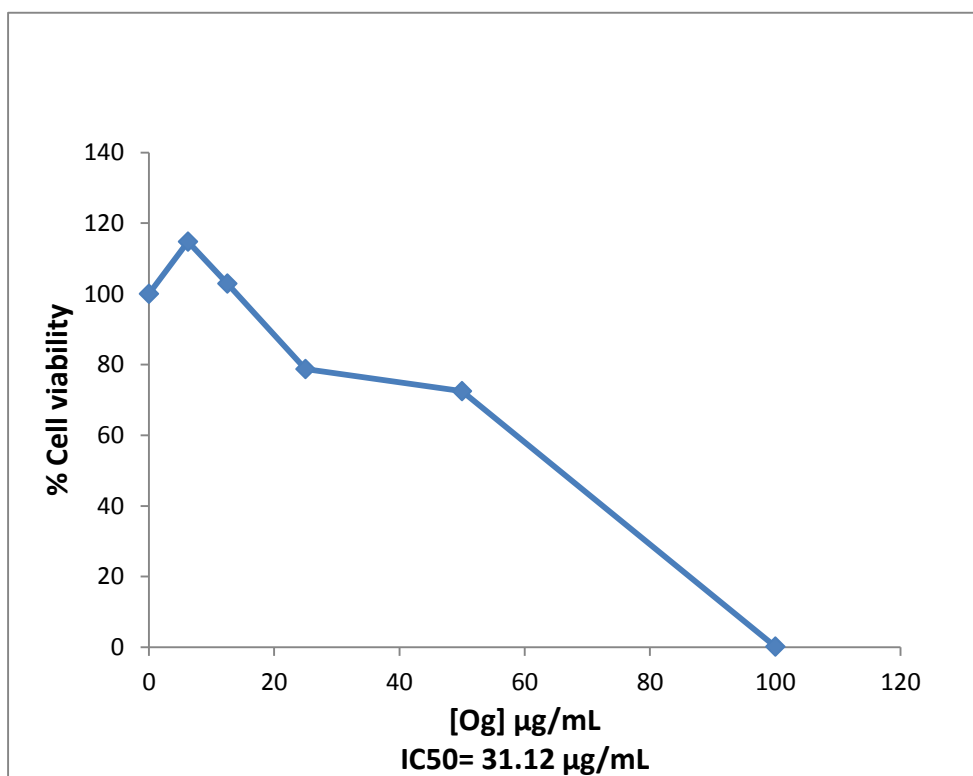


Figure 4.18 Cell viability curve of petroleum ether fraction of *Ocimum gratissimum*.

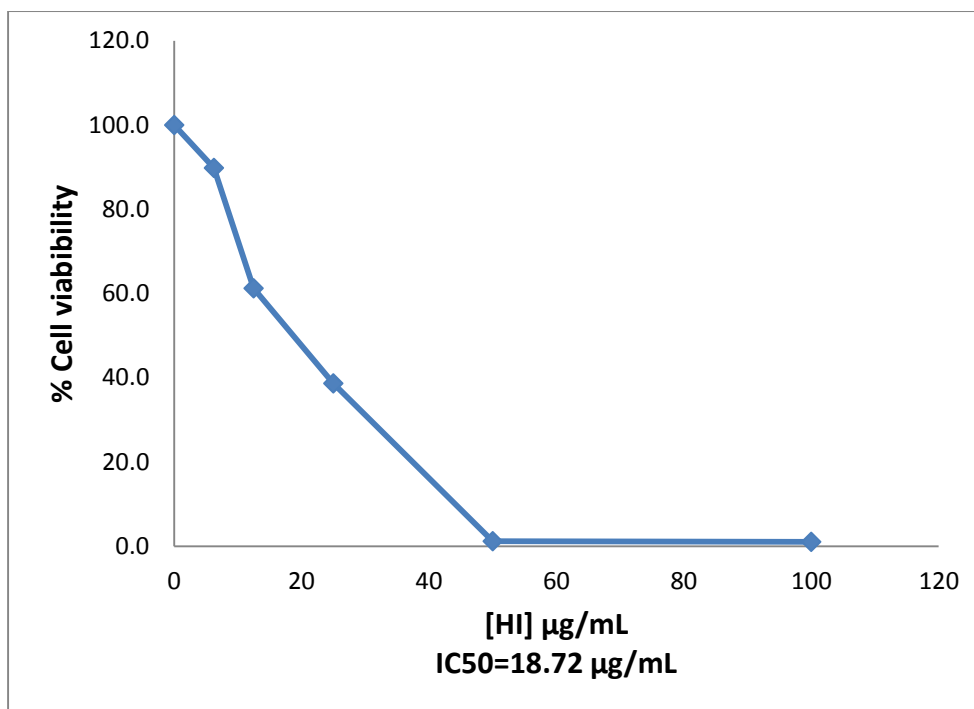


Figure 4.19 Cell viability curve of petroleum ether fraction of *H. indicum*.

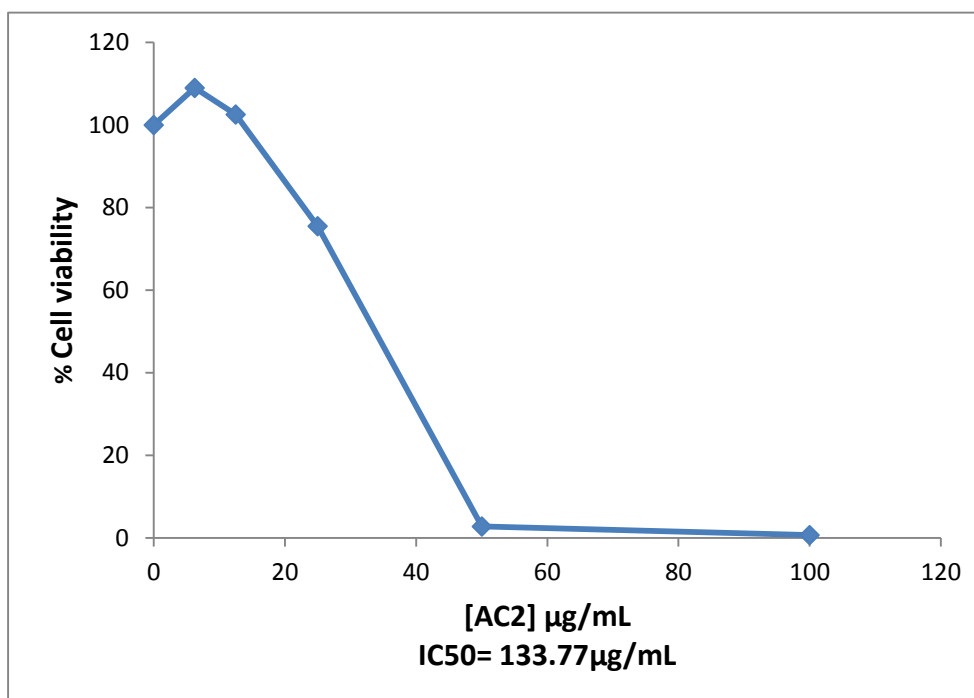


Figure 4.20 Cell viability curve of petroleum ether fraction of *Alchornea cordifolia*.

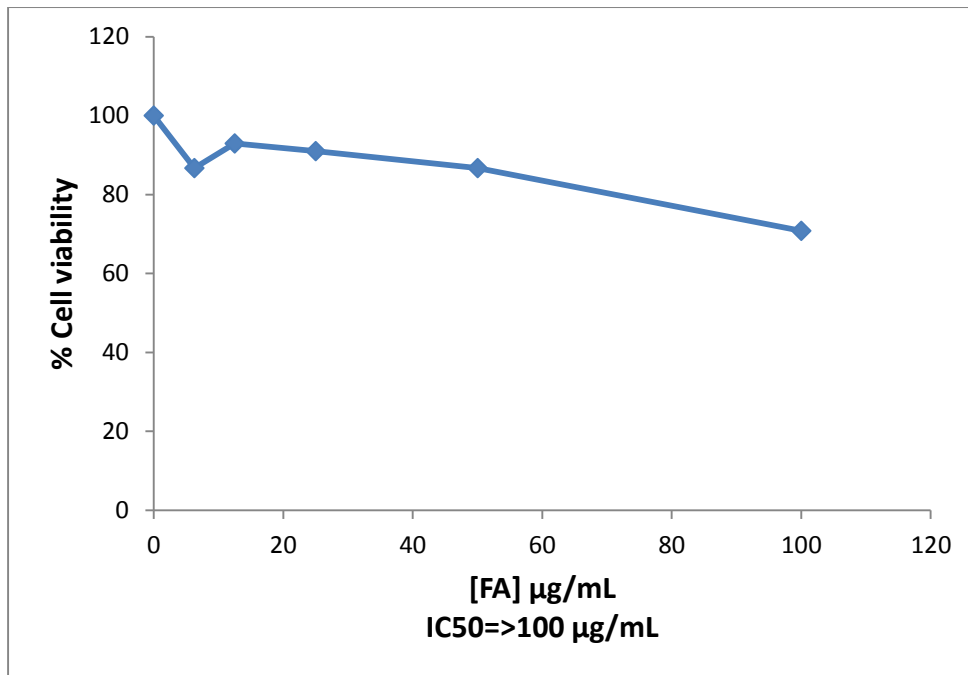


Figure 4.21 Cell viability curve of petroleum ether fraction of *Fleurya aestuans*.

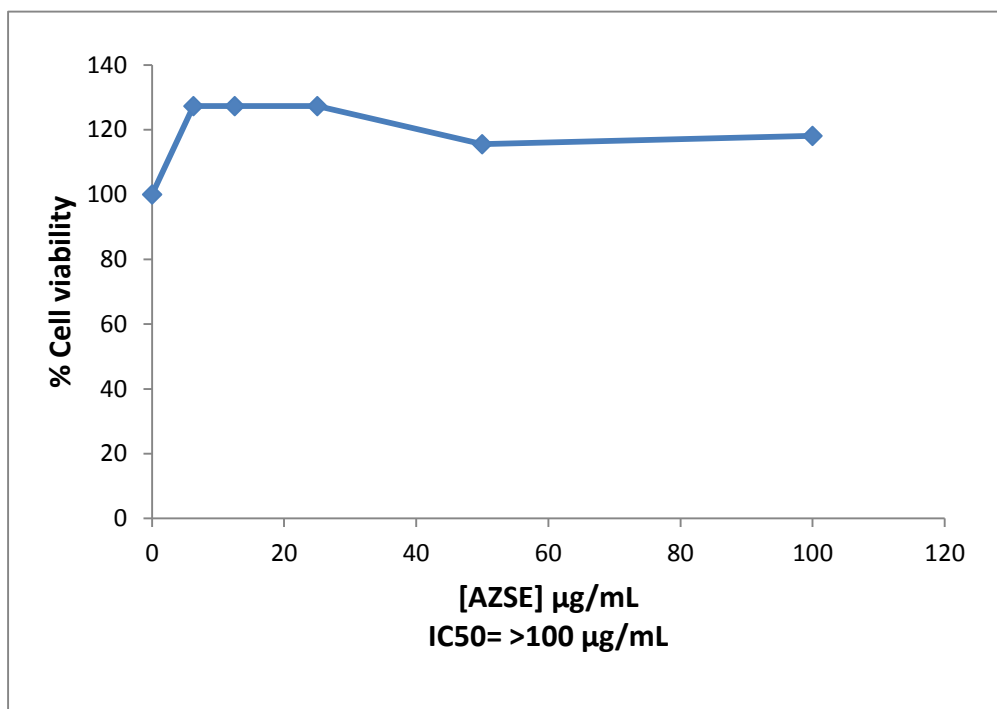


Figure 4.22 Cell viability curve of petroleum ether fraction of *Albizia zygia* stem bark.

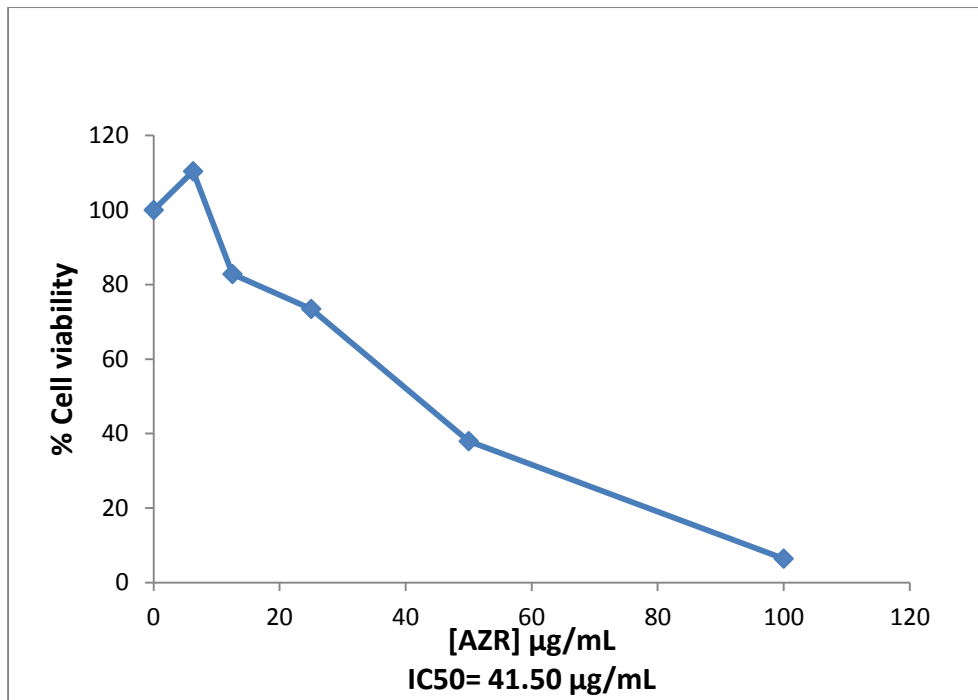


Figure 4.23 Cell viability curve of petroleum ether fraction of *Albizia zygia* root.

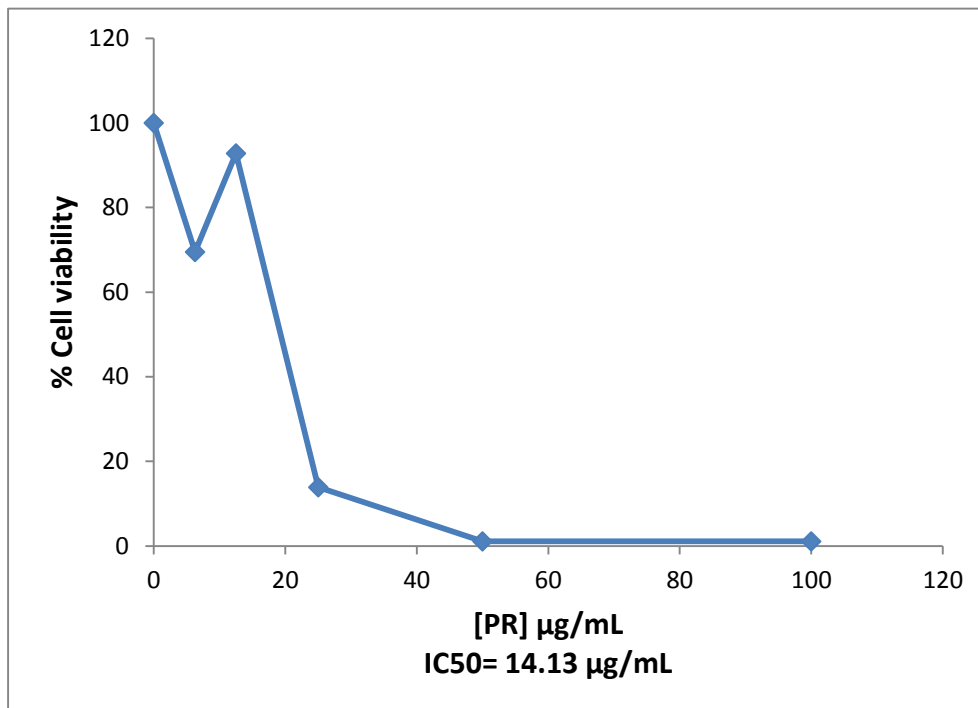


Figure 4.24 Cell viability curve of petroleum ether fraction of *P. macrocarpus* root.

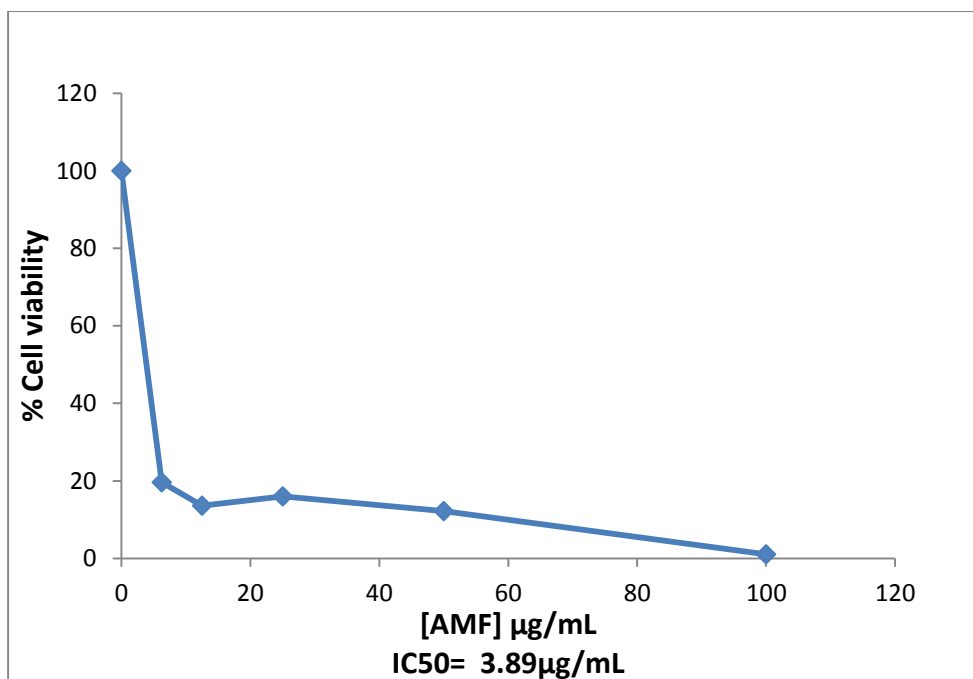


Figure 4.25 Cell viability curve of petroleum ether fraction of *Afromomum melegueta*.

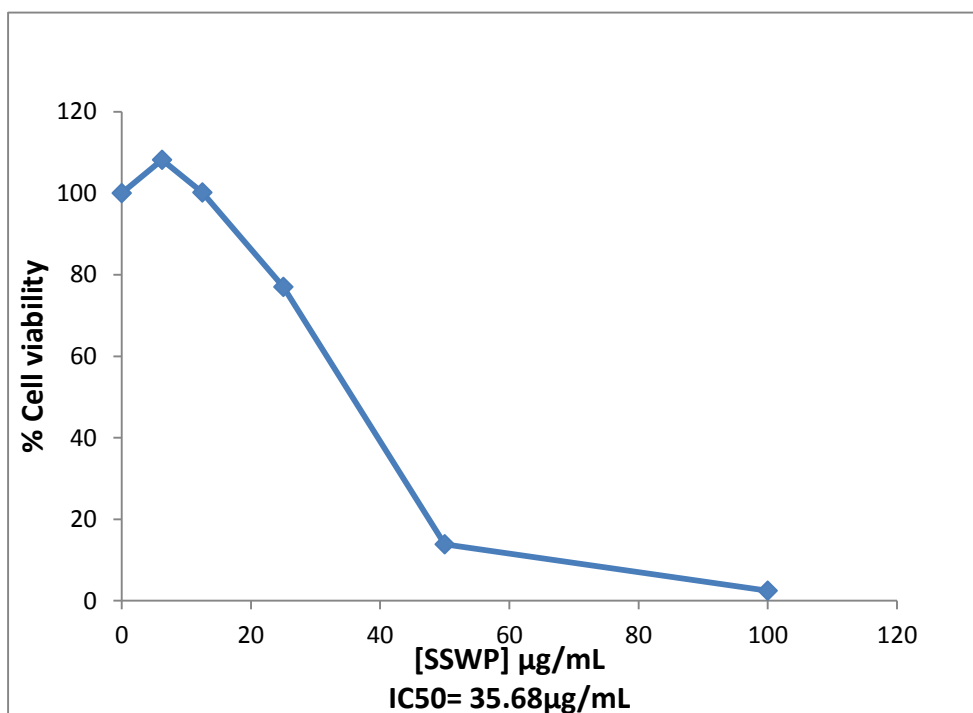


Figure 4.26 Cell viability curve of petroleum ether fraction of *S. rotundifolius*.

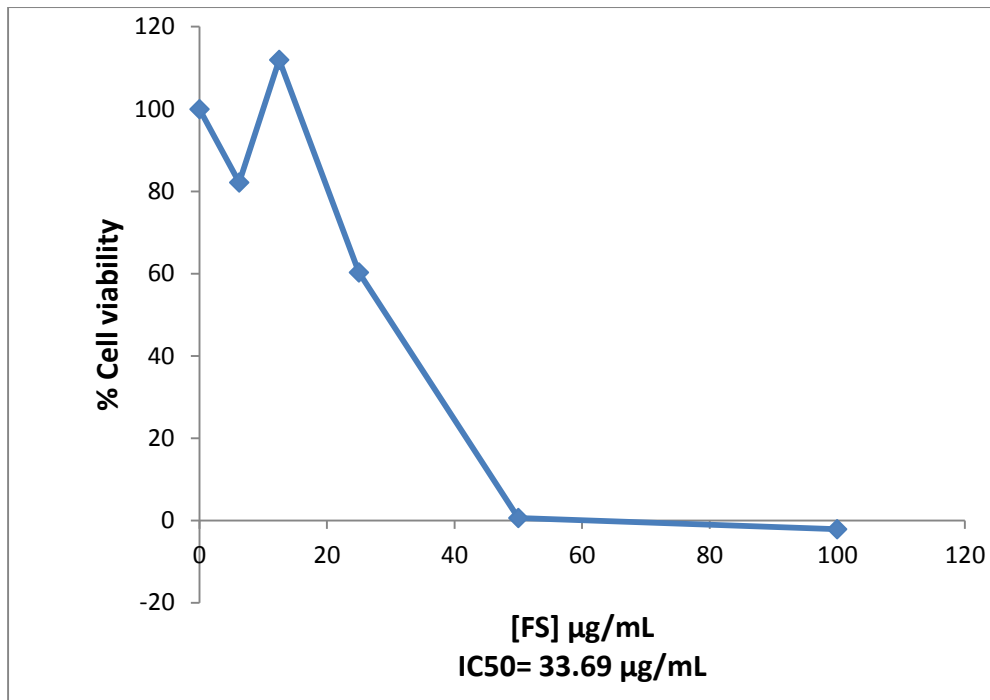


Figure 4.27 Cell viability curve of dichloromethane fraction of *Ficus exasperata*.

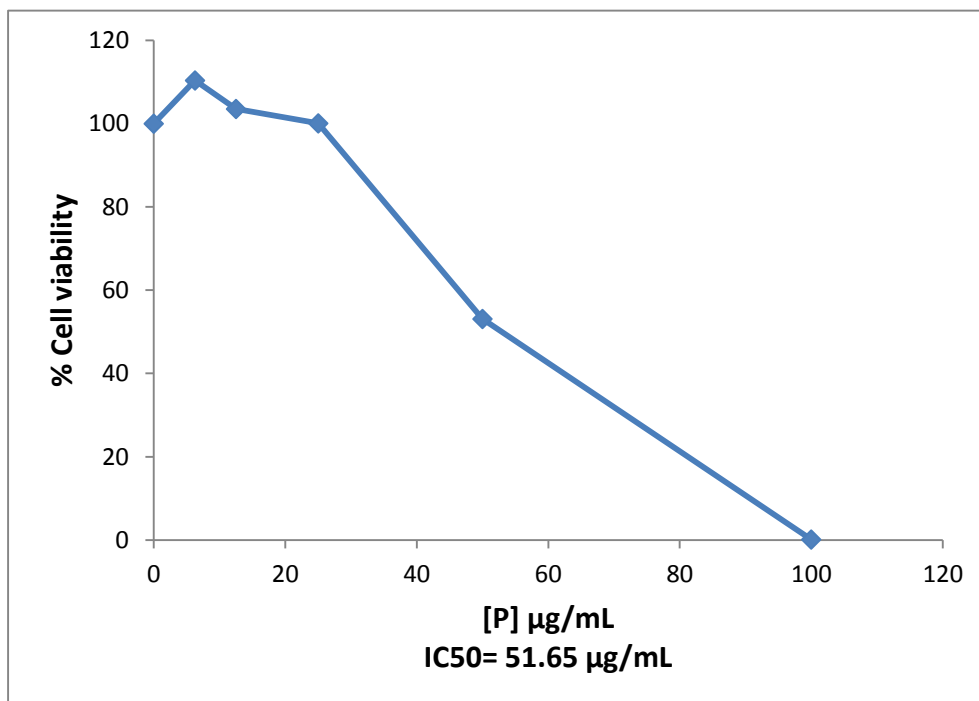


Figure 4.28 Cell viability curve of dichloromethane fraction of *P. macrocarpus* leaves.

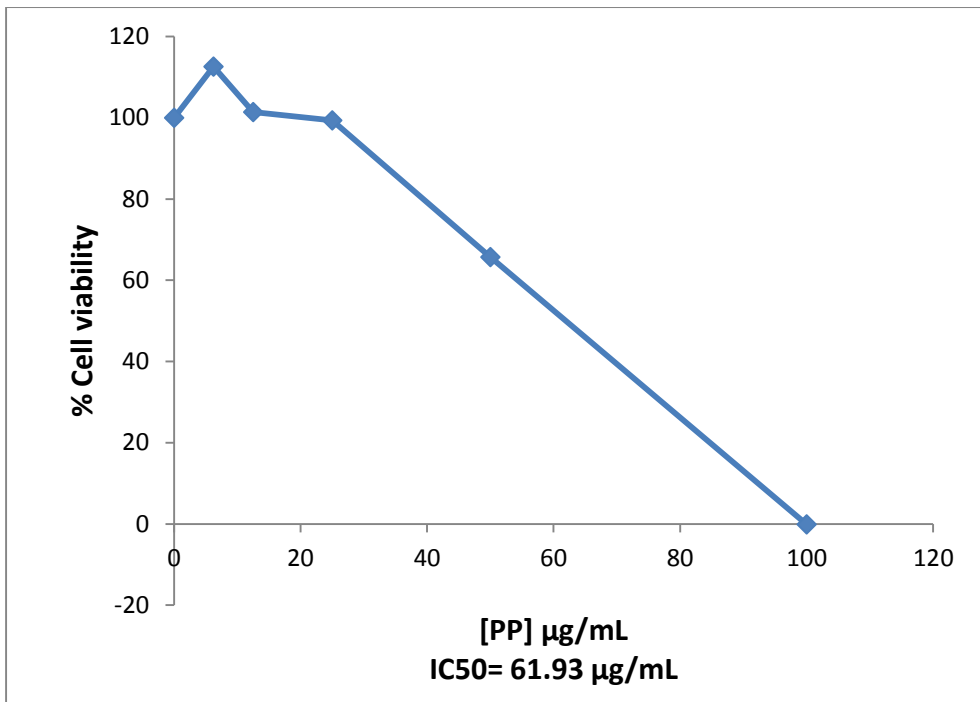


Figure 4.29 Cell viability curve of dichloromethane fraction of *Paullinia pinnata*.

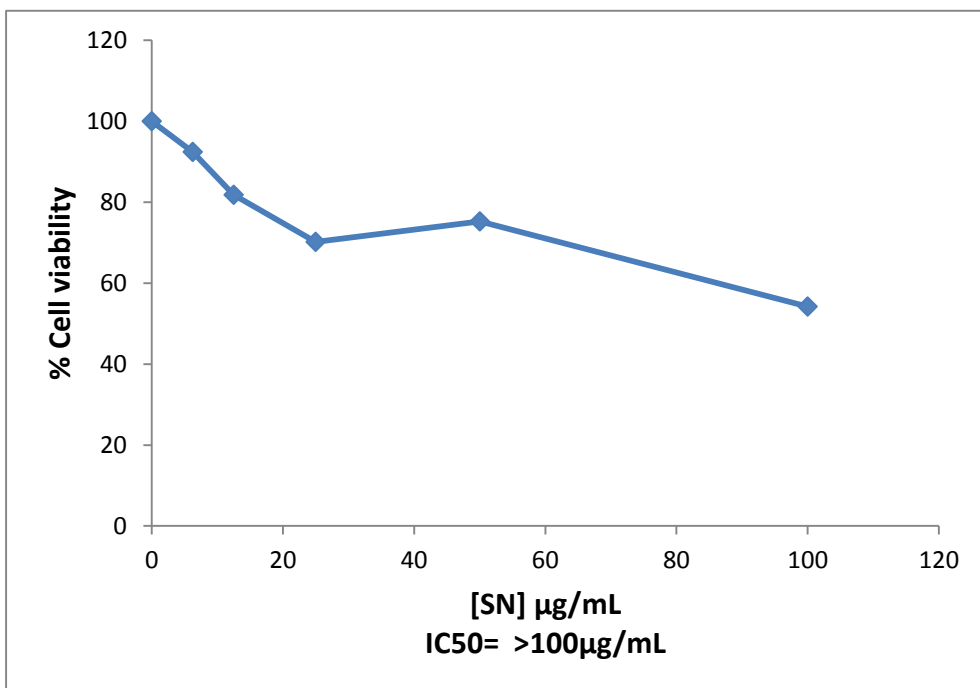


Figure 4.30 Cell viability curve of dichloromethane fraction of *Solanum nigrum*.

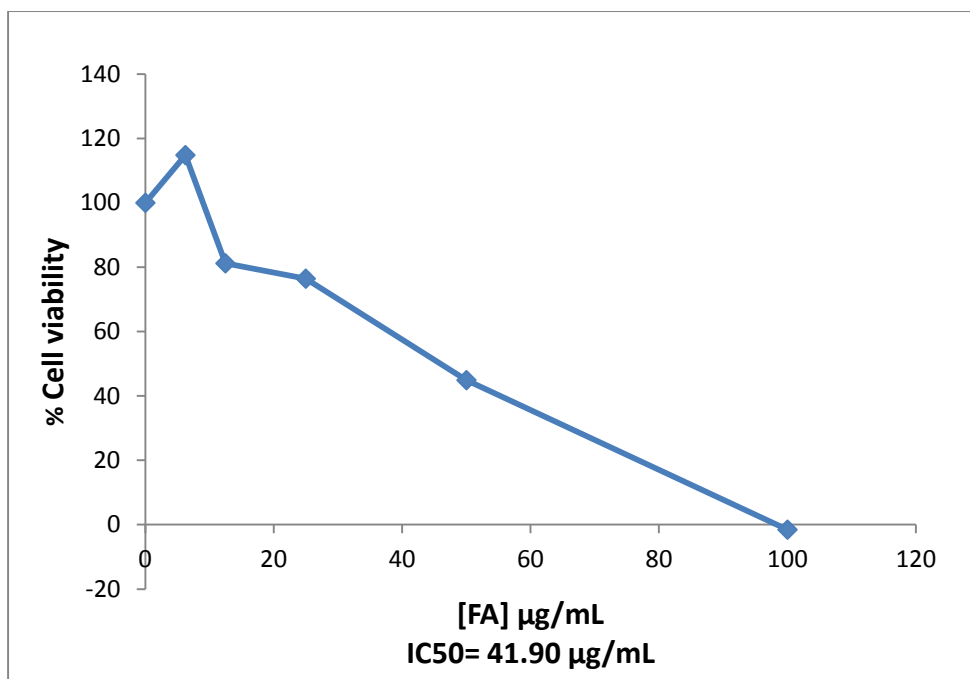


Figure 4.31 Cell viability curve of dichloromethane fraction of *Fleurya aestuans*.

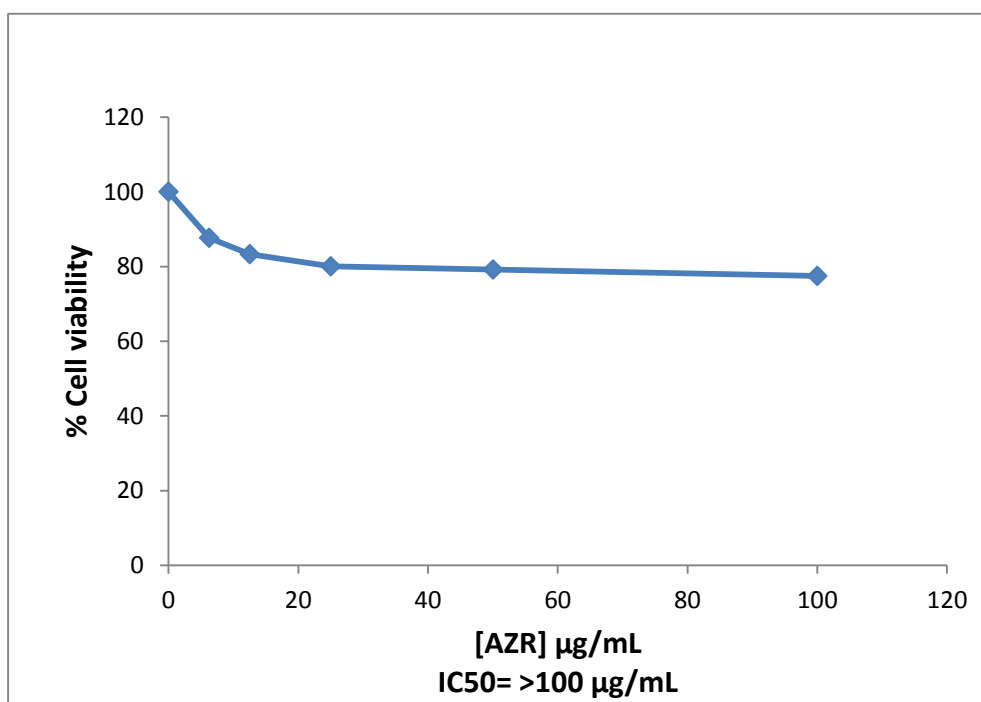


Figure 4.32 Cell viability curve of dichloromethane fraction of *Albizia zygia* root.

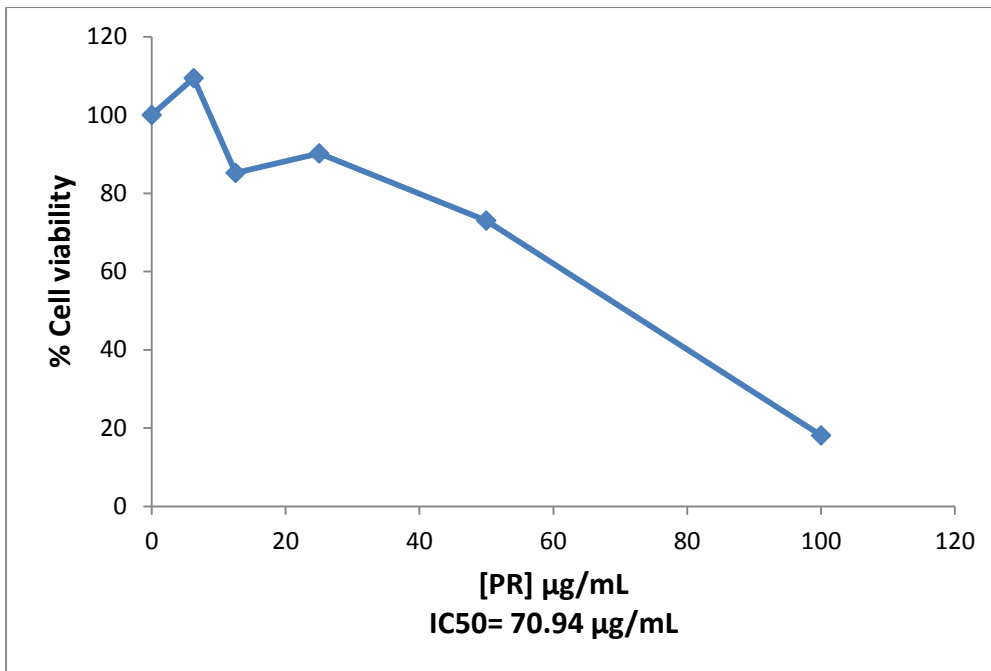


Figure 4.33 Cell viability curve of dichloromethane fraction of *P. macrocarpus* root.

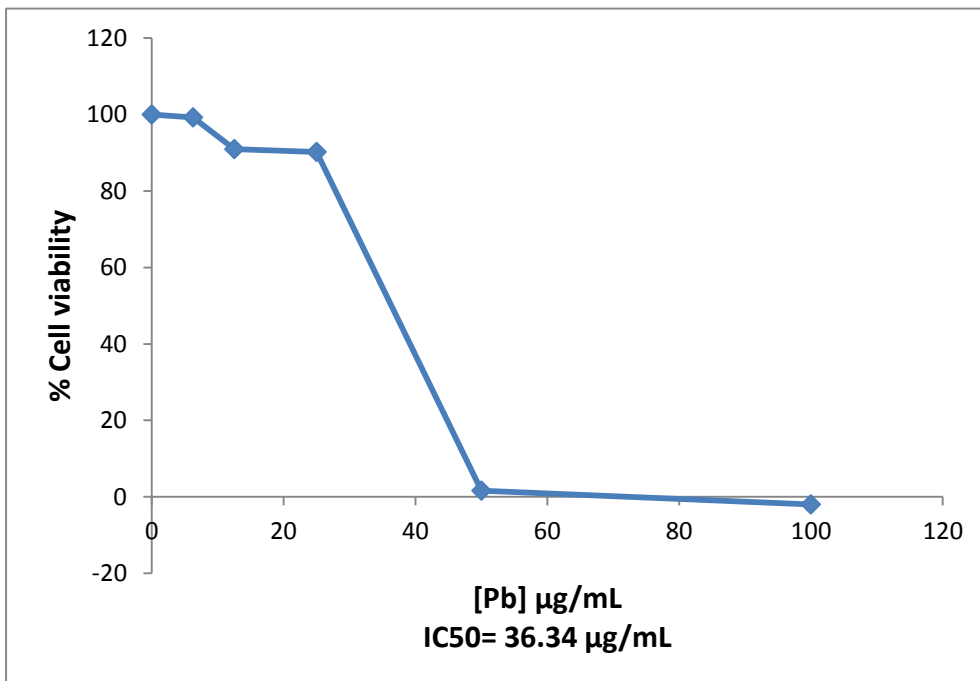


Figure 4.34 Cell viability curve of dichloromethane fraction of *P. macrocarpus* bark.

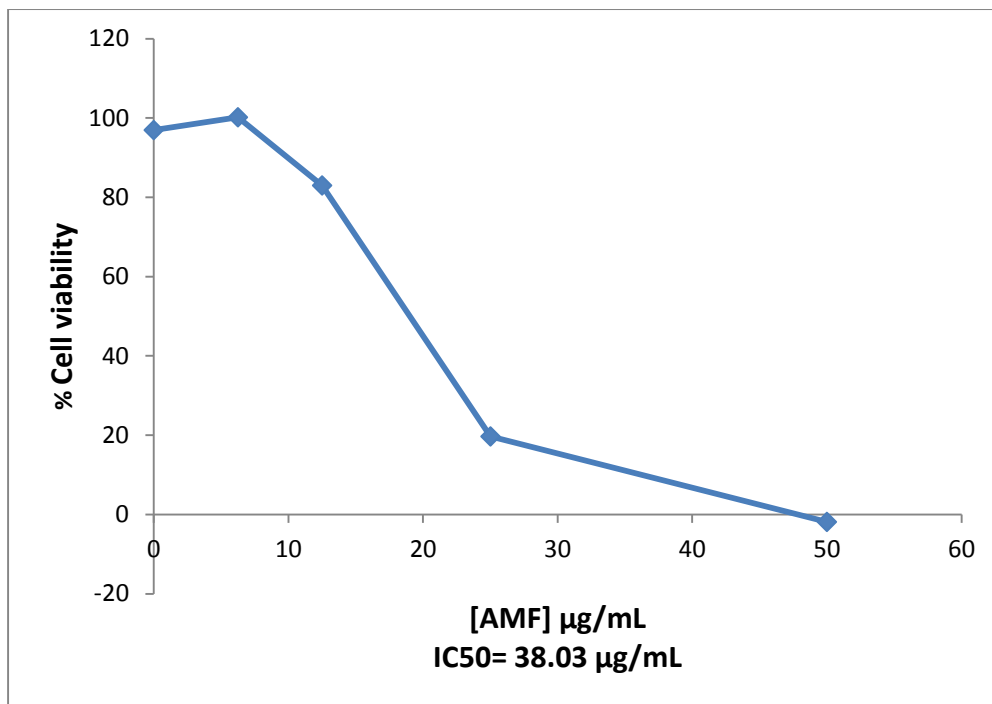


Figure 4.35 Cell viability curve of dichloromethane fraction of *Aframomum melegueta*.

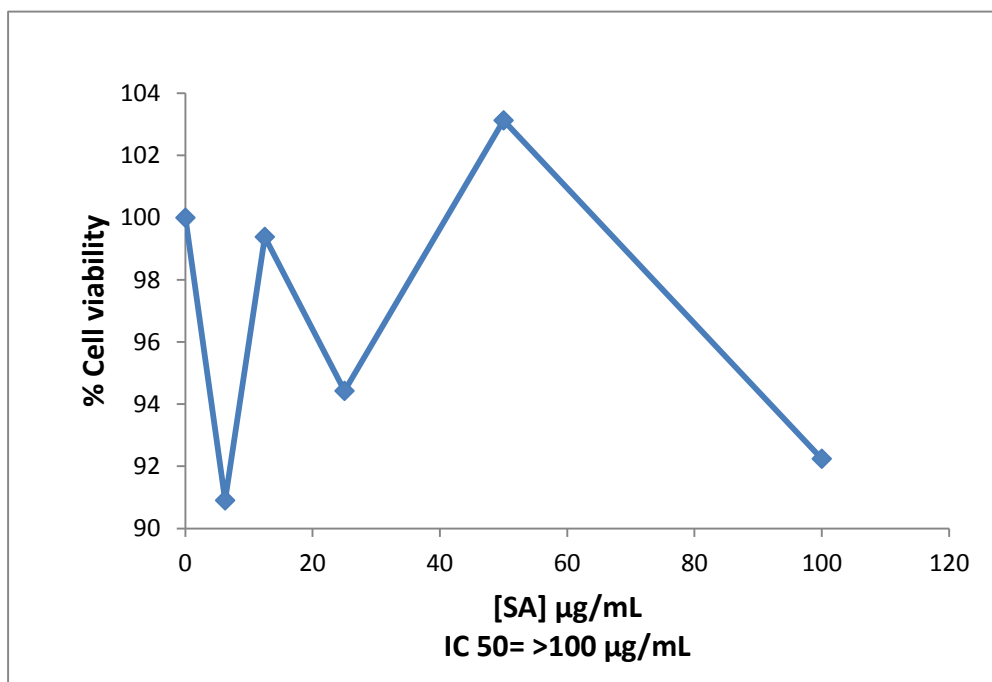


Figure 4.36 Cell viability curve of dichloromethane fraction of *Secamone afzelii*.

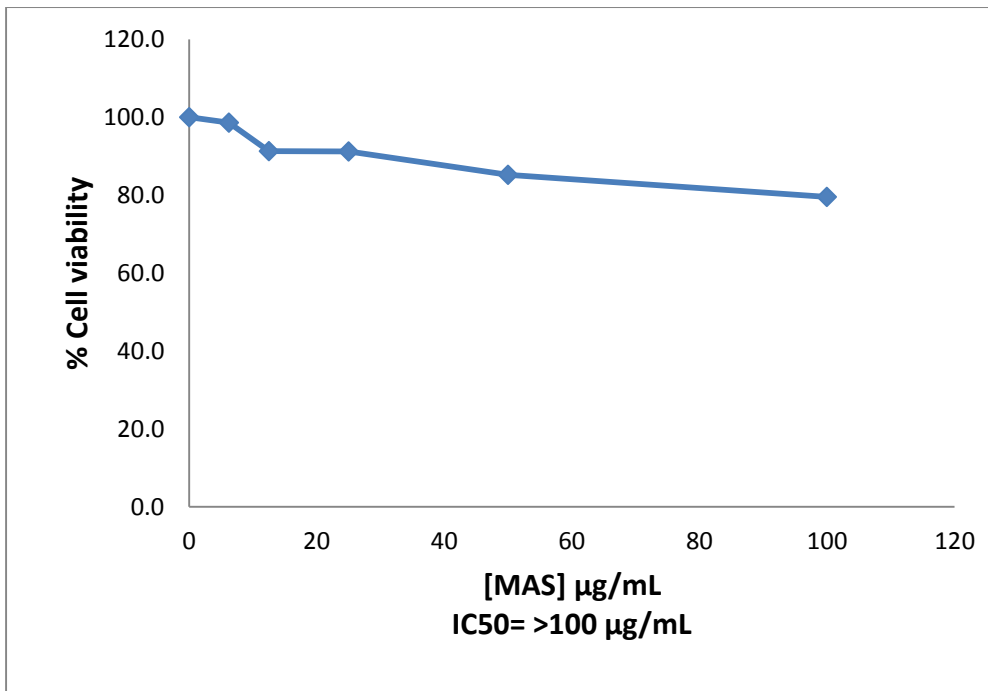


Figure 4.37 Cell viability curve of dichloromethane fraction of *M. oppositifolius*.

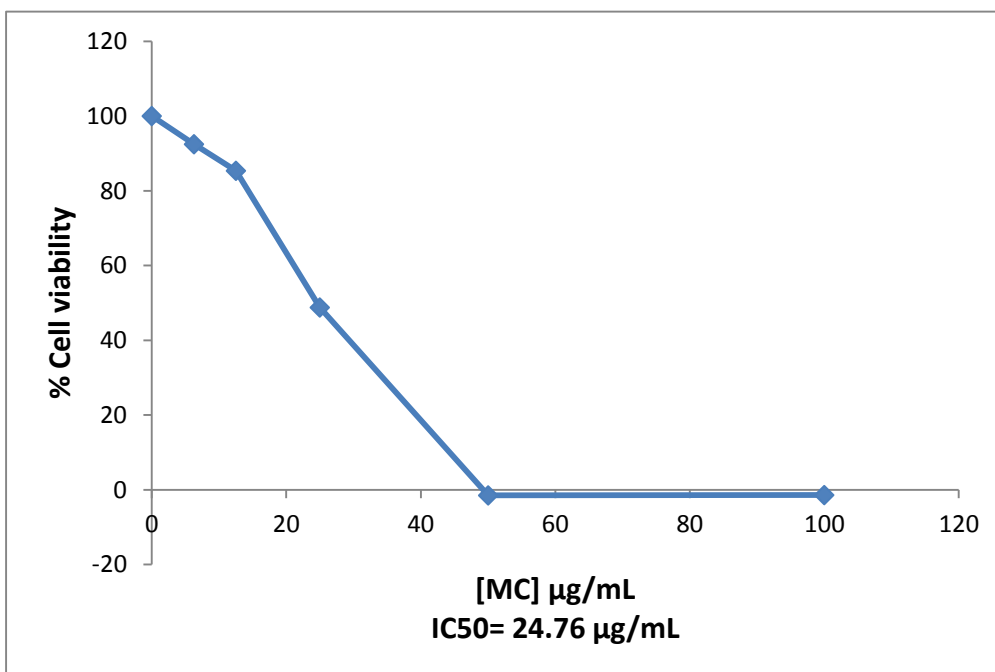


Figure 4.38 Cell viability curve of dichloromethane fraction of *Momordica charantia*.

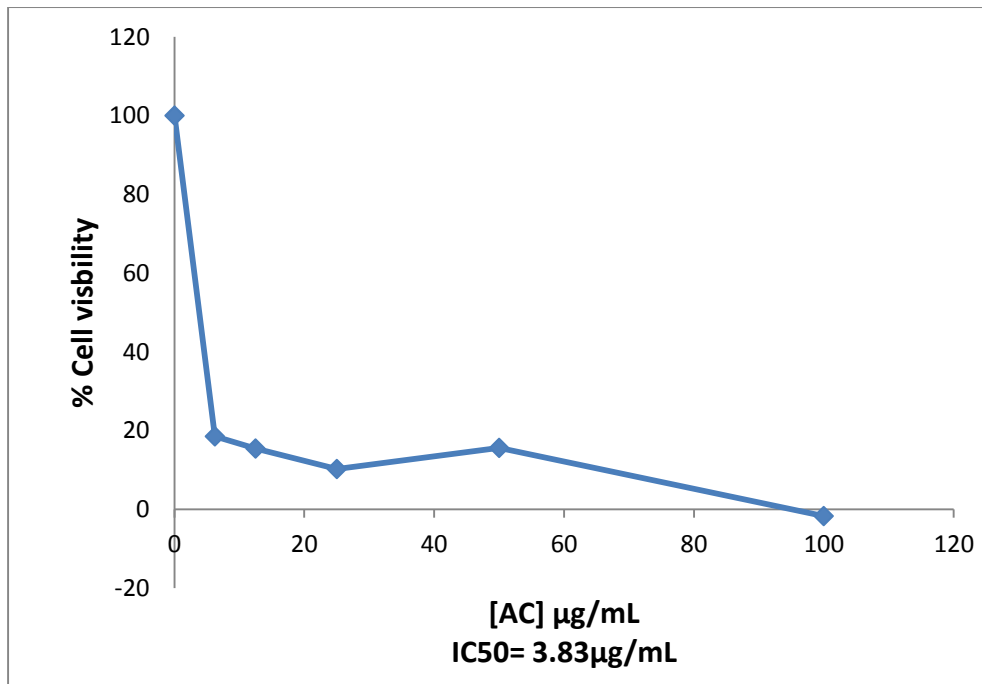


Figure 4.39 Cell viability curve of dichloromethane fraction of *Alchornea cordifolia*.

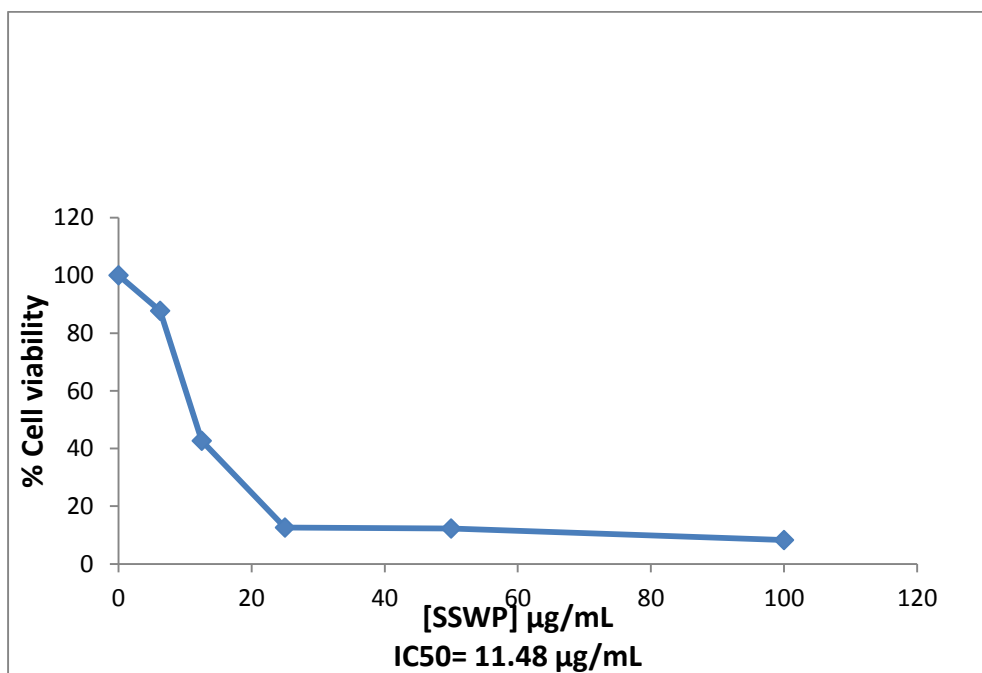


Figure 4.40 Cell viability curve of dichloromethane fraction of *S. rotundifolius*.

Table 4.1 Ethyl acetate fractions with IC₅₀ on MCF-7 breast cancer cells and PNT-2 normal prostate cells with their selectivity indices.

Plant Name	Extract Code	Plant Part	IC ₅₀ (µg/mL) ^a		Selectivity Index
			MCF-7	PNT-2	
<i>F. exasperata</i>	FS/FEE	leaves	>100	34.92 ± 1.31	<0.35
<i>A. cordifolia</i>	ACR /ArCE	root	>100	>100	<1 OR >1
<i>A. cordifolia</i>	AC/AICE	stem& leaves	41.60 ± 1.33	>100	>2.4
<i>M. charantia</i>	MC/MCE	whole plant	>100	>100	<1 or >1
<i>P. pinnata</i>	PP/PPE	leaves	>100	66.58 ± 1.46	<1
<i>O. gratissimum</i>	OG/OGE	leaves	51.19 ± 2.1	34.43	0.67
<i>H. indicum</i>	HI/HIE	whole plant	>100	>100	<1 OR>1
<i>O. canum</i>	OC/OCE	leaves	>100	>100	<1 OR>1
<i>S. nigrum</i>	SN/SONE	leaves	NA	NA	NA
<i>A. conyzoides</i>	AC2/AgCE	whole plant	NA	NA	NA
<i>F. aestuans</i>	FA/FAE	whole plant	>100	66.61 ± 1.52	<0.6
<i>A. zygia</i>	AZR/AZRE	root	>100	>100	<1OR>1
<i>A. zygia</i>	AZSE	stem bark	NA	NA	NA
<i>P. macrocarpus.</i>	PR/PRE	root	40.9 ± 1.81	49.68 ± 1.36	1.2
<i>P. macrocarpus</i>	P/PE	leaves	NA	NA	NA
<i>P. macrocarpus.</i>	Pb/PBE	bark	>100	NA	NA
<i>A. melegueta</i>	AMF/AME	fruit	98.3	>100	<1OR>1
<i>S. rotundifolius.</i>	SSWP/SOE	whole plant	>100	>100	<1OR>1
<i>S. afzelii</i>	SA/SAE	leaves	NA	NA	NA
<i>M. oppositifolius</i>	MAS/MOE	seed	NA	NA	NA

IC₅₀ < 30 µg/mL and SI ≥ 2 are significant

^a Data represent mean ± SEM of triplicates of an experiment

NA = sample unavailable

Table 4.2 Dichloromethane fractions with IC₅₀ for normal and cancer cells with selectivity indices.

Plant Name	Extract Code	Plant Part	IC ₅₀ (µg/mL) ^a		Selectivity Index
			MCF-7	PNT-2	
<i>F. exasperata</i>	FS/FEE	leaves	33.69 ± 1.34	12.17 ± 1.30	0.36
<i>A. cordifolia</i>	ACR /ArCE	root	NA	NA	NA
<i>A. cordifolia</i>	AC/AICE	stem& leaves	3.83 ± 1.22	7.19 ± 1.23	1.87
<i>M. charantia</i>	MC/MCE	whole plant	24.76 ± 1.32	66.63 ± 1.44	2.69
<i>P. pinnata</i>	PP/PPE	leaves	61.93 ± 1.41	62.17 ± 1.34	1
<i>O. gratissimum</i>	OG/OGE	leaves	NA	NA	NA
<i>H. indicum</i>	HI/HIE	whole plant	NA	NA	NA
<i>O. canum</i>	OC/OCE	leaves	NA	NA	NA
<i>S. nigrum</i>	SN/SONE	leaves	>100	>100	<1 OR >1
<i>A. conyzoides</i>	AC2/AgCE	whole plant	NA	NA	NA
<i>F. aestuans</i>	FA/FAE	whole plant	41.90 ± 1.33	82.21 ± 1.31	1.96
<i>A. zygia</i>	AZR/AZRE	root	>100	>100	<1OR>1
<i>A. zygia</i>	AZSE	stem bark	NA	NA	NA
<i>P. macrocarpus</i>	PR/PRE	root	70.94 ± 1.27	NA	NA
<i>P. macrocarpus.</i>	P/PE	leaves	51.65 ± 1.29	NA	NA
<i>P. macrocarpus</i>	Pb/PBE	bark	36.34 ± 1.34	86.79 ± 1.38	2.39
<i>A. melegueta</i>	AMF/AME	fruit	38.03 ± 1.39	50.93 ± 1.33	1.34
<i>S. rotundifolius</i>	SSWP/SOE	whole plant	11.48 ± 1.22	11.19 ± 1.41	0.97
<i>S. afzelii</i>	SA/SAE	leaves	>100	11.32 ± 1.22	<0.1
<i>M. oppositifolius</i>	MAS/ MOE	seed	>100	NA	NA

IC₅₀ < 30 µg/mL is significant and SI ≥ 2 is significant

^a Data represent mean ± SEM of triplicate of an experiment

NA = sample unavailable

Table 4.3 Petroleum ether fraction with IC₅₀ for cancer and normal cells with their selectivity indices.

Plant Name	Extract Code	Plant Part	IC ₅₀ ($\mu\text{g/mL}$) ^a		Selectivity Index
			MCF-7	PNT-2	
<i>F. exasperata</i>	CFS/FEE	Leaves	16.30 ± 1.44	24.71 ± 1.33	1.5
<i>A. cordifolia</i>	ACR/ArCE	Root	NA	NA	NA
<i>A. cordifolia</i>	AC/AICE	stem & leaves	NA	8.56 ± 1.22	NA
<i>M. charantia</i>	MC/MCE	whole plant	103.89 ± 2.30	28.34 ± 1.24	0.27
<i>P. pinnata</i>	PP/PPE	Leaves	19.30 ± 1.61	>100	>5
<i>O. gratissum</i>	OG/OGE	Leaves	31.12 ± 1.41	36.57 ± 1.38	1.17
<i>H. indicum</i>	HI/HIE	whole plant	18.72 ± 1.21	23.93 ± 1.46	1.28
<i>O. canum</i>	OC/OCE	Leaves	NA	NA	NA
<i>S. nigrum</i>	SN/SONE	Leaves	NA	NA	NA
<i>A. conyzoides</i>	AC2/AgCE	whole plant	133.77 ± 1.44	16.70 ± 1.33	0.12
<i>F. aestuans</i>	FA/FAE	whole plant	>100	37.63 ± 1.29	<0.38
<i>A. zygia</i>	AZSE	stem bark	>100	NA	NA
<i>A. zygia</i>	AZR/AZRE	Root	41.50 ± 1.25	>100	>2.4
<i>P. macrocarpus</i>	PR/PRE	Root	19.20 ± 1.37	NA	NA
<i>P. macrocarpus</i>	P/PE	Leaves	42.21 ± 1.93	37.71 ± 1.41	0.89
<i>P. macrocarpus</i>	Pb/PBE	Bark	NA	NA	NA
<i>A. melegueta</i>	AMF/AME	Fruit	3.89 ± 1.21	39.68 ± 1.26	10.2
<i>S. rotundifolius</i>	SSWP/SOE	whole plant	35.68 ± 1.39	8.56 ± 1.35	0.23
<i>Secamone afzelii</i>	SA/SAE	Leaves	NA	37.71 ± 1.24	NA
<i>Mallotus oppositifolius</i>	MAS/ MOE	Seed	NA	NA	NA

IC₅₀ < 30 $\mu\text{g/mL}$ and SI ≥ 2 are significant

^a Data represents mean \pm SEM of triplicate of an experiment

NA = sample unavailable

This section shows the results for test for apoptosis as a mechanism of induction of cytotoxicity. Figure 4.1 shows the nuclear morphology of the MCF-7 cells treated with petroleum ether fraction of *A. melegueta*. The condensed nucleus and fragmented DNA of some of the cells is indicative of induction of apoptosis in the cells as a mechanism of cytotoxicity. The number of cells with condensed nucleus in (d) is more than that in (c), followed by (b) and then (a) which is the negative control.

Figure 4.2 equally demonstrates a concentration dependent activity of petroleum ether fraction of *A. melegueta* on the MCF-7 breast cancer cell line. When the concentration of *A. melegueta* was increased from 0 to 4 $\mu\text{g/mL}$, the early apoptotic cells and late apoptotic cells increased, respectively, shown in graphs (a), (b) and (c). Graph (d) is the positive control, curcumin at 2.9 $\mu\text{g/mL}$ concentration and demonstrated the highest number of early apoptotic and late apoptotic cells. The negative control contained 1% DMSO as the 0 $\mu\text{g/mL}$ concentration. This lends evidence to the induction of apoptosis as a possible mechanism of action of cytotoxicity by the *A. melegueta* fraction.

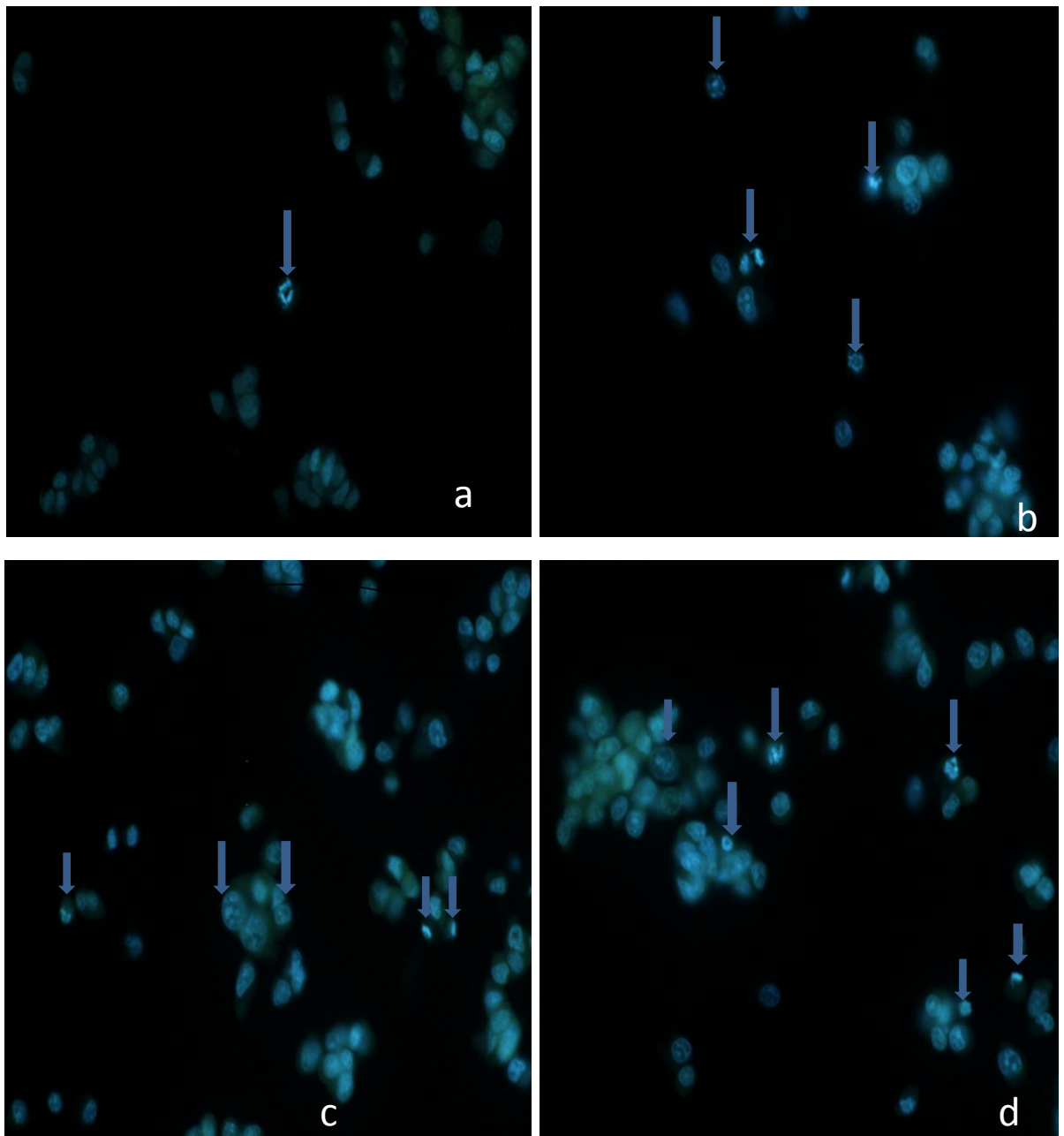


Figure 4.41 Nuclear morphology of cells in early apoptosis using Hoechst stain.

Apoptotic cells are indicated with arrows showing condensed and fragmented nucleus. Fig. a, b and c represent negative control (0 µg/mL), 2 µg/mL and 4 µg/mL respectively of petroleum ether fraction of *A. melegueta*. Fig. d is urosoic acid used as positive control.

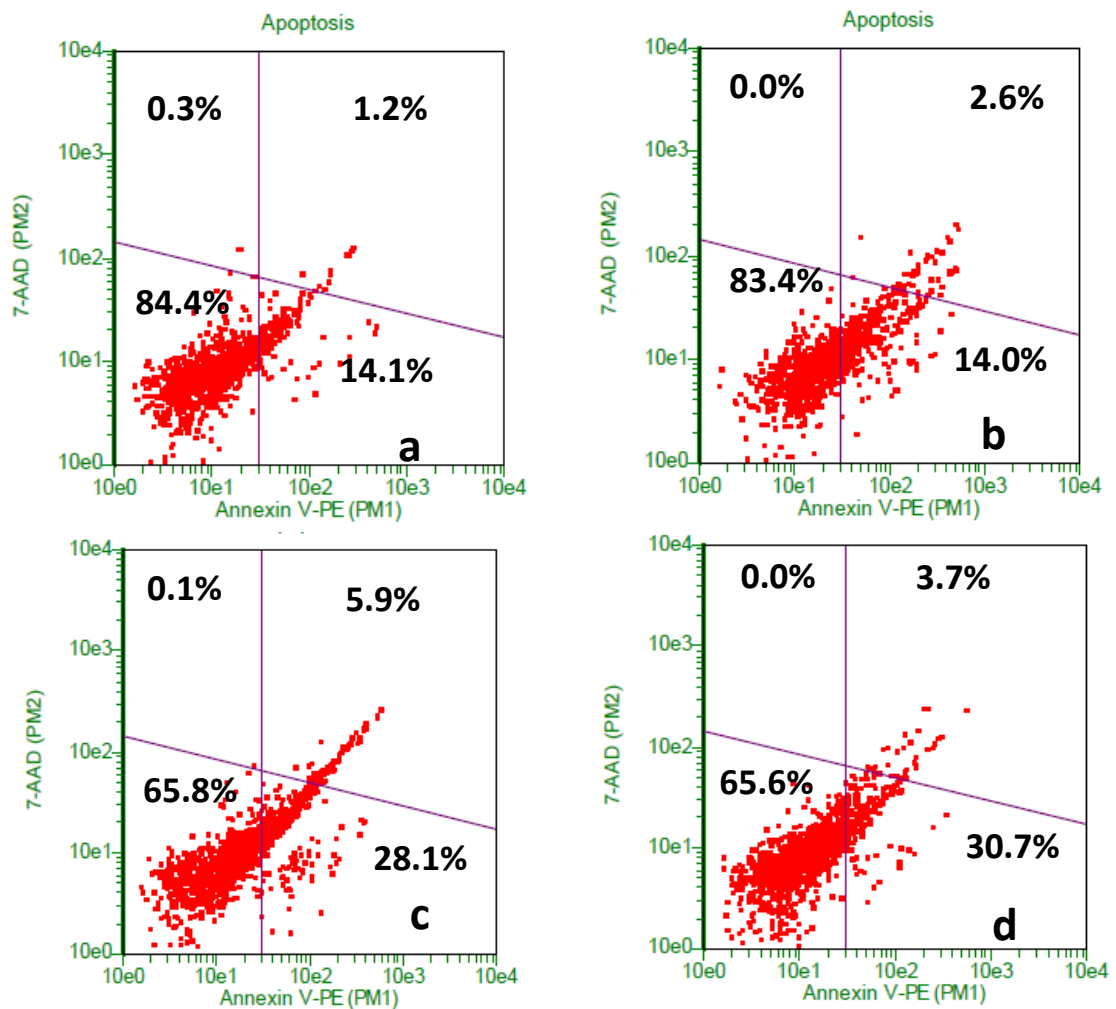


Figure 4.42 Contour diagram of FITC-Annexin V /7-AAD flow cytometry of MCF-7 breast cancer cells after treatment with petroleum ether fraction of *A. melegueta*.

The graphs a, b and c are the negative controls (0 $\mu\text{g/mL}$), 2 $\mu\text{g/mL}$, and 4 $\mu\text{g/mL}$, respectively. Graph d is curcumin at 2.9 $\mu\text{g/mL}$ concentration (positive control). The lower left and right of the quadrant represents viable cells and early apoptotic cells, respectively and the upper left and right of the quadrant represent necrotic and late apoptotic cells, respectively.

Table 4.4 Yield of crude extracts of *Moringa oleifera* leaves

Extracts	Mass (g)	% Yield
Aqueous	15.3	30.5
50% Ethanolic	7.2	71.8

Yield was calculated as w/w of the leaves

Table 4.5 Total phenolic content of *Moringa oleifera* leaf extracts.

The total phenolic content, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to the standard curve ($y = 1.4168x + 0.0623$)

Extracts	mg GAE/g	P-value
Aqueous	184.6 ± 24.5	< 0.005
50% Ethanolic	177.4 ± 17.4	<0.005

Values are reported as mean ± the standard deviation of three experiments and compared with standard gallic acid

Table 4.6 Antioxidant activity of the *Moringa oleifera* leaf extracts expressed as the EC₅₀

Extract	EC ₅₀ (mg/mL)	P-value
Aqueous	3.1 ± 0.3	< 0.0005
50% Ethanolic	3.0 ± 0.2	< 0.0005
BHT (control)	0.053 ± 0.002	< 0.0001

Values are mean ± standard deviation of triplicates and compared with positive control p<0.0005 is significant.

This section shows the results for cytotoxic activity of the crude extracts of *M. oleifera* on the MCF-7 breast cancer cell line. The graph in Figure 4.43 shows a concentration dependent activity of the crude extracts on the breast cancer cell line. There is an initial sharp decline in % cell viability as the concentration of the extract is increased followed by a steady decrease in % cell viability as the concentration increases. The IC₅₀ value is > 1000 µg/mL indicative of weak activity of the *M. oleifera* extracts on the MCF-7 breast cancer cell line. The IC₅₀ and SI could not be calculated due to the weak antiproliferative activity of the *M. oleifera* leaf extracts. Figure 4.44 is the positive control, curcumin used for the experiment with IC₅₀ = 4.53 µg/mL.

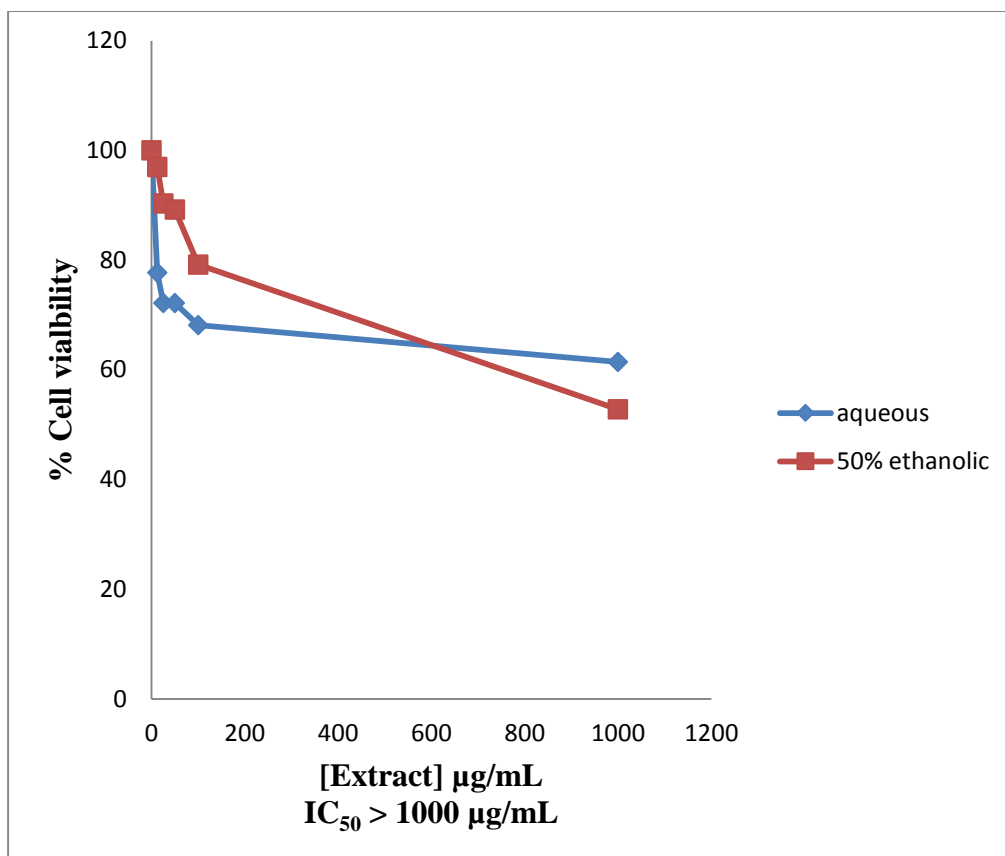


Figure 4.43 Cell viability assay showing the cytotoxic effect of *Moringa oleifera* leaf extracts on MCF-7 breast cancer cells.

The extracts exhibited a dose dependent activity on the MCF-7 breast cancer cell line however the IC₅₀ was high (> 1000 µg/mL)

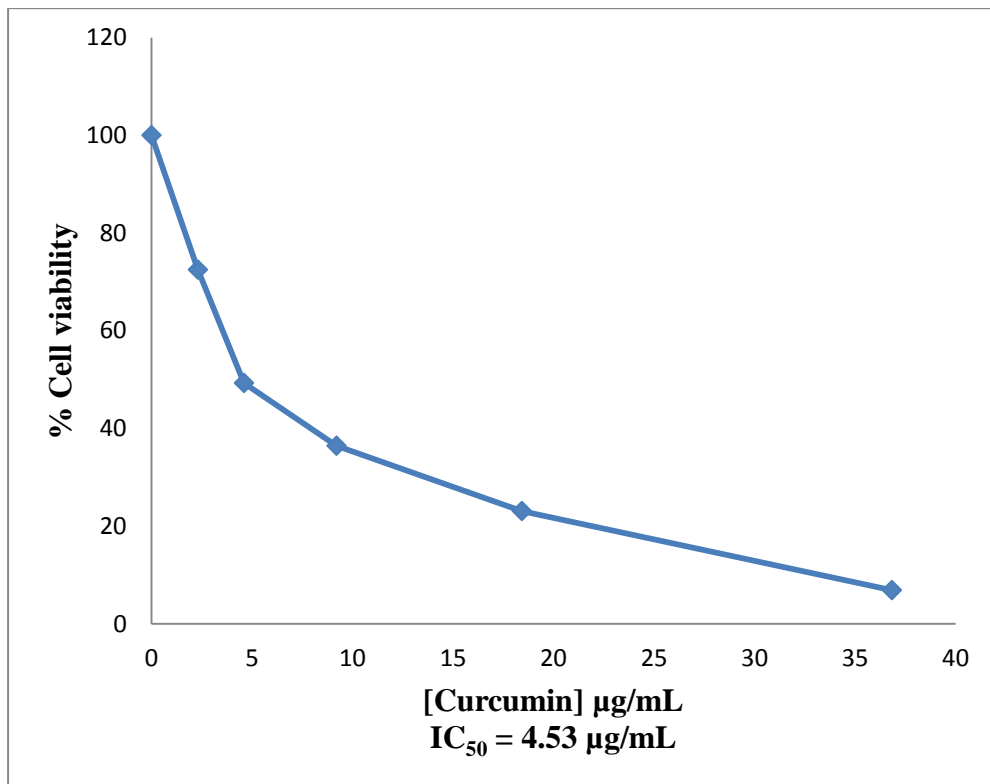


Figure 4.44 Cell viability curve showing a dose dependent activity of the positive control curcumin on MCF-7 breast cancer cells.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study sought to investigate the anti-breast cancer potential of some Ghanaian Traditional Medicinal plants on MCF-7 breast cancer cell line using MTT assay. The MTT assay together with other cytotoxicity assays have been employed in the study of antiproliferative activity of plant extracts (Adekunle & Ikupamayi, 2006; Vega-Avila & Pugsley, 2011; Fred-Jaiyesimi & Anthony, 2011; Jafarian *et al.*, 2014). Preliminary studies on some of the medicinal plants have indicated their cytotoxicity towards pathogenic bacteria and other cancer cell lines (Carceres *et al.*, 1992; Bharali *et al.*, 2003; Mensah *et al.*, 2006; Bayor *et al.*, 2007; Jafarian *et al.*, 2014). Bayor and others (2007) examined some traditional medicinal plants and identified their cytotoxicity towards MCF-7 cells in addition to other cancer cell lines. This study evaluated the antiproliferative potential of a total of 40 fractions comprising 14 fractions of ethyl acetate, 14 fractions of dichloromethane and 12 fractions of petroleum ether from 16 Ghanaian Traditional Medicinal plant species employed in treating cancers and other diseases for decades. The results of the cell viability graphs indicates a concentration dependent activity of the dichloromethane fractions against the MCF-7 breast cancer cell line and the PNT-2 prostate normal cells *in vitro*. Similar patterns were observed for the other fractions of ethyl acetate, dichloromethane and petroleum ether (refer to Figures 4.1 to 4.40). Thus as the concentration of the fraction increases, the percentage viability of the MCF-7 breast cancer cell line decreases. There is strong evidence of antiproliferative activity of the plant species and their parts as demonstrated by the cell viability graphs. The fractions with strong cytotoxic activity are comparable to the positive control used which is curcumin with an established cytotoxicity against

cancerous cells. The results obtained are comparable to that observed in literature (Bayor *et al.*, 2007). The established phytochemicals isolated from the medicinal plants could also account for the observed cytotoxicity on the breast MCF-7 cells (Olugbade *et al.*, 2000; Umukoro & Ashorobi, 2007; Ukeh *et al.*, 2009). Such phytochemicals have been reported to induce cytotoxic activity on other cancer cell lines (Heo, 2004).

From Table 4.1, the IC₅₀ values estimated for the ethyl acetate fractions were > 30 µg/mL, for all the fractions, with majority (79%) recording IC₅₀ values > 100 µg/mL. The American National Cancer Institute (NCI) stipulated that the IC₅₀ value for potent cytotoxic effect is IC₅₀ < 30 µg/mL (Itharat *et al.*, 2004). Since, the IC₅₀ values obtained for the ethyl acetate fractions were greater than the stipulated value, the fractions could be considered to have weak cytotoxic effect. Apart from *P. macrocarpus*, *A. cordifolia* and *O. gratissimum* with IC₅₀ values of 40.9 µg/mL, 41.6 µg/mL and 51.19 µg/mL, respectively, the remaining plant species recorded larger values. This suggests that the ethyl acetate solvent was not effective at extracting bioactive principles present in the plant species (Aliyu *et al.*, 2014). Additionally, the cytotoxic effect of the ethyl acetate fractions on the PNT-2 normal prostate cell line is insignificant indicated by a high IC₅₀ values for the normal prostate cells (>100 µg/mL). Thus the ethyl acetate fractions of the various plant species do not contain potent antiproliferative compounds and so do not exhibit promising anticancer activity. According to Aliyu *et al.* (2014) the compounds that are present in the ethyl acetate fraction are mostly tannins, flavonoids, steroids/terpenes. The possibility of these compounds present would be useful in other reactions but unable to induce a cytotoxic effect on the MCF-7 breast cancer cells. Hence they cannot offer as possible leads for the discovery of active plant components for drug research since they are unable to induce effective antiproliferative activity.

On the other hand, potent antiproliferative activities were exhibited by the dichloromethane and petroleum ether fractions as observed in Tables 4.2 and 4.3, respectively. Some of the fractions demonstrated promising bioactivity on the MCF-7 breast cancer cells. The dichloromethane fractions for some plant species had IC_{50} values $< 30 \mu\text{g/mL}$ which falls within the range stipulated by the NCI. For example the IC_{50} values for *A. coridifolia*, *M. charantia*, and *S. rotundifolius* were $3.83 \mu\text{g/mL}$, $24.76 \mu\text{g/mL}$ and $11.48 \mu\text{g/mL}$, respectively, will be of great interest since at these concentrations, fifty percent of the cancer cells are killed by the active compounds present in the fractions. This also suggests that the solvent was effective at extracting the active principles present in the plant extracts. Thus, the three fractions exhibited potent cytotoxic effect on the breast cancer cells. The fraction for *A. cordifolia* was the most bioactive followed by *S. rotundifolius* and then by *M. charantia*. The selectivity indices for the three most bioactive fractions were however not good for *S. rotundifolius* with a value of approximately one. Nevertheless further research can be conducted on the three plant species and fractions to study the effects of pure compound isolated from these plants on both cell lines. A promising candidate for potential drug development and pharmacognosy must have a selectivity index (SI) value that is ≥ 2 in order to prevent excessive cytotoxic activity on the normal cells. The dichloromethane fractions of *S. rotundifolius* and *M. charantia* could be investigated for cytotoxicity on other cancer cell lines. These plants may pose a challenge due to their SI values being less than two suggesting that the fractions possess deleterious activity on the normal cells. In order to address this challenge further research on isolation and characterization must be done.

The petroleum ether fractions demonstrated cytotoxicity that was comparable to that of dichloromethane with different plant species involved. This suggests that the petroleum

ether solvent was effective at extracting the active principles present in the extracts. Fractions from five plants namely *F. exasperata*, *P. pinnata*, *H. indicum*, *P. macrocarpus*, and *A. melegueta* had shown cytotoxicity with IC₅₀ values < 30 µg/mL and varying SI values. With the exception of *P. pinnata* and *A. melegueta*, the other bioactive fractions had SI values less than two which suggests toxic effect on normal cells. In order to employ them as drug candidates, the extreme cytotoxicity on the normal prostate cells must be assuaged. The SI value for *P. macrocarpus* could not be calculated as there was no IC₅₀ value of the fraction on normal PNT-2 cell line. In the case of *P. pinnata* and *A. melegueta*, their activities on the normal human cell line yielded SI values >5 and 10.2, respectively. Thus, they can serve as potential candidates for further research and isolation to fully characterize the active principles as possible drug leads.

The test of apoptosis has been employed to study the mechanism of action of bioactive compounds (Xing *et al.*, 2011; Koopman *et al.*, 1994). The process involves a change in refractive index of the cell followed by cytoplasmic shrinkage and nuclear condensation, blebbing of the cell membrane and formation of “apoptotic bodies” (Kerr *et al.*, 1972; Hengartner, 1997). In this study, two hallmarks of apoptosis which is represented by chromatin condensation and nuclear fragmentation is observed in the results of the Hoechst solution staining of the MCF-7 breast cancer cells. In Figure 4.41 the nuclear condensation reaction using the Hoechst stain demonstrated a concentration dependent activity of the *A. melegueta* fraction on the MCF-7 breast cancer cells as well as apoptosis for the possible mechanism of drug action. In addition, the cells in early apoptosis are 14% and 28.1% for the 2 µg/mL and 4 µg/mL of petroleum ether fraction of *A. melegueta*, respectively as shown in Figure 4.42. Careful examination of the figure reveals a concentration dependent activity of the bioactive fraction on the cells, as the

higher concentration has more cells in early apoptotic stage than the lower concentration. The results obtained in the study are comparable to that of the positive control and that reported by Xing and others (2011). The positive control (curcumin at 2.9 µg/mL) also exhibited a concentration dependent activity. Curcumin recorded 30.7% of the cells in early apoptosis being the highest percentage of cells in early apoptosis. The reason being that curcumin was a pure compound with established cytotoxicity unlike the petroleum ether fraction of *A. melegueta*. The pathway involved in the induction of apoptosis could be any of the two established processes; extrinsic or intrinsic involving mitochondrial membrane through activation of caspases and transduction of the signal downstream (Brentnall *et al.*, 2013). The extrinsic pathway could involve the interaction of the active principle with death ligand receptors on the MCF-7 breast cancer cell membranes which could transduce signals downstream to activate caspase 8 leading to downstream activation of other factors (Launey *et al.*, 2005). Further research can be advanced in this regard to establish the detailed pathways involved in the induction of apoptosis.

Several methods have been employed in the extraction of plant extracts for studies (Sreelatha & Padma, 2009; Bayor *et al.*, 2007; Jafarian *et al.*, 2014). Sreelatha and Padma (2009), for example obtained a yield of 10% for the aqueous extracts and 19.6% was recorded by Jafarian *et al.* (2014) for the hydromethanolic extracts. The extraction of *M. oleifera* in this study yielded a high amount of the crude extracts of 30% and 71.8% of the aqueous hydroethanolic extracts, respectively. These values are higher than those observed in literature. This suggests that the method is effective at giving higher yield. However, some heat sensitive polyphenols like anthocyanins may be lost as a result of the heating process during the aqueous extraction (Dai & Mumper, 2010). These heat sensitive polyphenols are lost at temperatures beyond 70°C (Dai & Mumper,

2010). Several studies have estimated the antioxidant properties of leaf extracts of *M. oleifera* (Ramachandran *et al.*, 1980; Sreelatha & Padma 2009; Jafarian *et al.*, 2014). The antioxidant assays enable the estimation of free radical scavenging ability of compounds (Sreelatha & Padma, 2009). The effective concentration values obtained for the aqueous and hydroethanolic extracts demonstrated the ability of the extracts to readily scavenge reactive oxygen species and offer protection to living tissues during physiologic processes (Sreelatha & Padma, 2009; Ramachandran *et al.*, 1980). The antioxidant activities of the *M. oleifera* extracts were lower compared to that of BHT, as the EC₅₀ of BHT was 56 times lower than that of the extracts which recorded 3.0 mg/mL and 3.1 mg/mL for the aqueous and hydroethanolic extracts, respectively. This clearly shows that the antioxidant activity of BHT was much stronger than that of the extracts. Earlier study has recorded EC₅₀ value of 0.018 mg/mL for matured *M. oleifera* leaf extracts (Sreelatha & Padma, 2009). This was lower than the estimated value in the current experiment suggesting that the indicated value in the preliminary study has a better DPPH scavenging potential than the value obtained in this study. The difference in geographical location may account for the observed difference as well as the methods employed in the extraction of the aqueous extracts. In the previous study a soxhlet extractor was used in the aqueous extraction process without prior heating to 80°C. Additionally, the total phenolic content indicates the ability to mop up free radicals due to the presence of many phenolic groups in the extracts (Dai & Mumper, 2010). The total phenolic content was lower than that of plants with known antioxidant activities and total phenolic content (Huang *et al.*, 2010; Zaidi *et al.*, 2014).

Several studies have shown that phenolic compounds are implicated in the induction of cytotoxic activity by plants (Mensah *et al.*, 2006; Sreelatha & Padma, 2009; Jafarian *et al.*, 2014). In Figure 4.43 the cell viability assay showed a mild antiproliferative activity

of the *M. oleifera* leaf extracts on the MCF-7 breast cancer cells. However, a concentration dependent activity was observed. The aqueous extract exhibited a higher cytotoxicity than the hydroethanolic extract. In a previous study, Jafarian *et al.* (2014) reported cytotoxicity of *M. oleifera* extracts on Hela cancer cell lines with IC₅₀ value of 260 µg/mL. Additionally, an unpublished study using *M. oleifera* extracts have shown cytotoxicity towards other cancer cell lines. The high IC₅₀ value for the *M. oleifera* leaf extracts (>1000 µg/mL) in this study cannot be estimated as a result of the weak antiproliferative activity. The higher value of IC₅₀ of *M. oleifera* leaf extract in this study than that observed in literature may be explained by the breast cancer cells being resistant to the mechanism of induction of cytotoxicity of the extracts. Although the IC₅₀ value of the Hela cell line was higher compared to NCI standards, it was lower than that of the MCF-7 breast cell line obtained in this study. The SI value could also not be calculated for *M. oleifera* due to the weak antiproliferative activity of the extracts.

5.2 Conclusion

The petroleum ether and dichloromethane fractions of the various plant species exhibited varying dose dependent activity on the MCF-7 breast cancer cell line. The dichloromethane and petroleum ether fractions of plants namely stem *A. cordifolia* stem and leaves, *M. charantia*, *S. rotundifolius*, *F. exasperata*, *P. pinnata*, *H. indicum*, *A. melegueta* respectively, demonstrated excellent cytotoxicity towards the MCF-7 breast cancer cell lines *in vitro* with IC₅₀ values < 30 µg/mL. The selectivity indices of these fractions suggest that they are good lead candidates for further development. The results obtained lend support to the use of some of the medicinal plants from folk medicine as anticancer remedies. *M. oleifera*, *A. cordifolia*, *P. pinnata* and *A. melegueta*, have

widely been used in many folk medicines from antiquity and continue to serve as useful chemopreventive dietary plants in Ghana.

The aqueous and hydroethanolic extracts of *M. oleifera* do not possess any significant antiproliferative activity towards the MCF-7 breast cancer cell line. *M. oleifera* had antioxidant activity although it was weak. The antioxidant activity of *M. oleifera* was estimated to be 3.0 mg/mL and 3.1 mg/mL for the aqueous and 50% hydroethanolic extracts respectively suggesting a mild scavenging ability. The total phenolic content was estimated to be 177mgGAE/g and 168mgGAE/g for the aqueous and 50% hydroethanolic extracts, respectively. The phenolic content of the plant could have accounted partly for the observed antioxidant activity.

RECOMMENDATIONS

There is the need to carry out further research into the mechanism of action of the other petroleum ether and dichloromethane fractions that demonstrated high cytotoxicity towards the MCF-7 breast cancer cells with high selectivity indices as they have demonstrated promising bioactivity against the cancer cells. For example the petroleum ether fractions of *Paullinia pinnata*, *Heliotropium indicum* and *Ficus exasperata* can be studied for their mechanism of inducing cytotoxicity to establish them as potential candidates for anticancer drug development. The apoptosis mechanism can further be studied to establish the pathways involved in its induction. Additionally, there must be a repeat of the experiment to test the reproducibility of the results in the case of *Aframomum melegueta* so that it can be researched into to isolate the bioactive compound(s) and fully characterize it (them). The experiment involving *Moringa oleifera* must also be repeated for reproducibility of the results obtained or otherwise. Finally funding for such experiments should be improved upon to enable detailed study of the scientific bases for the use of the various traditional medicinal plants in folk medicine.

REFERENCES

- Abbiw, D. K. (1990). *Useful Plants of Ghana*, Royal Botanic Garden. Kew Edition.
- Abdalla, M. A., & Laastsch, H. (2012). Flavonoids from Sudanese *Albizia zygia* (Leguminosae, Subfamily Mimosodeae), a plant with antimalarial potency. *Journal of African Traditional, Complementary and Alternative Medicines*, 9(1), 56–58.
- Abotsi, Wonder, M. K., Woode, E., Ainooson, George K., Amo-Barimah, Ama K., & Boakye-Gyasi, E. (2010). Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* P. Beauv. (Moraceae). *Pharmacognosy Research*, 2(2), 89–97.
- Abourashed, E. A., Toyang, N. J., Choinski, J., & Khan, I. A. (1999). Two new flavone glycosides from *Paullinia pinnata*. *Journal of Natural Products*, 62(8), 1179–1181.
- Adebayo, E. A., & Ishola, O. R. (2009a). Phytochemical and antimicrobial screening of crude extracts from the root, stem bark, and leaves of *Terminalia glaucescens*. *African Journal of Pharmacy and Pharmacology*, 3(5), 217–221.
- Adebayo, E. A., & Ishola, O. R. (2009b). Phytochemical and antimicrobial screening of crude extracts from the root, stem bark and leaves of *Bridelia ferruginea*. *African Journal of Biotechnology*, 8(4), 650–653.
- Adebolu, T. T., & Oladimeji, S. A. (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in Southwestern Nigeria. *African Journal of Biotechnology*, 4(7), 682–684.
- Adekunle, A. A., & Ikumapayi, A. M. (2006). Antifungal property and phytochemical screening of the crude extracts of *Funtumia elastica* and *Mallotus oppositifolius*. *West Indian Medical Journal*, 55 (4): 219 – 223.
- Agnaniet, H., Makani, T., Akagah, A., Menut C., & J. M. Bessiere (2005). Volatile constituents and antioxidant activity of essential oils from *Lippia multiflora* Mold growing in Gabon. *Flavour Fragrance Journal*, 20, 34–38.
- Ahmad, B., Naeem A. K., & Ghufuran, A., (2005). Innamudin Pharmacological Investigation of *Cassia sophera*, Linn. Var. *purpurea*, Roxb. *Medical Journal of Islamic World Academy of Sciences*, 15(3), 105–109.
- Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essays*, 2(5), 163–166.

- Akpan, E. J., & Udoh, A. P. (2004). Effect of the leaves of (*Fleurya aestuans*) on the food quality of the corm of cocoyam (*Xanthosoma sagittifolium* (L) (Schott). *Global Journal of Pure and Applied Sciences*, *10*(2), 287–290.
- Aliyu, M. M., Musa, A. I., Kamal, M. J. & Mohammed, M. G. (2014). Phytochemical screening and anticonvulsant studies of ethyl acetate fraction of *Globimetula braunii* on laboratory animals. *Asian Pacific Journal of Tropical Biomedicine*, *4*(4), 285–289.
- Aljadi, A. M., & Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry*, *85* 513–518.
- Amankwaa-Frimpong, E., Yeboah, F. A., Nguah, S. B. & Afriyie, O. O. (2014). Response to chemotherapy and association with three tumour markers in breast cancer patients in Ghana. *International Journal of Cancer Therapy and Oncology*, *2*(3), 1–7.
- Anderson, O. B., Shyyan, R., Eniu, A., Smith, R. A., Yip, C. H., Bese, N. S., Chow, L. W., ... & Carlson, R. W. (2005). Breast cancer in limited-resource countries: an overview of the breast health global initiative 2005 guidelines. *The Breast Journal*, *12* Suppl 1:S3-15.
- Andhiwal, C. K., Has, C., Varshney, R. P. (1985). Chemical and pharmacological studies of *Heliotropium indicum*. *Indian Drugs*, *22*, 567–569.
- Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research*, *21*(1), 17–25. doi:10.1002/ptr.2023.
- Aprioku, J. S., & Obianime, A. W. (2008). Antioxidant activity of the aqueous crude extract of *Ocimum gratissimum* LINN. leaf on basal and cadmium-induced serum levels of phosphatases in male guinea-pigs. *Journal of Applied Sciences and Environmental Management*, *12*(4), 33–39.
- Areekul, S., Sinchaisri, P., & Tigvatananon, S. (1988). Effect of Thai plant extracts on the oriental fruit fly. II. Repellency test. *Kasetsart Journal of Natural Science*, *22*, 56–61.
- Arribas, J., Baselga, J., Pedersen, K., & Parra-Palau, J. L. (2011). p95HER2 and breast cancer. *Cancer Research*, *71*, 1515–1519.
- Ayisi, N. K., Appiah-Opong, R., Gyan, B., Bugyei, K., & Ekuban, F. (2011). Plasmodium falciparum: Assessment of selectivity of action of chloroquine, *Alchornea cordifolia*, *Ficus polita*, and other Drugs by a Tetrazolium-Based Colorimetric Assay. *Malaria Research Treatment Journal*. Article ID 816250, doi: 10.4061/2011/816250.

- Babu, N. P., Pandikumar, P., & Ignacimuthu, S. (2009). Anti-inflammatory activity of *Albizia lebbek* Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. *Journal of Ethnopharmacology*, *125*(2), 356–360. doi:10.1016/j.jep.2009.02.041.
- Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences*, *78*(5), 431–441. doi:10.1016/j.lfs.2005.09.012.
- Barok, M., Joensuu, H., & Isola, J. (2014). Trastuzumab emtansine : mechanisms of action and drug resistance *Breast Cancer Research*, *16*, R22.
- Bassole, I. H. N., Nebie, R., Savadogo, A., Ouattara, C. T., Barro, N., & Traore, S. A. (2005). Composition and antimicrobial activities of the leaf and flower essential oils of *Lippia chevalieri* and *Ocimum canum* from Burkina Faso. *African Journal of Biotechnology*, *4*(10), 56–64.
- Bayor, M .T., Ayim, J. S. K., Phillips, R. M., Shnyder, S. D., & Wright, C. W. (2007). The evaluation of selected Ghanaian medicinal plants for cytotoxic activities. *Journal of Science and Technology*, *27* (3), 16–22.
- Bears, D. J., Hurley, L. H. & Von Hoff, D. D. (2000). Telomere maintenance mechanisms as a target for drug development. *Oncogene*, *19*, 6632–6641.
- Bharali, R., Tabassum, J., & Azad, M. R. (2003). Chemomodulatory effect of *Moringa oleifera*, Lam on hepatic carcinogen enzymes, antioxdant parameters and skin papillomagenesis in mice. *Asian Pacific Journal of Cancer Preview*, *4*, 131–135.
- Blain, S. W., Montalvo, E., & Massague, J. (1997). Differential interaction of the cyclin dependent kinase (Cdk) inhibitor p27Kip1 with cyclin A-Cdk2 and cyclin D2-Cdk4. *Journal of Biological Chemistry*, *272*, 25863–25872.
- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*, 1199–1200.
- Boguski, M. S., & McCormick, F. (1993). Proteins regulating Ras and its relatives *Nature*, *366*, 643–654.
- Brakohiapa, E. K., Armah, G. E., Clegg-Lampthey, J. N. A. & Brakohiapa, W. O. (2013). Patterns of breast cancer diseases in Accra: Review of mammography reports. *Ghana Medical Journal*, *47*(3), 101–106.
- Brentnall, M., Rodriguez-Menocal, L., De Guevara, R. L., Cepero, E., & Boise, L. H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC Cell Biology*, *14*(1), 32. doi:10.1186/1471-2121-14-32.

- Buniyamin, A. A., Eric, K. I. Q., & Fabian, C. A. (2007). Pharmacognosy and Hypotensive evaluation of *Ficus exasperata* Vahl (moraceae) leave. *Acta Poloniae Pharmaceutica-Drug Research*, 64(6), 543–546.
- Burkill, H. M. (1985) *The useful plants of West Tropical Africa. Volume 1*. 2nd edition. Royal Botanic Gardens, Kew.
- Butler, M. S. (2004). The role of natural product chemistry in drug discovery. *Journal of Natural Products*, 67(12), 2141–2153. doi:10.1021/np040106y.
- Caceres, A., Saravia, S., Rizzo, L., Zabala, E., Deleron, F., & Nave, F. (1992). Pharmacological properties of *Moringa oleifera*: screening of antispasmodic and antiinflammatory diuretic activity. *Journal of Ethnopharmacology*, 36, 233–236.
- Casey, P. J. (1989). p21 ras is modified by a farnesyl isoprenoid *Proceedings of the National . Academy.of Sciences. USA*, 86, 8323–8327.
- Chen, P. L., Riley, D. J., Chen-Kiang, S., & Lee, W. H. (1999). Retinoblastoma protein directly interacts with and activates the transcription factor NF-IL6. *Proceedings of the National Academy of Sciences USA*, 93, 4325–4329.
- Cheng, M., Olivier, P., Diehl, J. A., Fero, M., Roussel, M. F., Roberts, J. M., & Sherr, C. J. (1999). The p21(Cip1) and p27(Kip1) CDK “inhibitors” are essential activators of cyclin D-dependent kinase in murine fibroblasts. *EMBO Journal*, 18, 1571–1583.
- Chou, F., Lin, H., Tseng, H., Wang, C., Lin, J., & Loa, C. (2008). Hepatoprotective effects of *Solanum nigrum* Linn extract against CCl₄ induced oxidative damage in rats. *Chemico-Biological Interactions*. 171, 283–293.
- Clegg-Lampsey, J. N., Edusa, C., Ohene-Oti, N. & Tagoe, J. A. (2007). Breast cancer risk in patients with breast pain in Accra, Ghana. *East Africa Medical Journal*, 84 (5), 215–218.
- Clegg-Lampsey, J. & Hodasi, W. (2007). A study of breast cancer in Korle bu Teaching Hospital: assessing the impact of health education. *Ghana Medical Journal*, 41, 40–49.
- Coruh, N., Celep, A. G. S., & Ozgokce, F. (2007). Antioxidant properties of *Prangos ferulacea* (L) Lindl., *Chaerophyllum macropodum* Boiss, and *Heracleum persicum* Desf. From Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione S-transferase. *Food Chemistry*, 100, 1237–1242.

- Dai, J. & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*, 7313–7352. doi:10.3390/molecules15017313.
- Dalgarno, D. C., Botfield, M. C., & Rickles, R. J. (1997). SH3 domains and drug design: ligands, structure, and biological function. *Biopolymers*, *43*(5), 383–400. doi:10.1002/(SICI)1097-0282(1997)43:5<383::AID-BIP4>3.0.CO;2-R.
- Dhellot, J. R., Matouba, E., Maloumbi, M. G., Nzikou, J. M., Dzondo, M. G., Linder, M., Parmentier, M., & Desobry, S. (2006). Extraction and nutritional properties of *Solanum nigrum* L seed oil. *African Journal of Biotechnology*. *5*(10), 987–991.
- Dokosi, O. B. (1998). *Herbs of Ghana*. Ghana Universities Press.
- Dongo, E., Hussain, H., Miemanang, R. S., Tazoo, D., Schulz, B., & Krohn, K. (2009). Chemical constituents of *Klainedoxa gabonensis* and *Paullinia pinnata*. *Records of Natural Products*, *3*, 165–169.
- Earnshaw, W. C., Martins, L. M., & Kaufmann, S. H. (1999). Mammalian caspases : structure, activation, substrates, and functions during apoptosis. *Annual Review of Biochemistry*, *68*, 383–424.
- Ekundayo, O., Laakso, I., & Hiltunen, R. (1989). Constituents of the volatile oil from leaves of *Ocimum canum* Sims. *Flavour and Fragrance Journal*, *4*, 17–18. doi 10.1002/ffj.2730040104.
- Evgeny, N. I., & Kaido, P. H. (2004). Mechanisms of breast cancer. *Drug Discovery Today: Disease Mechanisms* *1* (2), 235–245.
- Faizi, S., Siddiqui, B. S., Saleem, R., Saddiqui, S., & Aftab, K. (1994). Isolation and structural elucidation of new nitrile and mustard oil glycosides from *Moringa olieifera* and the effect on blood pressure. *Journal of Natural Products*, *57*: 1256–1261.
- Fakae, B. B., Campbell, A. M., Barrett, J., Scott, I. M., Teesdale-spittle, P. H., Liebau, E., & Brophy, P. M. (2000). Inhibition of glutathione S-transferase (GTS) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. *Phytotherapy Research* *14*, 608– 611.
- Fan, L., Liedke, P. E. R., Isakoff, S. J., St Louis, J., Ryan, P. D., & Goss, P. E. (2014). Intermittent letrozole therapy for metastatic breast cancer: case reports and literature review. *Clinical Breast Cancer*, *14* (2), e41- e45.
- Ferlay, J., Bray, F., Pisani, P., & Parkin, D. M. (2002). GLOBOCAN: Cancer incidence, mortality and prevalence worldwide. *IARC Cancer base no. 5*, version 2.0. Lyon, France: IARC Press, 2004; available at <http://www-dep.iarc.fr/>.

- Ferreira, J. F. S., Luthria, D. L., Sasaki, T., & Heyerick, A. (2010). Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*. doi:10.3390/molecules15053135.
- Flemington, E. K., Speck, S. H., & Kaelin, W. G. Jr. (1993). E2F-1 mediated transactivation is inhibited by complex formation with the retinoblastoma susceptibility gene product. *Proceedings of the National Academy of Sciences USA*, *90*, 6914–6918.
- Fred-Jaiyesimi, A. A. & Anthony, O. (2011). Larvicidal activities of the extract and fractions of *Paullinia pinnata* Linn leaf. *Pharmacognosy Communications*, *1*(2), 37–40.
- Gamani, K., Wambebe, C., Amupitan, J., Hussaini, I.M., Amos, S., Awodogan, A., Dunah, A.W., Ekuta, J.E., Akeju, M.O., Usman, H., & Enwerem, N. (1997). Active column fractions of *Nauclea latifolia* on *Plasmodium berghei* and rabbit ileum. *Journal of Pharmaceutical Research and Development*, *2*, 44–47.
- George, N. J., Obot, I. B., Ikot, A. N., Akpan, A. E., & Obi-Egbedi, N. O. (2010). Phytochemical and Antimicrobial properties of leaves of *Alchonea cordifolia*. *Journal of Chemistry*, *7*(3), 1071–1079.
- Girard, F., Strausfeld, U., Fernandez, A., & Lamb, N. J. (1991). Cyclin A is required for the onset of DNA replication in mammalian fibroblasts. *Cell*, *67*, 798–806.
- Glew, R. S., Amoako-Atta, B., Ankar-Brewoo, G., Presley, J., Lu-Te, C., Millson, M., Smith, B. R., & Glew, R. H. (2009). "Non-cultivated plant foods in West Africa: Nutritional analysis of the leaves of three indigenous leafy vegetables in Ghana. *Food* *3*, *1*, 39–42.
- Glück, S. (2014). Extending the clinical benefit of endocrine therapy for women with hormone receptor positive metastatic breast cancer: differentiating mechanisms of action. *Clinical Breast Cancer*, *14* (2), 75-84.
- Goldman, R., Ben Levy, R., Peles, E., & Yarden, Y. (1990). Heterodimerization of the erbB1 and erbB2 receptors in human breast carcinoma cells: A mechanism for receptor transregulation. *Biochemistry*. *29*, 11024–11028.
- Gonzalez-Angulo, A. M., Morales-Vasquez, F., & Hortobagyi, G. N. (2007). Overview of resistance to systemic therapy in patients with breast cancer. *Advanced Experimental Medical Biology*, *608*, 1-22.
- Guidry, J. J., Aday, L. A., Zhang, D., & Winn, R. J. (1997). Transportation as a barrier to cancer treatment. *Cancer Practice*. *5*(6), 361–366.

- Gullett, N. P., Ruhul-Amin, A. R., Bayraktar, S., Pezzuto, J. M., Shin, D. M., Khuri F. R., Aggarwal, B. B., Surh Y. J., & Kucuk, O. (2010). Cancer prevention with natural compounds. *Seminars in Oncology*, 37(3), 258–281.
- Harborne, J. B. (1973). *Phytochemical Methods*, Chapman and Hall, Ltd. Pp 49–188.
- Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., & Elledge, S. J. (1993). The p21 Cdk-interactin protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell*, 75, 805–816.
- Hengartner, M. O. (2000). The biochemistry of apoptosis. *Nature*, 407, 770–776.
- Hengartner, M. O. (1997). Programmed Cell Death. In: *C. elegans II*. Riddle, D.L. et al. eds. 383–496. (Cold Spring Harbor Laboratory Press, Plainview, N.Y.).
- Heo, K. S., Lee, S. J., Ko, J. H., Lim, K., Lim, K. T. (2004). Glycoprotein isolated from *Solanum nigrum* L. inhibits the DNA-binding activities of NF- κ B and AP-1, and increases the production of nitric oxide in TPA-stimulated MCF-7 cells. *Journal of Toxicology in vitro*. 18(6), 755–763.
- Hiyama, K & Hiyama, E. (2003). Telomerase as tumor marker. *Cancer Letters* 194, 221–233.
- Hostettmann, K., Marston, A., Ndjoko, K., & Wolfender, J.-L. (2000). The Potential of African Plants as a Source of Drugs. *Current Organic Chemistry*, 4(10), 973–1010. doi:10.2174/1385272003375923.
- Hu, W., Feng, Z., & Levine, A. J. (2012). The Regulation of Multiple p53 Stress Responses is Mediated through MDM2. *Genes & Cancer*, 3(3-4), 199–208. doi:10.1177/1947601912454734.
- Huang, H. C., Syu, K.Y., & Lin, J. K. (2010). Chemical composition of *Solanum nigrum* Linn extract and induction of autophagy by leaf water extract and its major flavonoids in AU565 breast cancer cells. *Journal of Agricultural and Food Chemistry*, 58(15), 8699–8708.
- Hunter, T. (2000). Signaling — 2000 and Beyond. *Cell*, 100, 113–127. doi:10.1016/S0092-8674(00)81688-8.
- Hussain, H., Grove, L., Griffiths, S. J., & Busby, J. A. (1994). A seven-gene operon essential for formate-dependent nitrite reduction to ammonia by enteric bacteria. *Molecular Microbiology*, 12, 153–163.
- Igwe, S. A., Emeruwa, I. C., & Modie, J. A. (1999). Ocular toxicity of *Aframomum melegueta* (alligator pepper) on healthy Igbos of Nigeria. *Journal of Ethnopharmacology*, 65(3), 203–206. doi:10.1016/S0378-8741(98)00188-3.

- Irvine, F.R., (1961). *Woody plants of Ghana*. Oxford University Press, London.
- Itharat, A., Houghton, P. J., Eno-Amooquaye, E., Burke, P. J., Sampson, J. H., & Raman. A. (2004) In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *Journal of Ethnopharmacology*, *90*, 33–38. doi:10.1016/j.jep.2003.09.014.
- Jafarian, A., Asghari, G., & Ghassami, E. (2014). Evaluation of cytotoxicity of *Moringa oleifera* Lam. callus and leaf extracts on Hela cells. *Advanced Biomedical Research*, *3*, 194–199. doi: 10.4103/2277-9175.140668.
- Jansen, P. C. M. (2008) *Solanum nigrum* L. Record from Protabase. Schmelzer, G. H., Gurib-Fakim, A. (Eds) PROTA (Plant Resources of Tropical Africa/Ressources végétales de l’Afrique tropicale), Wageningen, Netherlands.
- Jemal, A., Bray, F., & Ferlay, J. (2011). Global Cancer Statistics, *61*(2), 69–90. doi:10.3322/caac.20107.
- Jimoh, F. O., Sofidiya, M. O., & Afolayan, A. J. (2007). Antioxidant properties of the methanol extracts from the leaves of *Paullinia pinnata*. *Journal of Medicinal Food*, *10*(4), 707–711.
- Johnson, G. R., Kannan, B., Shoyab, M., & Stromberg, K. (1993). Amphiregulin induces tyrosine phosphorylation of the epidermal growth factor receptor and p185erbB2. *Journal of Biological Chemistry*. *268*, 2924–2931.
- Kato, J., Matsushime, H., Hiebert, S. W., Ewen, M. E., & Sherr, C. J. (1993). Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. *Genes and Development*, *7*, 331–342.
- Kell, D. B. (2013). Finding novel pharmaceuticals in the systems biology era using multiple effective drug targets, phenotypic screening and knowledge of transporters: Where drug discovery went wrong and how to fix it. *FEBS Journal*, *280*(23), 5957–5980. doi:10.1111/febs.12268.
- Kerharo, J., & Adams, J. (1974). *La Pharmacopée Senegalaise Traditionnelle. Plantes médicales et Toxiques*. Edition Vigot et Frères, Paris.
- Kerr, J. F. R., Wyllie, A. H., & Currie, A. R. (1972) Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer*, *26*, 239–57.
- Khazaie, K., Schirmacher, V., and Lichtner, R. B. (1993). EGF receptor in neoplasia and metastasis. *Cancer and Metastasis. Review*, *12*, 255–274.

- Kim, M. O., Lee, M. H., Oi, N. Kim, S. H., Bae, K. B., ... & Dong, Z. (2014). [6]-Shogaol inhibits growth and induces apoptosis of non-small cell lung cancer cells by directly regulating Akt1/2. *Carcinogenesis*, 35(3), 683–691.
- Kohn, K. W. (1999). Molecular interaction map of the mammalian cell cycle control and DNA repair systems. *Molecular Biology of the Cell*, 10, 2703–2734.
- Kokila, K., Priyadarshini, S. D., & Sujatha, V. (2013). Phytochemical properties of *Albizia* species: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 70–73.
- Koopman, G., Reutelingsperger, C., Kuijten, G., Keehnen, R., Pals, S., & Oers, M. van. (1994). Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood Journal*, 84(5), 1415–1420.
- Kugelman, M., Lui, W-C, Axelrod, M., McBride, T., J., Rao, K., V. (1976). Indicine-N-oxide: the antitumor principle of *Heliotropium indicum*. *Lloydia*, 39, 125–128.
- Kukuia, K. K. E., Mante, P. K., Woode, E., Elvis O. Ameyaw, E. O., & Adongo, D. W. (2014). Antidepressant effects of *Mallotus oppositifolius* in acute murine models. *International Scholarly Research Notices: Pharmacology*, Article ID 324063, <http://dx.doi.org/10.1155/2014/324063>.
- Lasisi, A. A., Ayinde, B. W., Adeleye, A. O., Onocha, P. A., Oladosu, I. A., & Idowu, P.A. (2015). New triterpene isovanilloyl and antibacterial activity of constituents from the roots of *Paullinia pinnata* Linn (*Sapindaceae*). *Journal of Saudi Chemical Society*, 19, 117–122.
- Launay, S., Hermine, O., Fontenay, M., Kroemer, G., Solary, E., & Garrido, C. (2005). Vital functions for lethal caspases. *Oncogene*, 24(33), 5137–5148. doi:10.1038/sj.onc.1208524.
- Leopold, W. R., Bridges, A., Decker, S., Fry, W. D., Kraker, A., & Sebold-Leopold, J. (2002). Growth factor and signal transduction targets for cancer therapy [in Anticancer Drug Development], Academic Press 31–41.
- Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. *Cell*, 88, 323–331.
- Lu, Y., Zi, X., Zhao, Y., Mascarenhas, D., & Pollak, M. (2001). Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *Journal of the National Cancer Institute*, 93, 1852–1857.
- Malumbres, M., & Barbacid, M. (2005). Mammalian cyclin-dependent kinases. *Trends in Biochemical Sciences*, 30(11), 630–641. doi:10.1016/j.tibs.2005.09.005.

- McCormick, F. (1993). Signal transduction: how receptors turn Ras on *Nature*, 363, 15–17.
- Mengome, L-E., Akue, J. P., Souza, A., Tchoua, F. G. R. & Emvo, E. N. (2010). In vitro activities of plant extracts on human *Loa loa* isolates and cytotoxicity for eukaryotic cells. *Parasitology Research*, 107(3), 643–650.
- Mensah, A. Y., Houghton, P. J., Agyare, C., Komlaga, G., Mensah, M. L. K., Fleicher, T. C., & Sarpong, K. (2006). Investigation of activities related to wound healing of *Secamone afzelii*. *Journal of Science and Technology*, 26(3), 83–89.
- Miele, M., Mumot, A. M., Zappa, A., Romano, P., & Ottaggio, L. (2012). Hazel and other sources of paclitaxel and related compounds. *Phytochemistry Reviews*. doi:10.1007/s11101-012-9234-8.
- Morimitsu, Y., Hayashi, K., Nakagawa, Y., Fujii, H., Horio, F., Uchida, K., & Osawa, T. (2000). Antiplatelet and anticancer isothiocyanates in Japanese domestic horseradish, wasabi. *Mechanisms of Ageing and Development*, 116, 125 –134. Retrieved from <http://ac.els-cdn.com/>
- Morin, G. B. (1989). The human terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell*, 59, 521–529.
- Motoyama, A. B., Hynes, N. E., & Lane, H. A. (2002). The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. *Cancer Research*, 62, 3151–3158.
- Murakami, T., Emoto, A., Matsuda, H., & Yoshikawa, M. (2001). Medicinal food stuffs. Part XXI. Structures of new cucuritanone type triterpene glycosides, goyaglycosides -a,-b,-c,-d,-e,-f,-g, and -h, and new oleanane- type triterpene saponins, goyasaponins I, II and III. From the fresh fruit of Japanese *Momordica Charantia* L. *Chemical and Pharmaceutical Bulletin*, 49, 54–63.
- Nagata, Y., Lan, K. H., Zhou, X., Tan, M., Esteva, F. J., Sahin, A. A., ... & Yu, D. (2004). PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell*, 6, 117–127.
- Nagy, P., Friedlander, E., Tanner, M., Kapanen, A. I., Carraway, K. L., Isola, J., & Jovin, T. M. (2005). Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Research*, 65, 473–482.
- Nakagawa, T., Zhu, H., Morishima, N., Li, E., Xu, J., Yankner, B. A., & Yuan, J. (2000). Caspase-12 mediates endoplasmic reticulum-specific apoptosis and cytotoxicity by amyloid beta. *Nature*, 403, 98–103.
- Nam, N., & Parang, K. (2003). Current Targets for Anticancer Drug Discovery. *Current Drug Targets*, 4, 159–179.

- Neidle, S & Parkinson, G. (2002). Telomere maintenance as a target for anticancer drug discovery. *Nature Reviews Drug Discovery*, 1, 383–393.
- Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, 70, 461. doi:10.1021/np068054v.
- Ngassoum, M. B., Ousmaila, H., Ngamo, L. T., Maponmetsem, P. M., Jirovetz, L., & Buchbauer, G. (2004). Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. *Journal of Food Composition and Analysis*, 17(2), 197–204.
- Nilsson, I., & Hoffmann, I. (2000). Cell cycle regulation by the Cdc25 phosphatase family. *Progress in Cell Cycle Research*, 4, 107–114.
- Nowsheen, S., & Yang, E. S. (2012). The intersection between DNA damage response and cell death pathways. *Experimental Oncology*, 34(3), 243–254.
- Nyarko, A. K., Asare-Anane, H., Ofosuhene, M., & Addy, M. E. (2002). Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic β -islet cells. *Phytomedicine*, 9(4), 346–351.
- Oboh, G. (2008). Antioxidative Potential of *Ocimum gratissimum* and *Ocimum canum* leaf polyphenols and protective effects on some pro-oxidants induced lipid peroxidation in rat brain: An *in vitro* study. *American Journal of Food Technology* 3(5), 325–334.
- Oboh, G., Raddatz, H., & Henle, T. (2009). Characterization of the antioxidant properties of hydrophilic and lipophilic extracts of Jute (*Corchorus olitorius*) leaf. *International Journal of Food Sciences and Nutrition*, 60(2), 124–134.
- Odukoya, O. A., Ilori, O. O., Sofidiya, M. O., Aniunoh, O. A., Lawal, B. M., & Tade, I. O. (2005). Antioxidant activity of Nigerian dietary spices. *Elective Journal of Environmental and Agricultural Food Chemistry*, 4, 1086–1093.
- Odunbaku, O. A., Ilusanya, O. A., & Akasoro, K. S. (2008). Antibacterial activity of ethanolic leaf extract of *Ficus exasperata* on *Escherichia coli* and *Staphylococcus albus*. *Scientific Research and Essays*, 3(11), 562–564.
- Okunade, A. L. (2002). *Ageratum conyzoides* L. (Asteraceae). *Fitoterapia* 73, 1–16.
- Olugbade, T. A., Ogundaini, A., Birlirakis, N., Pais, M., & Martin, M-T. (2000). Petersaponins III and IV, triterpenoid saponins from *Petersianthus macrocarpus*. *Journal of Natural Products*, 63(5), 716–719.

- Omer, C. A.; Anthony, N. J. & Buser-Doepner, C. A. (1997). Farnesyl protein transferase inhibitors as agents to inhibit tumor growth. *Biofactors*, 6, 359–366. DOI:10.1002/biof.5520060306.
- Pande, M., & Pathak, A. (2009). Aphrodisiac activity of roots of *Mimosa pudica* Linn. ethanolic extract in mice. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 2(1), 477–486.
- Pal, S. K., & Mittal, B. (2004). Fight against cancer in countries with limited resources: the post-genomic era scenario. *Asian Pacific Journal of Cancer Prevention*, 5, 328–333.
- Palyi-Krekk, Z., Barok, M., Isola, J., Tammi, M., Szollosi, J., & Nagy, P. (2007). Hyaluronan-induced masking of ErbB2 and CD44-enhanced trastuzumab internalisation in trastuzumab resistant breast cancer. *European Journal of Cancer*, 43, 2423–2433.
- Pauletti, G., Dandekar, S., Rong, H., Ramos, L., Peng, H., Seshadri, R., & Slamon, D. J. (2000). Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *Journal of Clinical Oncology*, 18, 3651–3664.
- Pessoa, L. M., Morais, S. M., Bevilaqua, C. M. L., & Luciano, J. H. S. (2002). Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn and eugenol against *Haemonchus contortus*. *Veterinary Parasitology*, 109(1), 59–63.
- Prakash, A., N. G., T. B., & Tso, W.W. (2002). Purification and characterization of charantin, a napin like ribosome-inactivating peptide from bitter melon (*Momordica charantia*) seeds. *Journal of Peptide Research*, 59, 197–202.
- Ramachandran, C., Peter, K. V., & Gopalakrishnan, P. K. (1980). Drumstick (*Moringa oleifera*): A multipurpose Indian vegetable. *Economic Botany*, 34(3), 276–283. doi:10.1007/BF02858648.
- Raman, A., & Lau, C. (1996). Anti-diabetic properties and phytochemistry of *Momordica charantia* L. (Cucurbitaceae). *Phytomedicine*, 2(4), 349–362.
- Rao, K. V. (1993). Taxol and related taxanes. I. Taxanes of *Taxus brevifolia* bark. *Pharmaceutical Research*, 10(4), 521–524. doi:10.1023/A:1018937700459.
- Rich, T., Watson, C. J. & Wyllie, A. (1999). Apoptosis: The germs of death. *Nature Cell Biology*, 1, E69–E71.
- Riss, T. (2005). Selecting cell-based assays for drug discovery screening. *Cell Notes* 13, 16–21.

- Sainsbury, M., & Sofowora, E. A. (1971). Essential oil from the leaves and inflorescence of *Ocimum gratissimum*. *Phytochemistry*, *10*(12), 3309–3310.
- Sarker, S. D., Latif, Z., & Gray, A. I. (2005). *Natural Product Isolation: Methods in Biotechnology*. Human Press New Jersey 2nd Ed. Pp 1 – 20.
- Satyavati, G. V., Gupta, A. K., & Tandon, N. (1987). *Medicinal Plants of India* Vol. II. ICMR, New Delhi.
- Sawyer, T. K. (1998). Src homology-2 domains: Structure, mechanisms, and drug discovery. *Biopolymers*, *47*(3), 243–261. doi:10.1002/(SICI)1097-0282(1998)47:3<243::AID-BIP4>3.0.CO;2-P.
- Scaltriti, M., Eichhorn, P. J., Cortes, J., Prudkin, L., Aura, C., Jiménez, J., ... & Baselga J. (2011). Cyclin E amplification/over expression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. *Proceedings of the National Academy of Sciences, U. S. A.* *108*, 3761–3766.
- Sei-Jung, L., Kye-Taek, L. (2006). Apoptosis induced by glycoprotein (150-kDa) isolated from *Solanum nigrum* L. is not related to intracellular reactive oxygen species (ROS) in HCT-116 cells. *Journal of Cancer Chemotherapy and Pharmacology*, *57*(4), 507–516.
- Sherr, C. J. & Roberts, J. M. (1999). CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes and Development*, *13*, 1501–1512.
- Shieh, S. Y., Ikeda, M., Taya, Y., & Prives, C. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell*, *91*, 325–334.
- Shukla, S., Ohnuma, S., & Ambudkar, S. V. (2011). Improving cancer chemotherapy with modulators of ABC drug transporters. *Current Drug Targets*, *12*(5), 621–630.
- Silici, S., Sagdic, O., & Ekici, L. (2010). Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry*, *121*(1), 238–243. doi:10.1016/j.foodchem.2009.11.078.
- Singletary, S. E. (2003). Rating the risk factors for breast cancer. *Annals of Surgery*, *237*, 474–482.
- Stewart, B., & Kleihues, P. E. (2003). *World cancer report*. Lyon, France: IARC Press. Available at www.iarc.fr
- Stoddart, M. J. (2011). Cell viability assays: introduction. *Methods in Molecular Biology (Clifton, N.J.)*. doi:10.1007/978-1-61779-108-6_1.
- Sreelatha, S & Padma, P. R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition*, *64*, 303–311.

- Tennant, D. A., Durán, R. V., Boulahbel, H., & Gottlieb, E. (2009). Metabolic transformation in cancer. *Carcinogenesis*. doi:10.1093/carcin/bgp070.
- Tennant, J. R. (1964). Evaluation of the trypan blue technique for determination of cell viability. *Transplantation*. doi:10.1097/00007890-196411000-00001.
- Thomas, S. M., & Brugge, J. S. (1997). Cellular functions regulated by src family kinases. *Annual. Review of Cell and Developmental. Biology*, 13, 513–609.
- Togola, A., Diallo, D., Dembélé, S., Barsett, H., & Paulsen, B. S. (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali,(West Africa) in the regions Doila, Kolokani and Siby. *Journal of Ethnobiology and Ethnomedicine*, 1(1), 7.
- Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*, 00(00), 1–22. doi:10.3322/caac.21262.
- Ukeh, D. A., Birkett, M. A., Pickett, J. A., Bowman, A. S., & Jennifer Mordue, A. (2009). Repellent activity of alligator pepper, *Aframomum melegueta*, and ginger, *Zingiber officinale*, against the maize weevil, *Sitophilus zeamais*. *Phytochemistry*, 70(6), 751–758. doi:10.1016/j.phytochem.2009.03.012.
- Umukoro, S., & Ashorobi, B. R. (2007). Further pharmacological studies on aqueous seed extract of *Aframomum melegueta* in rats. *Journal of Ethnopharmacology*, 115(3), 489–493. doi:10.1016/j.jep.2007.10.019.
- Usami, Y., Nakagawa-Goto, K., Lang, J. Y., Kim Y., Lai C. Y., Goto M., ... & Lee K. H. (2010). Antitumor Agents. 282. 2'-(R)-O-acetylglauucarubinone, a quassinoid from *Odyendyea gabonensis* as a potential anti-breast and anti-ovarian cancer agent. *Journal of Natural Products*, 73(9), 1553-1558.
- Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160(1), 1–40. doi:10.1016/j.cbi.2005.12.009.
- Vega-Avila, E., & Pugsley, M. K. (2011). An overview of colorimetric assay methods used to assess survival or proliferation of mammalian cells. *Proceedings of the Western Pharmacology Society*, 54, 10–14. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/22423572>.
- Vermes, I., Haanen, C., Steffens-Nakken, H., & Reutelingsperger, C. (1995). A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *Journal of Immunological Methods*, 184(1), 39–51. doi:10.1016/0022-1759(95)00072-I.

- Vinogradov, S., & Wei, X. (2013). Cancer stem cells and drug resistance: the potential of nanomedicine. *Nanomedicine (Lond)*, 7(4), 597–615. doi:10.2217/nmm.12.22.
- Wada, T., Qian, X., & Green, M. I. (1999). Intermolecular association of the p185neu protein and EGF receptor modulates EGF receptor function. *Cell*, 61, 1339–1347.
- Wajant, H. *et al.* (2003). Death Receptors. In: *Essays in Biochemistry*. Cotter, T. *et al.* eds. 39, 53–71. (Cold Spring Harbor Laboratory Press, Plainview, N.Y.)
- Wannang, N. N, Anuka, J. A, Kwanashie H. O, Bichi L. A. (2004). Effects of *Solanum nigrum* Linn aqueous extracts on the behavioral activities in chicks. *Biological and Environmental Sciences Journal for the Tropics*, 1 (1), 139–142.
- Waterhouse, L. A. (2002). *Current protocols in food analytical chemistry*. John Wiley and Sons, New York.
- Waterhouse, N. J., Goldstein, J. C., Von Ahsen, O., Schuler, M., Newmeyer, D. D., & Green, D. R. (2001). Cytochrome c maintains mitochondrial transmembrane potential and ATP generation after outer mitochondrial membrane permeabilization during the apoptotic process. *Journal of Cell Biology*, 153(2), 319–328. doi:10.1083/jcb.153.2.319.
- Weaver, D. K., Dunkel, F. V., Ntezurubanza, L., Jackson, L. L., & Stock, D. T. (1991). The efficacy of linalool, a major component of freshly-milled *Ocimum canum* Sims (Lamiaceae), for protection against postharvest damage by certain stored product Coleoptera. *Journal of Stored Products Research*, 27(4), 213–220.
- WHO (2012). World Health Organization.
- WHO (2008). World Health Organization.
- Williamson, P., Eijnde, S., & Schlegel, R. A. (2000). Phosphatidylserine exposure and phagocytosis of apoptotic cells. *Methods in Cell Biology*, 66, 339–64.
- Wiredu, E., K., & Armah, H., B. (2006). Cancer mortality patterns in Ghana: a 10-year review of autopsies and hospital mortality. *BMC Public Health*, 6, 159.
- Xie, Z., Schendel, S., Matsuyama, S. & Reed, J. C. (1998). *Biochemistry*, in press.
- Xing, Z-B., Yao, L., Zhang, G-Q., Zhang, Y-X., & Pang, D. (2011). Fangchinoline inhibits breast adenocarcinoma proliferation by inducing apoptosis. *Chemical and Pharmaceutical Bulletin*, 59, 1476 – 1480.

- Xu, X., Doshi, A., Lei, M., Eck, M. J. & Harrison, S. C. (1999). Crystal structures of c-Src reveal features of its autoinhibitory mechanism, *Molecular Cell*, 3(5), 629–638.
- Yesilada, E., Gurbuz, I., & Shibata, H. (1999). Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity. *Journal of Ethnopharmacology*, 66(3), 289–293.
- Yin, L., Wei, Y., Wang, W., Wang, Y., Xu, Y., & Yang, Y. (2014). Simultaneous application of BrdU and WST-1 measurements for detection of the proliferation and viability of airway smooth muscle cells. *Biological Research*, 47(75), 1–5.
- Yoshida, K., & Miki, Y. (2004). Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Science*, 95(11), 866–871. doi:10.1111/j.1349-7006.2004.tb02195.
- Zaidi, K. S., Md. Hoda, N., Tabrez, S., Ansari, S. A., Jafri, M. A., Khan, M. S., ... & Banu, N. (2014). “Protective effect of *Solanum nigrum* leaves extract on immobilization stress induced changes in rat’s brain,” Evidence-Based Complementary and Alternative Medicine, Article ID 912450, 7 doi:10.1155/2014/912450.
- Zamble, A., Carpentier, M., Kandoussi, A., Sahpaz, S., Petrault, O., Ouk, T., & Martin-Nizard, F. (2006). *Paullinia pinnata* extracts rich in polyphenols promote vascular relaxation via endothelium-dependent mechanisms. *Journal of Cardiovascular Pharmacology*, 47(4), 599–608.
- Ziech, D., Franco, R., Georgakilas, A. G., Georgakila, S., Malamou-Mitsi, V., Schoneveld, O., ... Panayiotidis, M., I. (2010). The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. *Chemico-Biological Interactions*, 188(2), 334–339. doi:10.1016/j.cbi.2010.07.01.

APPENDICES

APPENDIX I

Preparation of reagents used for MTT assay

DMEM

A mass of 4.75 g of DMEM powder was weighed and 300 mL of sterile distilled water added and stirred for 30 min. Sterile distilled water was added to top up to 500 mL and transferred into an autoclave flask for autoclaving. After autoclaving, the content was cooled to 37°C and 5 mL of 10% NaHCO₃, 5 mL of PSG, and 5 mL of L-glutamate are added under sterile conditions. The medium was stored at 4°C for later use.

Acidified Isopropanol

A volume of 1.7 mL of 37% HCl was added to 500 mL of isopropanol in a reagent bottle and mixed thoroughly and labelled and stored at 4°C. Five millilitre of the solution was pipetted off and replaced with 5 mL of triton X. The solution was mixed well to obtain a homogenous mixture and stored at room temperature.

Phosphate buffered saline (PBS)

Four grams of NaCl, 0.1 g of KCl, 0.72 g of Na₂HPO₄ and 0.12 g of KH₂PO₄ were weighed and dissolved with 450 mL of distilled water in a 500 mL beaker. The pH was adjusted to 7.4 using NaOH and HCl. The solution was transferred into a 500 mL measuring cylinder and topped up to the 500 mL mark. The solution was then transferred into a 1 L autoclave bottle and sterilised. After cooling, it was labelled and stored at 4°C.

MTT solution

A mass of 0.12 g of MTT was weighed in the dark with the tube covered with aluminium foil. A volume of 50 μ L of PBS was added and mixed to dissolve completely the MTT salt. After that the solution was filtered using a 50 mL syringe barrel and 0.45 μ M Millipore filter. It was labelled and wrapped in a 50 mL centrifuge tube container with aluminium foil and stored at 4°C.

APPENDIX II

Preparation of solutions for antioxidant assay

0.5mM DPPH

After calculating the mass needed using the formula $m = C \times Mr \times V$, where m is the mass, C is the concentration, Mr is the molecular weight and V is the volume.

A mass of 0.0044g of DPPH was weighed and dissolved in 1 mL of methanol.

10mM BHT

A mass of 0.0016 g of BHT was calculated and weighed using the formula above and dissolved in absolute methanol.

Standard Gallic acid curve used in the estimation of Total Phenolic content of *M. oleifera* leaf extracts.

