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Phytochemical, Antioxidant and Anticancer Properties of *Elaeis guineensis* and *Elaeis oleifera*

By

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil, and that to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Oil palm (*Elaeis* spp) is largely known for its economic values but it has also been used in folklore for the treatment of a wide range of diseases including cancer. This study was aimed at assessing and comparing the antioxidant and anticancer potentials as well as evaluating the phytochemical constituents of aqueous and hydroethanol extracts of leaves from two species of oil palm, *Elaeis guineensis* and *Elaeis oleifera*. Aqueous and hydroethanolic extracts were prepared from dried leaves of *Elaeis guineensis* and *Elaeis oleifera* and successively fractionated with petroleum ether, chloroform and ethyl acetate. Anti-proliferative activities of the extracts and fractions were determined using the tetrazolium-based colorimetric, 3-(4, 5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Jurkat, MCF-7, and Chang liver cell lines. Antioxidant potential of crude extracts was assessed by 2, 2- diphenyl-1picryl hydrazyl (DPPH) and reducing power assays. Total phenol and flavonoid contents were determined by the Folin-Ciocalteu and aluminum chloride spectrometric methods, respectively. The presence of tannins, alkaloids, terpenoids and saponins were qualitatively screened for in the crude aqueous and ethanolic extracts. The study revealed that both *E. guineensis* and *E. oleifera* contained tannins, flavonoids, alkaloids, terpenoids and saponins. *E. oleifera*, however, had higher quantities of total phenolic content than *E. guineensis*. The *E. oleifera* hydroethanolic extract had total phenolic content of 34.99 ± 3.92 g GAE/100g, whereas that of the aqueous extract gave 21.05 ± 1.21 mg GAE/100g. The total flavonoid content of hydroethanolic extract of E. oleifera was 100.40 ± 1.39 mgQE/100g, while that of the aqueous extract was 82.15 ± 3.03 mgQE/100g. E. guineensis and E. oleifera showed significant antioxidant properties comparable to the standards, butylated hydroxyl toluene (BHT) and ascorbic acid. The aqueous *E. oleifera* extract showed higher antioxidant activity of 0.072 ± 0.010 mg/mL and 0.045 ± 0.006 mg/mL for the hydroethanol extract, as well as the ferric reducing ability of the aqueous extract being 0.597 ± 0.014 mg/mL and 0.120 ± 0.010 mg/mL for the hydroethanol extract. *E. guineensis* and *E. oleifera* both exhibited cytotoxicities against Jurkat, MCF-7 and Chang liver cells. Jurkat cells were the most sensitive and MCF-7, the most resistive. The hydroethanol *E. oleifera* extracts exerted the most cytotoxicty with the lowest IC_{50} of 87.54 ± 4.27µg/mL against Jurkat cell lines with selectivity index of 2.87 whilst on the MCF-7 cell lines, it was 476.32 ± 82.74 µg/mL with selective index of 0.77. The cytotoxicity evaluations of the extracts from successive solvent system fractionations of the crude extracts with petroleum ether, chloroform and ethyl acetate revealed the ethyl ether fractions of *E oleifera* hydroethanol extract to be the most cytotoxic exhibiting IC_{50} of 18.88 ± 0.12 µg/mL against the jurkat cells with a selective index of 1.87. Ethyl acetate fraction of aqueous *E. oleifera* showed the most cytotoxicity against MCF-7 of 27.26 ± 1.26 µg/mL with a selective index of 36.15. The study showed that both *E. guineensis* and *E. oleifera* leaf extracts possess significant antioxidant and anticancer properties; however, E. oleifera had a better potential for therapeutic applications.

DEDICATION

To my lovely children: Manuel Korley, Theophilus Atteh and Theodore Lawyer OssomKenney, and my beloved, Catherine Odeibea Ossom.

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This MPhil journey has been a long one that has had a number of persons supporting, encouraging and pushing me along. Without you, I may have given up, considering the obstacles and challenges I had to surmount.

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LIST OF ABBREVIATIONS

BHT	-	Butylated hydroxyl toluene
DMEM	-	Dulbecco‘s Modified Eagles Medium
DMSO	-	Dimethyl sulphoxide
DPPH	-	2,2-diphenyl-1-picyl hydrazyl
EC_{50}	-	Half- maximal effective concentration
FBS	-	Foetal bovine serum
GSH	-	Glutathione
IC_{50}	-	Half-maximal inhibitory concentration
LADH	-	Lactate dehydrogenase
MCF-7	-	Michigan Cancer Foundation-7
MTT3	-	(4,5-dimethylthiazol-2 yl)-2,5-didiphenyl tetrazolium bromide
PBS	-	Phosphate buffer saline
PSG	-	Penicillin steptromycin L-glutamine
ROS	-	Reactive oxygen species
SI	-	Selective Index
SOD	-	Superoxide dismutase
TFC	-	Total flavonoid content
TNF	-	Tumour necrosis factor
TPC	-	Total phenol content
TRAIL	-	Tumour necrosis factor -regulated apoptosis –inducing ligand

CHAPTER ONE

1.0. INTRODUCTION

Cancer is globally considered as a significant cause of mortality across all ages, race and sex (Mathers *et al.*, 2008). The cancer load is on the ascendancy in developing countries, most probably due to aging populations and the appropriation of cancer-related style choices like substance abuse, physical latency and ―westernized ―diets (Jemal *et al.*, 2011).

Based on the GLOBOCAN (2008) estimates, an estimated 12.7 million cancer cases with 7.6 million related deaths was projected to have occurred in 2008; 56% of these cases and 64% of the deaths reportedly occurred in the second and third world countries. Cancer is characterized by unregulated growth, replication and migration of cells and if it is unchecked, it can lead to imminent patient death.

The most widely used standard treatment, chemotherapy is based on the premise that anticancer drugs inhibit cell growth or induce their death (Dantu *et al.*, 2012). Notwithstanding this, cancer chemotherapy is still fraught with several challenges, notably drug resistance, toxicity, excessive hair loss, nausea and low selectivity of currently available drugs. Recent studies are now focusing on development of pharmacological agents that inhibit malignant cells but not normal cells. The utilization of natural products in therapies, especially those from plant sources cannot be over emphasized.

Undoubtedly, medicinal plants are utilised globally as sources of drugs or herbal extracts for various chemotherapeutic purposes (Farombi, 2004; Hammad *et al.*, 2014). In addition, the use of natural compounds derived from plants, as part of herbal preparations continues to be important in the general wellness of people globally (Farombi, 2004). The African continent has a significant rich biodiversity, with an abundance of plants of economic and medicinal importance, one of these is the oil palm (Farombi, 2004).

Oil palm of *Elaeis* spp. is a plant widely used by natives of oil palm growing areas, particularly West Africa. The oil palm is of enormous economic importance to people in palm growing areas, with numerous health applications associated with most parts of the plant. Crude red palm oil processed from palm fruit mesocarp, is administered as an antidote to poison and used with other herbs in the treatment of skin ailments (Iwu, 2014). Crude palm kernel oil from the kernel of palm fruit is also used to regulate body temperature of convulsive children. The fruit husk is utilised in medicinal soaps for treating skin infections. The leaves, fruit husk and roots of oil palm are used for the treatment of skin infection, gonorrhoea, menorrhagia and bronchitis (Gill, 1992; Sasidharan *et al.*, 2011). The fresh sap from the palm tree is utilised as a laxative, and partially-fermented palm wine is administered to nursing mothers to enhance lactation (Gill, 1992; Syahmi *et al.*, 2010). Juice extracts of the leaves is put on wounds to advance healing (Sasidharan *et al.*, 2011; Yin *et al.*, 2013). Ethnobotanical studies have uncovered cases of oil palm being used to treat tumours, pains , rheumatism and as an aphrodisiac, diuretic and liniment (Yin *et al.*, 2013; Irvin, 1985)

Numerous studies have indicated that other than the fruit of oil palm, fatty acids, essential oils as well as alkaloids , tannins, saponins, terpenoids and flavonoids are present in leaf extracts of oil palm (Sasidharan *et al.*, 2010; Chong *et al.*, 2012; Ibraheem *et al.*, 2012). These classes of compounds have wide range of biological and biochemical influences. For example, saponins are used in treating tuberculosis and inflammation; sterols are indicated for allergy, arthritis and coronary failure, alkaloids for increasing blood circulation, nutrient absorption, pain management and nerve stimulation due its narcotic effects (Yin *et al.*, 2013). Some tannins appears to exhibit anti-parasitic, antimicrobial, anti-secretolytic, anti-irritant , and antiphlogistic properties (Chooi, 2006). Some flavonoids are also reported to possess antiinflammatory, anti-microbial, anti-allergic, and anticancer properties (Cushnie and Lamb, 2005; Yin *et al.*, 2013).

The possibility of oil palm leaves having anticancer properties is boosted by the realization that polyphenols, tocotrienols and carotenoids are also very much present in palm oil leaf extracts (Tan *et al.*, 2011)

1.1. PROBLEM STATEMENT OF STUDY

Conventional methods for cancer treatment including chemotherapy, radiography, and surgery amongst others are plagued with significant challenges such as side-effects, development of resistance by cancer cells to cancer drugs, recurrence of cancer cells and the high financial cost of treatment. Here in Ghana, first line cancer drugs such as Geldanamycin and its analogues (Bedin *et al*., 2004) are very expensive and beyond the means of the average Ghanaian. For example, the average cost of first line treatment of breast cancer patients, as at 2012 was estimated to be GH₵ 6,008.09 (Hughes *et al.*, 2013). The Ghana National Health Insurance scheme covers only limited aspects of cancer treatment and the cancer chemotherapeutic drugs are not readily available at most pharmacies, even at points of prescription.

Local traditional healers, who use available plants for treatment of a vast array of ailments including cancer, at very low costs, are treating a significant number of cancer patients. Some of the plants reportedly used by these traditional healers in their herbal preparations are said to be potentially toxic, mutagenic and carcinogenic due to their crude nature. For instance, Isnard *et al*. (2004), implicated herbal remedies in 35% of all cases of renal ailments and that the efficacy, toxicity and safety of many plants used in herbal remedies are not scientifically confirmed.

1.2. JUSTIFICATION

Leaves of palm trees are mostly considered as waste by the oil palm industry. At most, it is a source of mulch, domestically used as feed for some rodents and ruminants, and as roofing materials for huts. However, it has some indigenous medicinal applications. It is widely noted for its wound healing properties and ethnobotanical studies reveals its varied use in the treatment of headaches, rheumatism and its consideration as an aphrodisiac, diuretic and liniment (Irvin, 1985).

Studies on leaf extracts of the *Elaeis guineensis* specie of oil palm reports it to be non-toxic and showing antimicrobial, antibacterial and antimalarial properties (Vijayarathna *et al.*, 2012; Anyanji *et al.*, 2013a). It is purported to have cardiovascular, anti-diabetic, anti-inflammatory properties as well as positive effects on liver and kidney functions (Abeywardena *et al.*, 2002; Jaffri *et al.*, 2011; Tan *et al.*, 2011; Anyanji *et al.*, 2013b).

The potential of oil palm leaves having antioxidant and anticancer properties has been under investigation in recent times and it is boosted by the realization that polyphenols such as flavonoids, tocotrienols and carotenoids are also very much present in palm oil leaf extracts (Tan *et al.*, 2011). Some studies have reported antioxidant properties and cytotoxic effects on mcf-7 cell lines by the leaf extracts of *Elaeis guineensis* (NgMei and ChooYuen, 2010). However, none of such reports has made mention of *Elaeis oleifera* and no comparative studies on their phytochemical profile, antioxidant and anti-cancer properties have been made.

1.3. MAIN OBJECTIVE

The general objective of this study was to compare the phytochemical profile, antioxidant and anticancer properties of aqueous and hydroethanol extracts of *E. guineensis* and *E. oleifera* leaves on leukemia and breast cancer cell lines *in vitro.*

1.3.1 Specific Objectives

This work specifically seeks to

1. Qualitatively profile the phytochemical constituents of crude aqueous and hydroethanol leaf extracts of *E. guineensis* and *E. oleifera*.
2. Evaluate antioxidant activity of the crude aqueous and hydroethanol leaf extracts of *Elaeis guineensis* and *Elaeis oleifera* using 1,1-diphenyl-2-picrylhydazyl (DPPH) and Reducing power assays.
3. Determine the total phenolic and flavonoid contents of the crude aqueous and hydroethanol leaf extracts of *E. guineensis* and *E. oleifera* by Folin-Ciocalteau and Aluminium chloride methods, respectively.
4. Evaluate the cytotoxic activity of crude aqueous and hydroethanolic leaf extracts of *E. guineensis* and *E. oleifera* as well as ethyl acetate, petroleum ether and chloroform solvent fractions of the extracts on leukaemia and breast cancer cell lines (Jurkat and MCF7, respectively), *in vitro*, using the tetrazolium-based colorimetric assay (MTT) assay.
5. Compare the phytochemical profile, cytotoxicity and antioxidant properties of crude aqueous and hydroethanol leaf extracts of *E. guineensis* and *E. oleifera* and their fractions.

CHAPTER TWO

2.0. LITERATURE REVIEW

Cancer is a generic term for diseases caused and characterised by the unregulated growth and replication of cells. The abnormal cells may invade neighbouring tissues via the blood and

lymph systems. The invasion, termed metastasis is of major concern in the management and treatment of cancer.

Cancers are known to have diverse causes but may be grouped into two factors; internal genetic susceptibility and external environmental factors (Stewart and Wild, 2014). The internal genetic factor is the inheritance of genetic mutations that predisposes a person to the growth of cancerous cells. The external factor refers to anything in the environment that interacts with the body. The external factor includes biophysical environment (exposure to factors such as carcinogens and radiations), lifestyle and behavioural choices such as reproductive behaviours, physical inactivity and tobacco smoking (Perera, 1997; Manton *et al.*, 2008). Infection by oncoviruses (Human papilloma, hepatitis B and C viruses), bacteria (*Helicobacter pylori*) and parasites (*schistosoma haematobium* and liver fluke) are also known causes of cancers (Parkin, 2006). These external factors may act together with other factors, or in sequence to initiate or promote the development of cancer.

Aging is another central component for the development and advancement of cancer (Siegel *et al.*, 2014). Inclination to cancer rises significantly with age, in all probability because of a build-up of risks for particular cancers diseases with age. The general risk aggregation is joined with the inclination for cell repair systems being less compelling as a person ages.

2.1.1 Incidence and Mortality Rates of Cancer

Cancer is a noteworthy ailment worldwide and its weight keeps on expanding to a great extent as a result of aging global populace with increasing appropriation of cancer-associated life-style including smoking and physical latency (Jemal *et al.*, 2011).

Estimated New Cases	Estimated

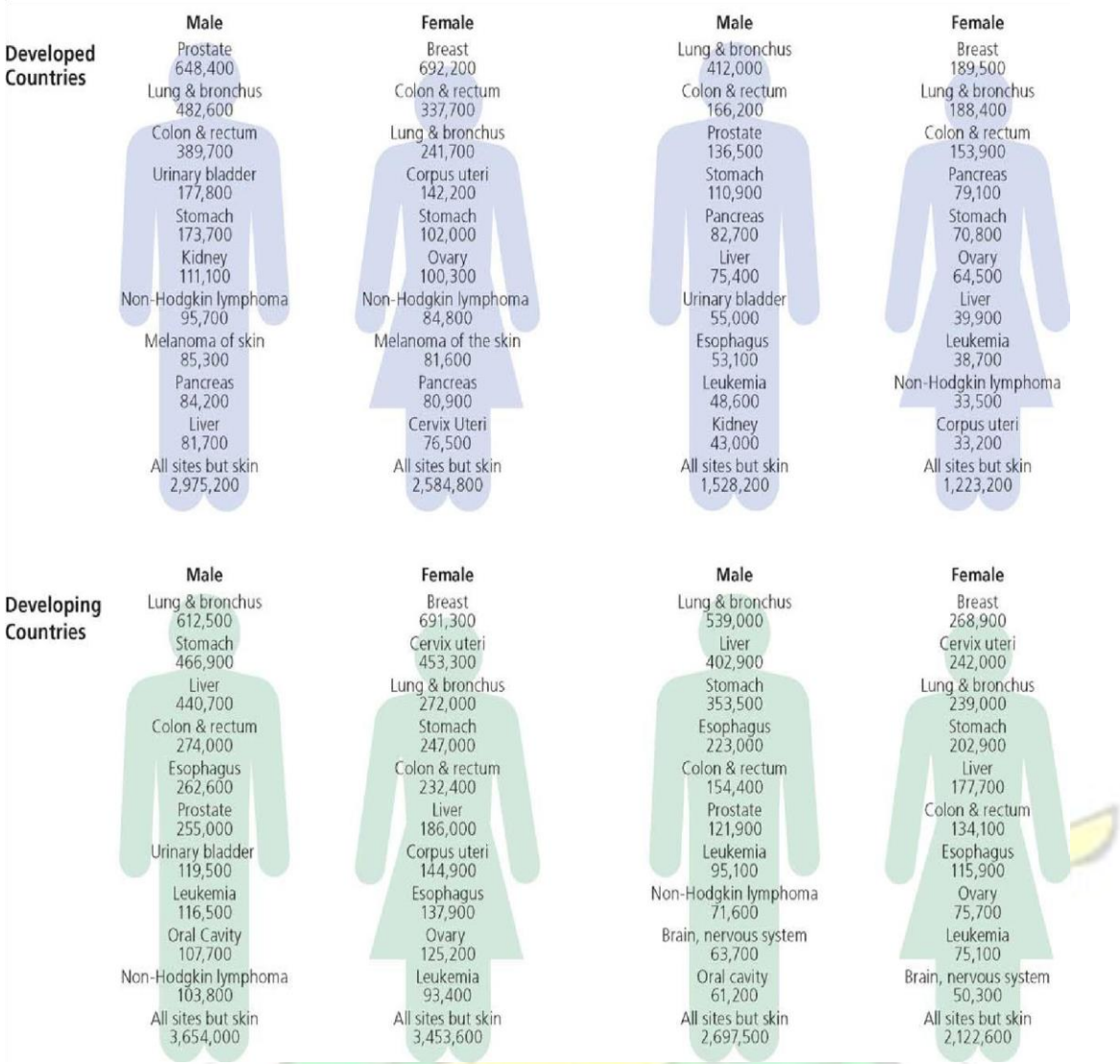


Figure 2.1: Estimates of cancer incidence and mortalities for developed and developing countries (Jemal *et al*., 2011)

Every year a huge number of individuals are diagnosed with cancer around the globe. As indicated by GLOBOCAN (2012), an expected 14.1 million new occurrences of cancer and 8.2 million cancer related demise happened in 2012. A projection in view of the GLOBOCAN 2012 report predicted a substantive increment to 19.3 million new cases every year by 2025 because of appropriation of cancer-associated life-style and aging of the worldwide populace (IARC and WHO, 2014).

In 2012, the most frequently cancer diagnosed, globally, were those of lung, breast and bowels (Stewart and Wild, 2014) while the most widely recognized cancer related death were those of lung, liver, and stomach. In economically developed world such as the United States of America and Europe, cancer is the main source of death and the second in developing world such as Africa and Asia (Stewart and Wild, 2014). About 60% of global cancer cases are recorded in Africa, Asia, South and Central America. These areas represented 70% of the globally recorded cancer-related deaths (Stewart and Wild, 2014).

Breast and lung cancers in females and males respectively; are the most reported, diagnosed and main source of cancer related-deaths globally (Jemal *et al.*, 2011). The global estimates of the occurance of various cancers and a ranking according to frequency, is presented in Figure 2.1. The increasing number of cancers disproportionately affects developing countries and the apparent cancer gap could be considered as an effect of aging populaces with the increasing appropriation of westernised lifestyles such as diet and the situation is exacerbated by the absence of timely diagnosis and poor access to treatment (Stewart and Wild, 2014).

2.1.2 Incidence and Mortality of Cancer in Ghana

Cancer is emerging as a significant health problem in Ghana, although infectious diseases and cardiovascular conditions still continue to be major cause of mortality. About 2.6% of all new hospital attendance at the Korle-Bu teaching hospital (KBTH) were diagnosed with a type of cancer and 5.6% of deaths reported have been attributed to cancer (Biritwum *et al.*, 2000). The top ten causes of cancer mortality in Ghana according to most frequently diagnosed cancers are cervix, breast, liver, haematopoietic organs, stomach, colorectal, ovary, bladder, with pancreas and Kaposi sarcoma at the 9th and 10th position in Females (Wiredu and Armah, 2006). For males, prostate, liver, haematopoietic organs, colorectal, bladder, Kaposi sarcoma, stomach,

lung, larynx and oral cavity cancers were the top ten (Ferlay *et al.*, 2005; Wiredu and Armah, 2006).

Table 2.1: Age-Standardized Cancer Ratio (ASCR) for cancer types responsible for patient mortalities at the Korle-Bu Teaching Hospital (KBTH)

Cancer Types	Females ASCR
Breast	17.24%
Haematopoietic organs	14.69%
Liver	10.97%
Cervix	8.47%

Cancer Types	Males ASCR
Liver	21.15%
Prostate	17.35%
Haematopoietic organs	15.57%
stomach	7.26%

Source (Wiredu and Armah, 2006)

Wiredu and Armah (2006) studied 3,659 cancer deaths over a ten-year period. They reported that the commonest cause of cancer-related death in females was that of the breast, haematopoietic organs, liver and cervix malignancies with a mean age of 46.5 years; and in males, cancers of the liver, prostate, haematopoietic organs and stomach (Table 1)

2.2. CARCINOGENESIS

Cancer is a complex disease that has been characterized with wide variations in it presentations, development and outcome. The incidence of cancer is the outcome of complex biological processes over a period of time, possible influenced by external agents, involving successive generations of cells progressively advancing towards cancerous growth (Foulds, 1975).

Carcinogenesis may be described by various ways. It may be described by its ―Hallmarks‖, which are fundamental properties required for its development ; self-sufficient proliferation, mitigation of anti-proliferation signals, mitigation of apoptosis signals, unregulated replication potential, tissue invasion and metasis (Hanahan and Weinberg, 2000; Rakoff-Nahoum, 2006). It could also be described as a multi-step process functionally designated as initiation, promotion and progression stages (Kinzler and Vogelstein, 1996; Hanahan and Weinberg, 2000; Rakoff-Nahoum, 2006).

2.2.1. Initiation

This encompasses all heritable changes that may occur within a cell resulting from irreversible mutations in one or more cellular genes controlling key regulatory pathways. It has been proven by various studies that, the evolution of a normal cell into a cancerous cell are characterized by changes at the genetic, epigenetic levels as well as abnormal cell divisions (Croce, 2008). To become cancerous, a cell must have several changes in oncogenes and tumour suppressor genes. These changes may occur when mutations occur in DNA sequences (Croce, 2008). Mutations in DNA sequences may involve point mutations, deletions, insertions, amplifications and translocations resulting in irreversible cellular changes (Rakoff-Nahoum, 2006). These genome alterations may result in the activation of proto-oncogenes such as Kras gene and the inactivation of tumor suppressor genes such as TP53 gene (Rakoff-Nahoum, 2006). These alterations may occur within a gene‘s sequences or in the promoter region of the gene. Mutation in a gene may stop the production of critical proteins or the production of abnormal levels of critical proteins. For example, the KRAS gene instructs the synthesis of kras protein. This protein is normally involved in the RAS/MAPK pathway delivering signals to the cell‘s nucleus for cell division (Gay *et al.*, 1999). A mutant KRAS gene produces a mutant kras protein which continuously transmit permanent cell division signals (Goodsell, 1999). Mutations

of the KRAS gene are implicated in a number of cancers such as colorectal cancer and adenocarcinomas of the lung (Wilson *et al.*, 2010).

Table 2.2: Common oncogenes and human suppressor genes involved in human cancer

ONCOGENES	
PDGF	Codes for platelet-derived growth factor involved in glioma
EGFR	Codes for epidermal growth factor receptor involved glioblastoma and breast cancer
HER-2 or ERBB2	Codes for a growth factor receptor involved in breast, salivary gland and ovarian cancers
RET	Codes for a growth factor receptor involved in thyroid cancer
KRAS	involved in lung, ovarian, colon and pancreatic cancers
NRAS	involved in leukaemia
MYCI	involved in leukaemia, breast, stomach and lung cancer
NMYC	involved in neuroblastoma and gastroblastoma
LMYC	involved in lung cancer
BCL2	Codes for a protein that is involved in follicular B cell lymphoma
TUMOUR SUPPRESSOR GENES	
APC	involved in colon and stomach cancers
DPC4	Codes for relay molecules in a signaling pathway that inhibits cell divsions involved in pancreatic cancer
NF-1	Codes for a protein that inhibit Ras proteins involved in neurofibroma , pheachromacytoma and myeloid leukaemia.
NF-2	involved in meningioma and schwannoma
CDKN2A or MTSI	codes for p16 proteins, a breaking component of the cell cycle clock
WT1	involved in Wims' tumour of the kidney
BRCA1	involved in breast and ovarian cancers
BRCA2	involved in breast cancers
VHL	involved in renal cell cancer

Source: (Wilson *et al.*, 2010)

A mutation in TP53 gene, which produces the p53 protein normally involved in the induction of apoptosis for cells with incomplete or aberrant DNA. A mutation in the TP53 proteins produces an incompetent p53 protein. Mutations of the TP53 genes are implicated in almost half of cancer cases (Olivier *et al.*, 2009; Suzuki and Matsubara, 2011). Such genes, whose

loss of function contributes to development of cancer cells are termed tumor suppressive genes (Olivier *et al.*, 2009).

2.2.2. Promotion

Promotion as a stage of carcinogenesis includes factors, which ultimately enable the cancerinitiated cell to survive and proliferate forming clonal growths. Promotion may be broadly categorized as specific and non-specific. Specific promotion involves interaction with receptors in and on target cells leading to the stimulation of unregulated proliferation of cancer-initiated cells, whilst non-specific promotion includes alteration of gene expression such as epigenetic events causing changes in gene expression without changes in DNA (Troll and Wiesner, 1985). Epigentic events refers to heritable changes that may affect expression of a gene not related to DNA mutations but from DNA methylation, RNA-mediated and silencing histone modification among others (Oey and Whitelaw, 2014). It encompasses mechanisms, which may alter the expression of the genetic code without a change in the underlying DNA sequence. DNA methylation critically controls the activity of genes and nucleus architecture of the cell. It does so by the covalent addition of a methyl group to the 5-carbon position of cytosine base preceding guanines (Esteller, 2007). These regions, referred to as CpGs islands are found in the regulatory regions of most genes and are not randomly placed in the genome (Herman and Baylin, 2003; Weber *et al.*, 2007). DNA methylation is typically, one of the several mechanisms that silences the expression of MASPIN and MAGE genes, as in malignant tissues

(Bodey, 2002; Futscher *et al.*, 2002). It also ensures the mono-allelic expression of one of the two parental alleles of a gene during genomic imprinting and the gene-dose reduction observed in inactivating an x-chromosome in females (Feinberg *et al.*, 2002).

Aberrant DNA methylation may encourage the development of cancer by hypo-methylation of oncogenes and hyper-methylation of tumor suppressing genes (Jones and Baylin, 2002; Feinberg and Tycko, 2004). Hypo-methylation refers to the loss of methylation resulting in low levels of DNA methylation in cancer cells compared to normal cells. The loss of methylation has been noted to promote the disruption of genomic imprinting such as that of the insulin-like growth factor, which is a risk factor for colorectal cancer (Cui *et al.*, 2003; Kaneda and Feinberg, 2005).The demethylation in the promoter region of CpG islands may influence the expression of genes that are normally repressed. For example, the PAX2 gene encoding transcription factors for proliferation and the Let-7a-3 miRNA gene are both activated when demethylation of their CpG ilands occurs. These two genes are implicated in endometrical and colon cancers (Wu *et al.*, 2005; Brueckner *et al.*, 2007) .

Hypermethylation of genes in their CpG islands of promoter regions represses the expression of such genes. Hypermethylation of genes involved in angiogenesis, apoptosis, cell-to-cell interaction, cell cycle, and DNA repair promotes the development of cancer. The inactivation of such genes have been implicated in many cancer cases (Herman and Baylin, 2003; Esteller, 2007) examples of such genes and cancer cases are shown in table 2.2.

Mitogenic events includes changes permiting the cell to proliferate excessively in an untimely manner, evade surveillance of the immune system, invade and influence other cells via metastases making them ungoverned by normal cell growth and behavior controls (Evan and Vousden, 2001).

The ability of cancer cells to outwit the immune system permits it to live independent and adopt aggressive and invasive behaviors, adapting themselves to new conditions such that they may resist attempts at eliminating them via either cytotoxic drugs or radiation treatments (Hanahan and Weinberg, 2000). To attain such status, a cell must be able to violate three fundament rules

that govern cell life and replication. Cells should divide only upon receiving explicit signals (Hanahan and Weinberg, 2000).

Table 2.3: Epigenetic Aberrations among different tumor types

Type of Cancer	Epigenetic Distruption
Colon cancer	CpG-island hypermethylation (hMLH1, p16INK4a, p14ARF, RARB2, SFRP1, and WRN), hypermethylation of miRNAs (miR-124a), global genomic hypomethylation, loss of imprinting of IGF2, mutations of histone modifiers (EP300 and HDAC2), diminished monoacetylated and trimethylated forms of histone H4
Breast cancer	CpG-island hypermethylation (BRCA1, E-cadherin, TMS1, and estrogen receptor), global genomic hypomethlation
Lung cancer	CpG-island hypermethylation (p16INK4a, DAPK, and RASSF1A), global genomic hypomethylation, genomic deletions of CBP and the chromatin-remodeling factor BRG1
Glioma	CpG-island hypermethylation (DNA-repair enzyme MGMT, EMP3, and THBS1)
Leukaemia	CpG-island hypermethylation (p15INK4b, EXT1, and ID4), translocations of histone modifiers (CBP, MOZ, MORF, MLL1, MLL3, and NSD1)
Lymphoma	CpG-island hypermethylation (p16INK4a, p73, and DNA-repair enzyme MGMT), diminished monoacetylated and trimethylated forms of histone H4
Bladder cancer	CpG-island hypermethylation (p16INK4a and TPEF/HPP1), hypermethylation of miRNAs (miR-127), global genomic hypomethylation
Kidney cancer	CpG-island hypermethylation (VHL), loss of imprinting of IGF2, global genomic hypomethylation
Prostate cancer	CpG-island hypermethylation (GSTP1), gene amplification of polycomb histone methyltransferase EZH2, aberrant modification pattern of histones H3 and H4
Esophageal cancer	CpG-island hypermethylation (p16INK4b and p14ARF), gene amplification of histone demethylase JMJD2C/GASC1
Stomach cancer	CpG-island hypermethylation (hMLH1 and p14ARF)
Liver cancer	CpG-island hypermethylation (SOCS1 and GSTP1), global genomic hypomethylation
Ovarian cancer	CpG-island hypermethylation (BRCA1)

(Source : Esteller, 2007)

To thwart this, cancer cells must permanently activate all the necessary signals from hormones and growth factors: which will normally activate cell division. When cells are faced with

stressful and/or unfavourable conditions for DNA replications, cells must activated apoptotic mechanisms so as not allow modification of genes (Hanahan and Weinberg, 2000). This mechanism is controlled by two genes Retinoblastic (RB1) (Downward *et al.*, 1984) and TP53 gene, which produces the P53 proteins (Burkhart and Sage, 2008; Olivier *et al.*, 2009). To outwit this rule, cancerous cells must be able to outwit the triggers of apoptosis

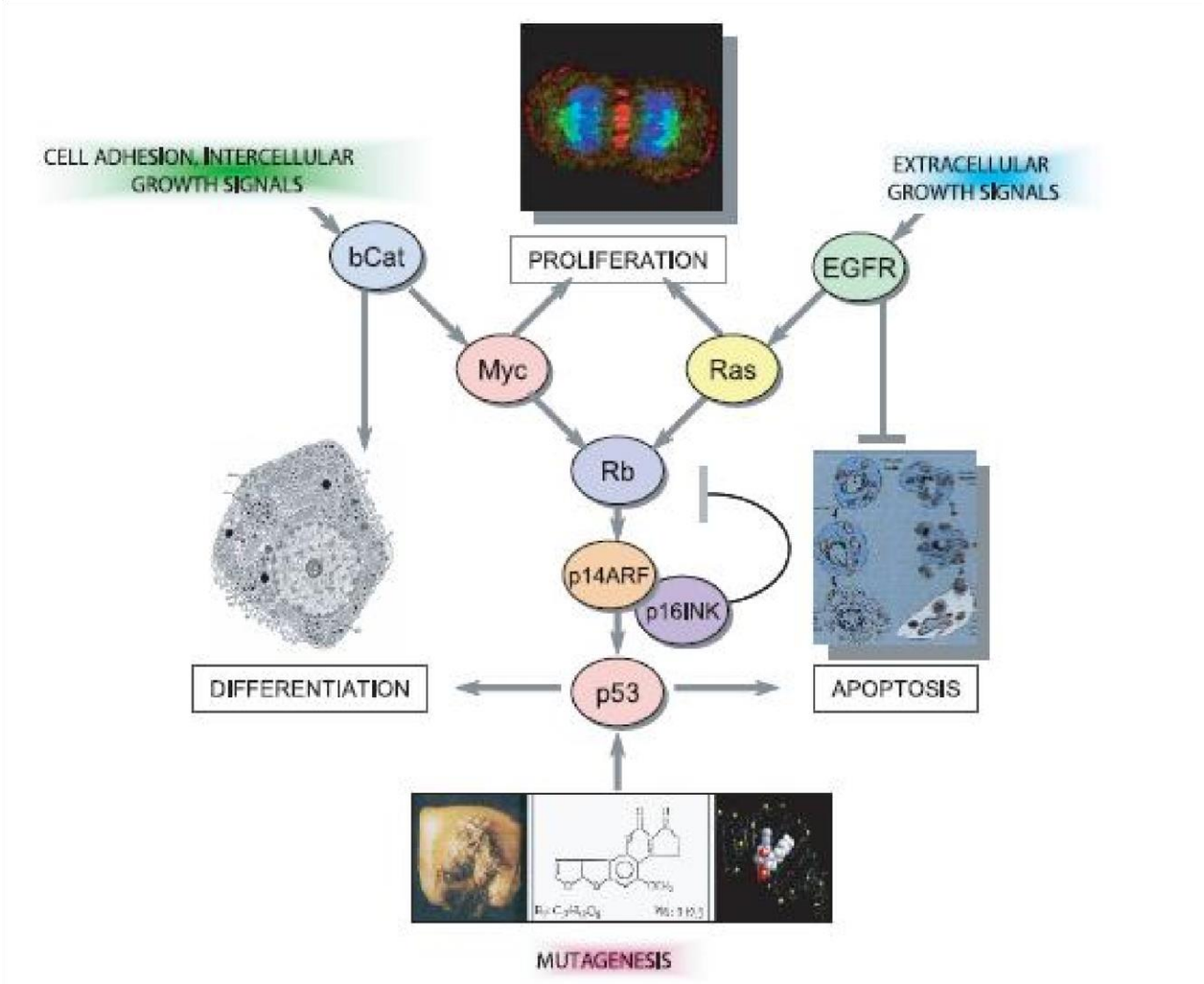


Figure 2.2: Illustration of cooperation of genes in cancer development

(source : Hanahan and Weinberg, 2000)

Normal cells are allowed a specific number of replications controlled by the telomere, small repeats of DNA sequence, which is at the end of each chromosome (Shay and Wright, 2000;

Blasco, 2005). The DNA repeat sequences erodes as the cell replicates and replication ceases when all repeats has been eroded. For a cancerous cell to replicate infinitely, it requires the impairment of the telomerase enzyme, which will enable the cells to continuously replicate well-beyond the supposed finite number of replications (Artandi and DePinho, 2010).

All cells irrespective of their intrinsic diversity follow a common scheme in the basic processes that control cell proliferation and death. Hence a number of oncogenes and tumour suppressors are commonly altered irrespective of the type and affected organ (table 2.x) these genes alltogether control cell replication, differentiation and survival. Fig 2.x presents the inter-play of gene and processes that must be altered in any cancer cell. It involves two growth-promoting processes and a growth suppressive mechanism.

Beta-carotene (bCat) and epidermal growth factor receptor (EGFR) are the effector proteins for growth-promoting processes (Kraus *et al.*, 1994; MacDonald *et al.*, 2009). bCat may be located inside the cytoplasm where, it forms a complex which receives proliferation and growth signals. Once it receives such signal, it moves into the nucleus stimulating the necessary genes required for proliferation (Kraus *et al.*, 1994; MacDonald *et al.*, 2009). In epithelial cancers such as breast, liver and colon, genes encoding bCat are often mutated (Forbes *et al.*, 2010). EGFR exists and operates on both internal and external sides of a cell (Herbst, 2004). It receives growth factors in intracellular spaces and from the blood, and activates a tyrosine kinase on the inside, which in-turn begins a cascade of intracellular signals ultimately activate cell proliferation via stimulation of progression of cell cycle (Yarden and Schlessinger, 1987; Herbst, 2004; Oda *et al.*, 2005). A mechanism to control cell proliferation, is initiated by the TP53 gene via its p53 protein. The anti-proliferation mechanism is initiated by the large accumulation of p53 proteins in the nucleus of cells in response to DNA damage and a failure to repair (Olivier *et al.*, 2009). This set-off an anti-proliferative mechanism that either induce apoptosis or block cell cycle progression forcing differentiation.

These three processes are connected via the CDKN2a gene (Hanahan and Weinberg, 2000). This gene directs the synthesis of two different proeins, p16 and p14ARF (Alternate reading frame). The p16 protein is cyclin –dependent kinase that ultimately induces cell cycle arrest, whiles p14ARF controls the intiation of p53 (Hanahan and Weinberg, 2000). Thus, mutation of the CDKN2a gene is noticed in almost every cancer.

2.2.3. Progression

Progression is an irreversible process that leads to cancer cell giving rise to increasingly malignant sub-populations. Progression of malignancy begins with and involves the conversion of pre-neoplastic cells into states of ―commitment to carcinogenesis‖. This involves continuous mutagenesis within the establishing pre-neoplastic cell clone (UNscear, 2000) due to genetic instability resulting from initiation and promotion. This confers on the pre-neoplastic dynamic cellular heterogeneity. In most cancer cases, transforming sequences in the cell genome are acquired during the progression from normalcy to cancerous cell. An elevated mutation rate established relatively early in carcinogenesis therefore provides for higher frequency generation of variant cells within a pre-malignant cell population. Such variant cells with the ability to outwit regulations that govern cell proliferation are mostly selected during carcinogenesis (unscear, 200).

As cancer progresses, carcinoma cells may embark on evasion and metastasis. Evasion and metastasis involves the alteration of cell shape and its detachment from malignant cancer cell mass to extracellular matrix such as blood and lymph. For example, the detachment of cancerous epithelial cells results from a mutational inactivation or down-regulation of Ecadherin protein, which is an adhesive molecule facilitating the adherens junctions between epithelial cells (Cavallaro and Christofori, 2004; Berx and Van Roy, 2009). Additionally, the

expression of other proteins that enhance attachment of cell to extracellular matrices are upregulated in extremely aggressive carcinomas (Cavallaro and Christofori, 2004). Detached cancerous cells upon attaching to extracellular matrix such as blood can then migrate from primary site of carcinogenesis to neighboring and distant tissues or organs, where a secondary carcinoma may be established.

The process of invasion and metastasis in humans carcinomas may be schematized as a multistep cell-biological process that begins with invasion of neighbouring cells (local invasion), entry and exit of cancer cells into (intravasation) and out-of (extravasation) hematogenous and lymphatic system and a subsequent establishment of secondary carcinomas. Carcinoma cells gain the capacity for evasion, metastasis and escape apoptosis by emulating some processes involved in embryonic morphogenesis and wound healing (Barrallo-Gimeno and Nieto, 2005; Klymkowsky and Savagner, 2009; Polyak and Weinberg, 2009; Thiery *et al.*, 2009; Yilmaz and Christofori, 2009). This ability is obtained via a couple of transcriptional factors such as Twist, Slug, Snail and Zeb1/3. These transcriptional factors normally regulate migratory processes during embryogenesis. When expressed by carcinoma cells, they elicit loss of adherens junctions and change of cell morphology (Yang and Weinberg, 2008; Schmalhofer *et al.*, 2009; Micalizzi *et al.*, 2010; Taube *et al.*, 2010).

The act of invasion may occur by anyone of three ways: ―inflammation enabled‖ invasion involves carcinoma cells secreting chemoattractants, which cause an assembly of inflammatory cells at cancer sites. These then facilitate invasion by producing enzymes that degrade extracellular matrix degrading enzymes enabling invasion (Joyce and Pollard, 2009; Kessenbrock *et al.*, 2010; Qian and Pollard, 2010). ―Amoeboid‖ invasion is the advancement of individual carcinoma cells across and into extracellular matrix facilitated by morphological plasticity enabling them to move through interstices(Madsen and Sahai, 2010; dos Santos

Menezes, 2011). ―Collective‖ invasion involves the advancement of a mass of carcinoma cells en-block into adjoining tissues. This is said to typically occur in squamous cell carcinoma (Friedl and Wolf, 2010).

Carcinoma cells requires angiogenesis to enable an adequate supply of much need nutrients, oxygen as well as evacuate carbon dioxide and metabolic wastes. Angiogenesis is typically regulated in normal tissues by an ―angiogenic switch‖ (Baeriswyl and Christofori, 2009). The ―angiogenic swtich‖ is a complex inter-play of counter-vailing regulators that either induce or inhibit angiogenesis (Hanahan and Weinberg, 2000; Baeriswyl and Christofori, 2009).

These regulators are mostly signaling proteins that stimulate or inhibit cell-surface receptors on vascular endothelial cells. Examples of such are thromnospondin-1 (TSP-1) as an inhibitor and vascular endothelial growth factor-A (VEGF-A) as an inducer. VEGF-A proteins are involved in the stimulation of new blood vessel growth in embryonic and postnatal development, endothelial cells as well as pathological and physiological situations of an adult (Bergers and Benjamin, 2003; Baeriswyl and Christofori, 2009).

Normally VGF signaling are executed via three tyrosine kinase receptors (VEGFR-1,2 and 3) and heavily regulated at multiple levels. However, it may be upregulated by oncogene signaling, hypoxia and fibroblast growth factor (FGF) signaling and these sustains carcinoma angiogenesis (Gabhann and Popel, 2008; Ferrara, 2009). TSP-1 proteins normaly binds the transmembrane receptors of endothelial cells evoking counterbalancing signals that suppresses proangiogenic stimulations (Kazerounian *et al.*, 2008).

Blood vessels produced by aberrant angiogenesis due to unregulated proangiogenic stimulation are enlarged and distorted allowing erratic blood flow. They are convoluted with excessive branching nourishing abnormal levels of endothelial cell proliferation (Baluk *et al.*, 2005; Nagy *et al.*, 2010).

On the activation of aberrant angiogenesis, carcinomas may exhibit diverse patterns of neovascularization. Aggressive carcinomas such as pancreatic ductal adenocarcinomas are noted to be highly hypo-vascularized, hence deemed anti-angiogenic whiles renal and pancreatic neuroendocrine carcinomas are highly pro-angiogenic and consequently hypervascularized (Turner *et al.*, 2003; Olive *et al.*, 2009).

2.2.4. Role of Anti-oxidants in Carcinogenesis and Signaling Pathways Targeted by Antioxidants

Reactive oxygen species (ROS) and other free radicals are produced in the body as a consequence of normal metabolic processes and also under the influence of external toxic situations and agents such as radiations and toxic chemicals (Devi, 2004). Reactive oxygen species (ROS) include hydroperoxides, superoxide anions, hydroxyl radicals, peroxy radicals amongst others. These ROS may interact with DNA and producing genomic mutations and chromosome aberrations, leading to cell transformation. They are noted to have a considerable role in the intiation of cancers (Devi, 2004). To nullify this effect, higher animals have developed defence mechanisms, including antioxidant proteins and phase II detoxification enzymes (Lee and Johnson, 2004). Studies designed to identify the regulatory element for the defence mechanism genes (of antioxidant proteins and phase II detoxification enzymes) have revealed a central role of the antioxidant responsive element (ARE) (Lee and Johnson, 2004). The ARE is a *cis*-acting regulatory element (enhancer sequence), which is found in promoter regions of genes encoding phase II detoxification enzymes and antioxidant proteins such as NAD(P)H quinone oxidoreductase 1, glutathione S-transferases, and glutamate-cysteine ligase (Lee and Johnson, 2004).

Many proteins have been suggested to regulate antioxidant responsive element, but the underlying antioxidant responsive element activation mechanism began to be elucidated with the identification of nuclear erythroid 2-related factor 2 (Nrf2) (Lee and Johnson, 2004). Nrf2, a basic leucine zipper transcription factor, was identified as a binding protein of locus control region of ß-globin gene. Nrf2 during ARE-driven gene expression, is sequestered in the cytoplasm by Keap1 and ARE activation signals (that is, protein kinase pathways and electrophiles), dissociates from Keap1 during oxidative stress and translocates into the nucleus, followed by dimerization with a Maf (musculoaponeurotic fibrosarcoma) protein, binding to ARE, and transcription of the gene (Ma *et al*., 2004).

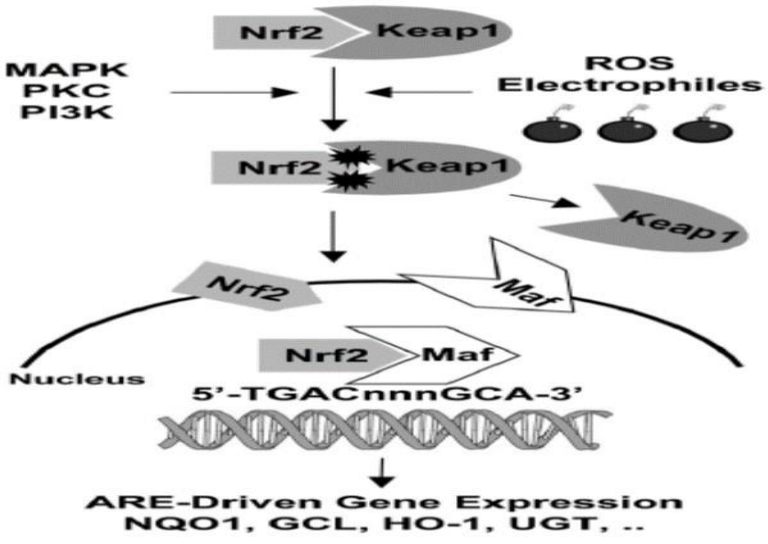


Figure 2.3 ARE-driven gene expression by Nrf2

(Source: Lee and Johnson, 2004)

One large group of these target genes is the phase II detoxification and antioxidant genes. By inducing these genes through the Nrf2/ARE pathway, chemopreventive agents could increase the detoxification of procarcinogens or carcinogens. Thus protecting normal cells from the DNA/ protein damage caused by electrophiles and reactive oxygen intermediates, thereby decreasing the incidence of tumour initiation and reducing the risk of cancer (Hu *et al*., 2006).

The role of Nrf2 in preventing tumorigenesis is further supported by studies in which Nrf2 knockout mice were much more susceptible to carcinogen-induced carcinogenesis and failed to respond to certain cancer chemopreventive agents which were effective in Nrf2 wild-type mice (Hu *et al*., 2006). Some studies have also suggested that Nrf2 might be involved in apoptosis signaling pathways. Overexpression of cleaved Nrf2 (C-terminal fragment) induced apoptosis in HeLa cells and caspase-3 (-like) proteases cleave Nrf2, inferring cleaved Nrf2 might have some role in the induction of apoptosis (Ohtsubo *et al*., 1999). In addition, Kotlo *et al*. (2003), reported that overexpression of Nrf2 protects cells from Fas-induced apoptosis, signifying an important role of Nrf2 in anti-apoptotic pathways. Work done by Li *et al*. (2002) identified the ARE-driven genes including NQO1 that were responsible for protecting IMR-32 human neuroblastoma cells from H_2O_2-induced apoptosis.

Moreover, the function of Nrf2 and its downstream target genes have been shown to be important for protection against oxidative stress-induced cellular damage. Chan and Kan (1999), showed that knockout ($Nrf2^{-/-}$) mice were extremely susceptible to the antioxidant, butylated hydroxytoluene (BHT), with the same doses of BHT tolerated by wild-type mice ($Nrf2^{+/+}$). Enomoto *et al*. (2001), also demonstrated that administration of acetaminophen induced more severe centrilobular hepatocellular necrosis in $Nrf2^{-/-}$ mice compared with $Nrf2^{+/+}$ mice. Studies by Lee *et al*. (2003a), on oligonucleotide microarray analysis revealed that, Nrf2 regulates the orchestrated gene expression of detoxification enzymes, antioxidant proteins, anti-inflammatory proteins, calcium homeostasis protein, and signaling molecules. For instance, Nrf2 coordinately up-regulates genes which are involved in maintenance (i.e. synthesis and regeneration) and utilization of glutathione. This orchestrated up-regulation of ARE-driven genes by Nrf2 appeared to be very efficient in increasing cellular detoxification and antioxidant capacity, implying an important role for Nrf2-ARE pathway as a cellular antioxidant defense.

Since the Nrf2-ARE pathway acts as a master regulator of many protective genes, it may serve as a therapeutic target for carcinogenesis, in which oxidative stress is involved.

2.3. MANAGEMENT OPTIONS FOR CANCER DISEASES

The primary objective of cancer therapy is to cure without significant damage to the body and ostensibly accord the patient some comfort and promote the best possible quality of life. Treatment programmes are presently dependent on early detection and establishment of diagnosis to ascertain the degree of malignancy.

There are many methods and techniques employed in the treatment of cancer. The most important treatment choices incorporate surgery, chemotherapy, radiation and targeted therapy. The choice of method depends on the type, location and level of malignancy as well as the patient‘s preferences and health status.

2.3.1 Surgery

Surgery is the physical removal of cancerous cells via an operation. This has been an essential basic treatment for some types of cancer. The purpose of surgery in relation to cancer management varies. It is frequently used to remove all or a percentage of the cancerous tissue after diagnosis; examples of such are mastectomy for breast cancer and prostatectomy for prostate cancer (Subotic *et al.*, 2012).

In some cases of cancer management, a biopsy is required for definitive diagnosis; hence a surgical biopsy is required. An incision biopsy for removal of some of the suspicious tissue or an excision biopsy to remove the entire suspicious area, if possible, is performed. Suspicious tissue is then examined and analysed for diagnosis (Zerbino, 1993).

Staging surgery may be required to determine extent of cancer spread and growth. This often includes removing compromised lymph nodes, tissues and cells surrounding cancerous cells.

Biopsy with the aid of physical examination and results of laboratory and imaging tests help plan the management of cancer, such as the kind of treatment and patient's prognosis.

Depending on the prognosis, curative surgery or tumour removal may be considered. This is the complete excision of suspicious cancerous tissue and some of the surrounding tissue. Curative surgery or tumour removal may be the main treatment, or it may be executed with chemotherapy, radiation therapy and other treatments, which might be given before or after surgery.

When complete removal of cancerous tissue might cause excessive damage to the body; the size of cancerous tissue is reduced by removing as much of it as possible in a procedure termed debulking. Other treatments (radiation therapy or chemotherapy) are used to shrink the remaining cancer.

2.3.2 Radiation Therapy

Radiation therapy of cancer involves the use of light emissions to annihilate cancer cells and mitigate genes that predisposes the patient (Dollinger *et al.*, 2002). Both healthy and cancerous cells are affected by exposure to high-energy radiations and the objective is to annihilate fewer normal, healthy cells as possible, hence the specificity required for radiation therapy. The therapy could be delivered externally from a linear accelerator outside the body, targeted at the specific cancer site or the radioactive material may be placed in the cancer or into tissues surrounding it (Joiner and van der Kogel, 2009).

Radiation therapy alone may be the main or only treatment employed to completely eliminate the cancerous cell, shrink tumours and in palliative care. It could also be employed as an adjuvant therapy, where it is used to complement other treatment therapies such as radio immunotherapy (Joiner and van der Kogel, 2009).

2.3.3 Chemotherapy

Chemotherapy refers to the use of drugs in the management of cancer. Anti-cancer drugs may be administered with curative intent or palliative intent and may be in conjunction with other treatment such as radiation therapy and surgery provided as adjuvant or neoadjuvant therapy (ACS, 2013).

Most cancer chemotherapeutic agents are cytotoxic, thus impairing mitosis by various mechanisms such as damaging DNA and inhibiting cellular mechanisms involved in cell division of fast-dividing cells and the induction of programmed cell death, apoptosis (Makin and Hickman, 2000; Malhotra and Perry, 2003; Kehe *et al.*, 2009).

2.3.3.1 Pathways targeted by chemotherapy

Apoptosis is a noteworthy control mechanism by which cells with damaged and unrepaired DNA are destroyed. Apoptosis is vital in controlling proliferation and growth as part of normal development (Lowe and Lin, 2000; Redondo *et al.*, 2009). Defects in apoptosis have been associated with the formation of immortal cells and cancers like leukemia (Tsujimoto *et al.*, 1985; Ghobrial *et al.*, 2005).

The amendment or regulation and stimulation of apoptosis via its pathways with chemotherapeutic agents as a way of curing or eliminating cancer cells is an important objective of numerous preclinical medication investigations (Ricci and Zong, 2006; Çiftçi *et al.*, 2014).

The two major signalling pathways that lead to apoptosis are intrinsic (mitochondria-mediated pathway) and the extrinsic (extracellular activated pathway) (Danial and Korsmeyer, 2004; Kroemer *et al.*, 2005).

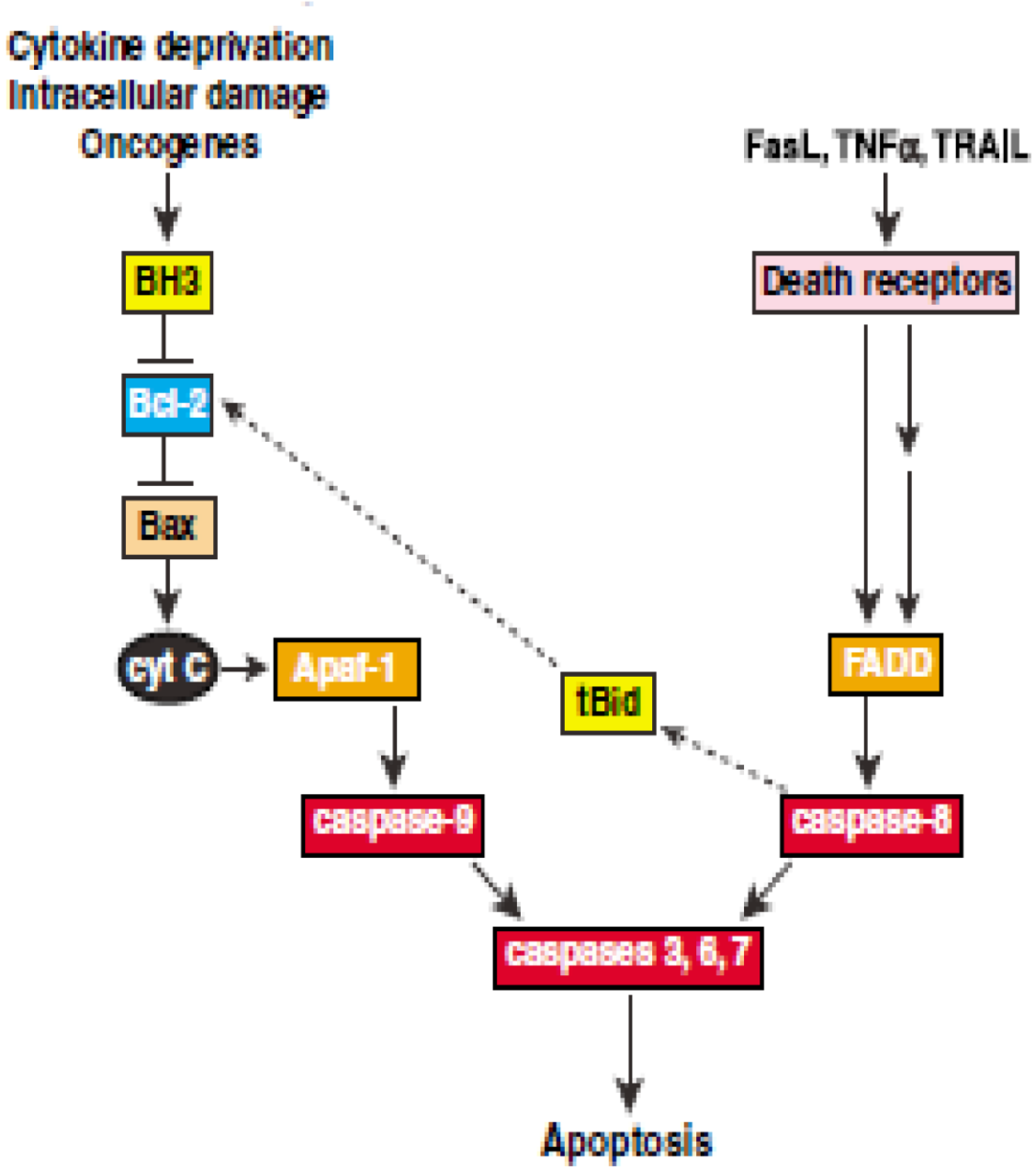


Figure 2.4: Pathways to apoptosis

(Source: Adams and Cory, 2007)

Intracellular stress signals, including DNA damage and reactive oxygen species (ROS), and in addition viral infection and initiation of oncogenes typically elicit the intrinsic pathway. Bcl-2 family of proteins primarily regulates this pathway. Intrinsic pathway, as illustrated in Fig 2.4, is launched by BH3 proteins that inactivate Bcl-2-like proteins preventing the restraining of

pre-forming proteins Bax and Bak. These proteins may permeabilize the mitochondria external membrane to discharge cytochrome C, which incites apoptotic protease-activating factor1(Apaf-1) to activate caspase-9 (Adams and Cory, 2007).

The extrinsic pathway is initiated by the activation of Fas death receptor, which stimulates caspase-8, 9 and 10, through adaptor proteins. Upon stimulation, the caspase launches downstream _effector caspases‘ such as caspases-3, 6 and 7 (Adams and Cory, 2007).

Thus, the intrinsic and extrinsic apoptotic pathways converge into a final path resulting in the promulgation of death signals by the activation of series of caspases. In the final apoptotic pathway, caspases 3, 6 and 7 are also involved (Mancini *et al*., 1998; Thornberry and Lazebnik, 1998). Both intrinsic and extrinsic pathways focalize on caspase-3 launching the caspaseactivated deoxyribonuclease, which invariably leads to nuclear apoptosis (Ghobrial *et al.*, 2005).

Some chemotherapeutic agents target the apoptotic machinery. Based on the apoptotic pathway that is targeted by these agents, they may be classified as extrinsic or intrinsic. Chemotherapeutic agents that target intrinsic pathway may act specifically on the mitochondrial inner membrane. For example, arsenic trioxide antagonise the anti-apoptotic members of the Bcl-2 family such as oblimersen sodium, and promote the activity of the proapoptotic members of the Bcl-2 family such as Bax (Lee and Tenniswood, 2004; Ghobrial *et al.*, 2005; Fulda *et al.*, 2010). Chemotherapeutic agents that target the extrinsic pathways are Tnf-regulated apoptosis-inducing ligand (TRAIL), monoclonal antibodies antagonistic to Dr5 and Dr4 and all trans retinoic acid (ATRA).

2.3.3.2 Challenges of cancer chemotherapy

Chemotherapy for the treatment of cancer has been a first line treatment option for the past half century. It has been successfully utilized for treating some tumours like testicular cancer and leukaemia, however the effectiveness in the treatment of tumours of the breast, colon and lung has been less successful (Johnstone *et al.*, 2002).

Generally, cancer chemotherapy is challenged by its attendant side effects such as development of resistance, recurrence of cancer cells and high financial cost of treatment.

Ideally, chemotherapeutic agents whether used in combination, as adjuvant or targeted therapy should be selective, specifically targeting only neoplastic cells and ought to diminish cancer load by inducing cytotoxicity with minimal impacts on normal cells. However, the reality is systematic toxicity as a result of inadequate specificity, rapid drug metabolism, intrinsic and developed drug resistance.

Issues of drug resistance in chemotherapy is still being studied, and it is the most flighty element influencing chemotherapy (Lage, 2008). Considering the adaptability of tumour cells, it appears likely that drug resistance will keep on being a critical issue, even in the age of targeted therapeutics and custom-made treatment regimes.

Management of cancer by chemotherapy, either in combination, as adjuvant or targeted therapy poses some side effects, which are dependent on the location and type of the cancer, type of drug and treatment dose, and patient‘s overall health. The effect on the cancer patient may range from pain, fatigue, nausea, diarrhoea, blood disorders, nervous system disorders, cognitive dysfunction, sexual and reproductive challenges, cardiac challenges and hair loss. The intensity of the side effect experienced is dependent on the type of therapy, the medication and treatment dose. For example, heart failures have been associated with trastuzumab and alemtuzumab (Monsuez *et al.*, 2010). Cetuximab and rituximab predisposes patients to hypertension, whiles bevacizumab may additionally promote venous thromboembolism. (Monsuez *et al.*, 2010).

In Ghana, geldanamycin and its analogues, associated with hepatoxicity, are first line drugs used for cancer chemotherapy (Bedin *et al.*, 2004). The cost associated with chemotherapy with these drugs is expensive and beyond the means of the ordinary Ghanaian. Fortunately, the Ghana National Health Insurance scheme covers treatment of some of the cancers. However, the availability of cancer chemotherapeutic drugs is another challenge, as they are not readily available at most pharmacies and even at the points of prescription.

These challenges have pushed for the search for locally and readily available medicinal plants with anticancer activity selective against cancer cells only.

2.4. MEDICINAL PLANTS

Natural products have been utilised in the treatment of various ailments, with records indicating their extensive use in Africa, China, India and Greece from ancient times (Samuelsson, 1992). In recent years, natural product sources have produced an impressive number of modern drugs used in the treatment of different diseases. The earliest records of drugs extracted from nature are the *Ebers* papyrus, an Egyptain pharmaceutical record dating 1500BC, indicating the extraction and use of over 700 drugs from plant sources, and the Chinese *Materia medica* of 1100BC also ascribing 600 medicinal plants (Samuelsson, 1992; Cragg *et al.*, 1997).

Natural products such as plants have continued to be of importance in health delivery. The initial use of these medicinal sources, as crude drugs, were in the forms of teas, powders, tinctures, poultices and powders. Their pharmacologically active components have been isolated and elucidated (Balick *et al.*, 1997; Balunas and Kinghorn, 2005) providing leads against various diseases including cancer, HIV/AIDS, Alzheimer‘s and malaria. A number of drugs derived from plants have been produced over a period and examples are nitisinone, arteether, galantamine and tiotropium (Balunas and Kinghorn, 2005).

Even though refined drugs are currently available for the management of different ailments including cancers, a substantial percentage of the world's population particularly in the developing world such as rural Africa, continue to utilise medicinal plants in their crude forms. Knowledge of these plants, their use and indications have been handed down to generations mostly verbally with minimal documentation. The preference of herbal remedies over ―refined drugs‖ may be because of the side effects of these drugs and attendant cost.

However, according to Fennell *et al.* (2004), a number of plants utilized in preparing herbal medicines are potentially toxic, mutagenic and cancer-causing (Akindele *et al.*, 2014). It has also been suggested that 35% of acute renal failures diagnosed in Africa are related to the use of traditional herbal remedies (Isnard Bagnis *et al.*, 2004). Despite these drawbacks, the high costs that come with undergoing chemotherapeutic treatment has meant that many patients in developing countries cannot afford conventional treatment. Many therefore rely on the herbal remedies for cancer treatment based on anecdotal evidence notwithstanding the fact that the efficacy, toxicity and safety of many of these plants are yet to be confirmed scientifically. A good understanding of the individual constituents of ―anticancer plants‖ and the synergistic interaction of various constituents would help in formulating and designing anticancer agents to attack the cancerous cells without harming the normal cells of the body (Larkin, 1983; Saxe, 1987; Merina *et al.*, 2012).

2.4.1 Anti Cancer Properties of Medicinal Plants used in Herbal Cancer Treatment Plants have been reportedly used in remedies for cancer related conditions for thousands of years. Most cancer designated drugs utilized in conventional chemotherapy are somehow derived from natural sources (Cragg and Newman, 2005; Manosroi *et al.*, 2012). Plants have continued

be an immense source of conventional drugs for cancer chemotherapy (Kaur *et al.*, 2011). The quest for anti-cancer agents began with discovery and advancement of vinca alkaloids and the elucidation of the cytotoxic podophyllotoxins. This prompted the search and discovery of other chemotypes, such as taxanes and camptothecins, exhibiting a range of cytotoxic activities (Cragg and Newman, 2005; Manosroi *et al.*, 2012).

The vinca alkaloids were extracted from *Catharenthus roseus*, and examples include vinblastine and vincristine, which have been in clinical use for decades and basically used with other drugs in the management of cancers such as leukemia, lymphomas, testicular cancer, breast and lung cancers (Kaur *et al.*, 2011). These alkaloids and their derivatives inhibit mitotic progression and cell proliferation of cancer cells. They act by suppression of microtubule treadmilling and dynamic instability which alter microtubule dynamics during mitosis, leading to metaphase arrest and resulting in apoptosis (Okouneva *et al.*, 2003; Jacobs *et al.*, 2004).

Podophyllotoxin, which is extracted from *Podophyllum peltatum L. (Berberidaceae)* was found to be too toxic in mice (El-Hallouty *et al.*, 2015). Hence, its derivatives were designed and the first clinically approved drug designed was etoposide. Epipodophyllotoxin, an isomer of popdophyllotoxin, has also been found as an active antitumor agent (Gordaliza *et al.*, 2004; Balakrishnan and Gandhi, 2013). Epipodophyllotoxins and their derivatives effect their anticancer properties by binding tubulin, causing DNA strand breaks during the gap two (G_2) phase of cell cycle by reversibly inhibiting DNA topoisomerase II (Gordaliza *et al.*, 2004). The taxanes include paclitaxel extracted and isolated from the stem back of *Taxus* species, specifically *Taxus brevifolia Nutt. (Taxaceae)* (Wall and Wani, 1996). The paclitaxel and its derivatives are significantly active against ovarian cancer and advanced breast cancer acting by binding tubulin without allowing depolymerisation or interfering with tubulin assembly (Horwitz, 2004).

Camptothecin from *Camptotheca acuminata* (Nyssaceae) is an important anticancer agent with potent antiproliferative activity that was initially withdrawn due to its attendant bladder toxicity (Balunas and Kinghorn, 2005; Rahier *et al.*, 2005). It's derivatives Topotecan is, however, used in the treatment of lung and ovarian cancer, whereas Irinotecan treats colorectal cancer (Rahier *et al.*, 2005; Latosińska and Latosińska, 2013). Camptothecin and the derivatives are able to stabilise the ―cleavable complex‖, where tyrosine residue of Topoisomerase I binds covalently to DNA via its phosphodiester backbone generating a single-strand break. The ternary complex formed between camptothecin, toppisomerae I and the cleaved DNA causes inhibition of DNA and RNA syntheses and prolonged exposure to the drug results in irreversible DNA damage initiating apoptosis (Holm *et al.*, 1989; Lavergne *et al.*, 1998).

2.4.2 Phytochemical Constituents of Medicinal Plants used for Cancer Treatment

Medicinal plants are generally significant in maintaining good health of individuals and communities. It is also an immense source of medicines in pharmacognosy. The medicinal value of these plants lies in a wide range of phytochemical compounds. Phytochemicals, universally present in plants might be classified as either primary or secondary metabolites (Croteau *et al.*, 2000). Primary metabolites such as organic acids, phytosterols, acyl lipids, amino acids and nucleotides are found substantially in most plants performing essential anabolic and catabolic roles required for plant cell maintenance and proliferation (Croteau *et al.*, 2000). Secondary metabolites on the other hand are present in specialised cells not necessarily required for basic metabolism but involved in plant fitness and survival in its environment (Lattanzio *et al.*, 2008; Cragg and Newman, 2013). These secondary metabolites include alkaloids, terpenoids, polyphenols, glycosides, tannins and saponins.

Plants present an inconceivable collection of secondary metabolites that are differentially distributed cross the plant kingdom (Macías *et al.*, 2007). The efficacy or therapeutic impact

of plant material are mostly due to the specific secondary metabolite present, its relative quantity and/or the combinations present in the plant or plant part that is utilised (Wink, 1999). Secondary metabolites are also noted for their complexicity of structures and biosynthetic pathways. In light of biosynthetic origins (Figure 2.5), secondary metabolites may be grouped as; terpenoids, alkaloids and phenylpropanoids with allied phenolic compounds (Mazid *et al*., 2011).

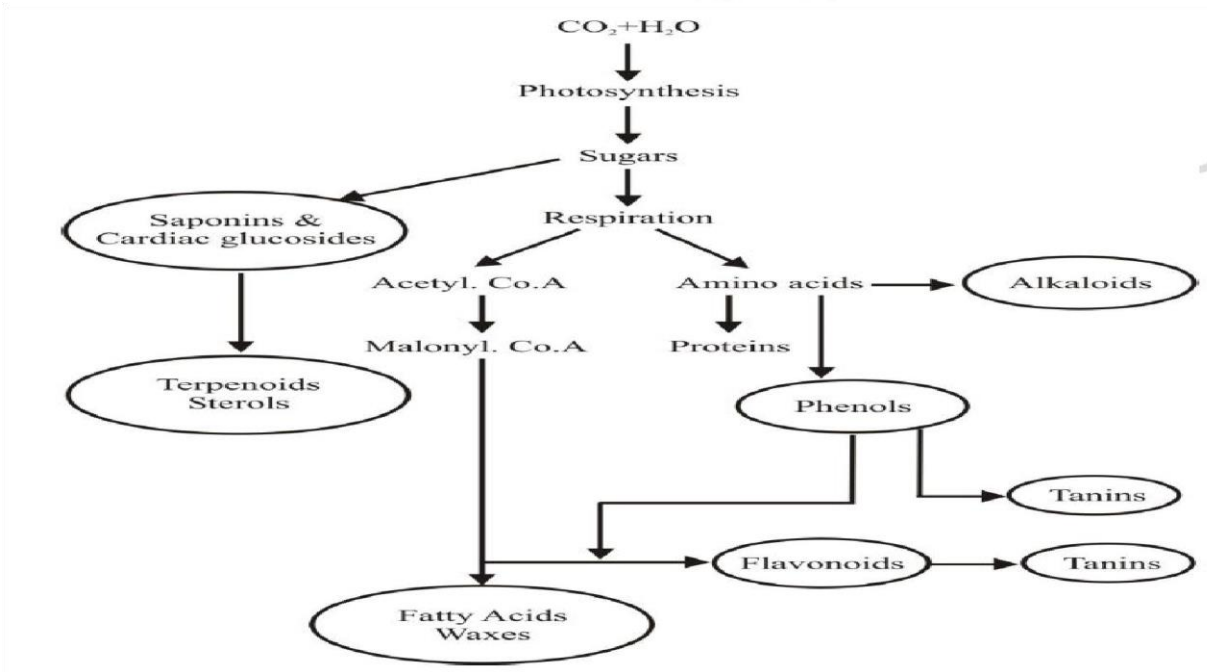


Figure 2.5: Biosynthetic relationship among some primary and secondary metabolite (Source: Mazid *et al*., 2011)

Some secondary metabolites (terpenoids, alkaloids and glycosides) provide protection against various pathogens and herbivory or growth regulatory molecules, they can serve as potential cancer chemotherapeutic agents (Kintzios, 2004).

2.4.2.1. Terpenoids

Terpenoids are metabolites basically made up of isoprene (2-methylbuta-1,3-diene) units . They are typically classified by the number of isoprene units. For example; monoterpenes (geraniol and carvone), diterpenes (trans-retinoic acid and retinol), triterpenes (ursolic acid and

betulinic acid), and tetraterpenes (α and β-carotene) (Rabi and Bishayee, 2009; Ashour *et al.*, 2010). The various differences across differing terpenoid structures complement and influence their functions (Thoppil and Bishayee, 2011). Various terpenoids have been found as the bioactive agents in host of medicinal plants with cancer-preventive and chemotherapeutic properties (Thoppil and Bishayee, 2011).

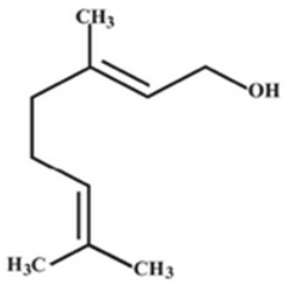
Several studies have cited monoterpenes in cancer therapy and management of various carcinomas such as prostrate, colon, mammary, lung and skin. For example Geraniol (Figure 2.6) , an acyclic dietary monoterpene has been proven to repress the development of HepG2 human hepatic carcinoma cells by limiting 3-hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, which is the significant rate-consuming enzyme in cholesterol biosynthesis in mammals (Polo and De Bravo, 2006; Thoppil and Bishayee, 2011)

Parthenolide (Figure 2.6) extracted from a medicinal plant, *Tanacetum parthenium,* popular for its anti-inflamatory properties, also has anticancer properties (Cragg and Newman, 2013). It sensitises cancer drug resistant cells to drugs such as tamoxifen and paclitaxel (Nakshatri *et al.*, 2004; Chadwick *et al.*, 2013).

A triterpene that exhibits anticancer activity is betulinic acid (Figure 2.6). Betulinic acid found in some plants like *Menyanthes trifoliate,* exhibits selective anticancer activities against human melanoma and neuroblastom cancer cell lines (Schmidt *et al.*, 1997; Cragg and Newman, 2013). The mechanism of action is by altering the integrity of membrane via loss of transmembrane potential (Fulda *et al.*, 1998).

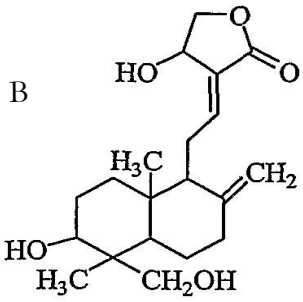
Tetraterpenes represent a class of terpenoids variously studied several times for their anticancer activities. Carotenoids, such as lycopene, β-carotene and α-carotene, and astaxanthin amongs others, are examples of tetraterpenes that are suggested to have potential anti-carcinogenic activity (Tanaka *et al.*, 2012). The carotenoids exert their anticancer activities through

interfering with pathways that lead to the activation of cell growth or cell death (Yasui *et al.*, 2011).



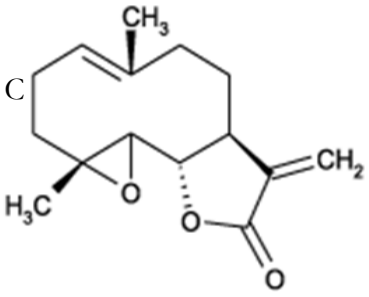
Geraniol

B



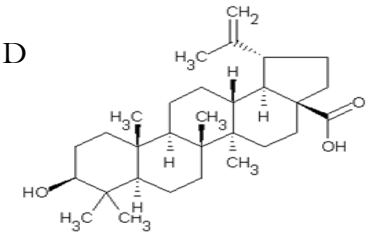
Andrographolide

C



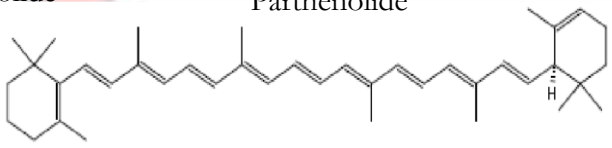
Parthenolide

D



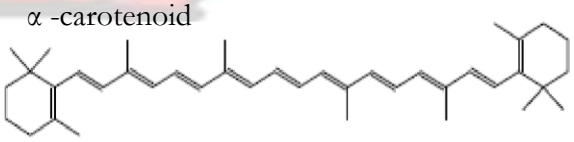
Butelinic Acid

E



α -carotenoid

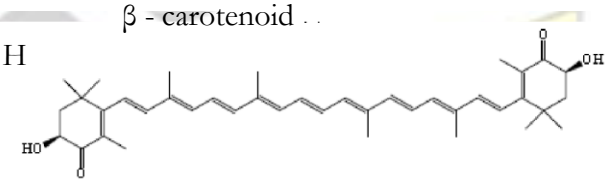
F



β - carotenoid

A

H



G

Lycopene

Astaxanthin

Figure 2.6: Structures of some Terpenoids

(Source: Schmidt *et al.*, 1997; Wang, 2007; Safaepour *et al.*, 2009; Tanaka *et al.*, 2012)

2.4.2.2. Alkaloids

Alkaloids have in recent times become a rich source for drug discoveries (Lu *et al.*, 2012). They are a diverse grouping of secondary metabolites characterized by a ring structure with a nitrogen atom, mostly in the heterocyclic ring structure. Alkaloids are mostly found in higher plants belonging to *Ranunculaceae*, *Leguminosae*, *Papaveraceae*, *Menispermaceae*, and *Loganiaceae* sub-families (Lu *et al.*, 2012). Some alkaloids have proven to possess significant medical applications, for example; the analgestic action of morphine, pain relieving activity of ephedrine, and the anticancer property of vinca alkaloids (Benyhe, 1994; Li *et al.*, 2007; Chen *et al.*, 2012; Lu *et al.*, 2012).

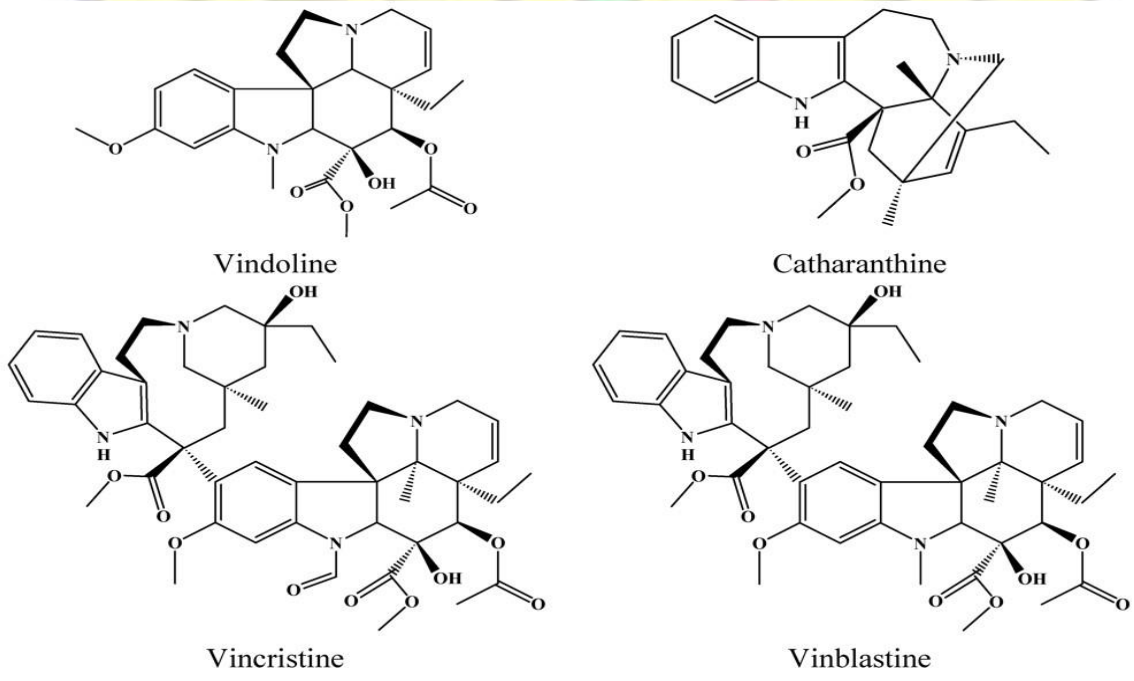


Figure 2.7: Structures of Vinca Alkaloids (Vincristine, Vinblastine, Vindoline and Catharanthine)

(source: (Mu *et al.*, 2012)

Alkaloids are important phytochemical constituents of natural herbs. Some alkaloids have been successfully isolated and effectively developed into chemotherapeutic drugs such as vinca alkaloids, that is, vinblastine, vincristine, vindesine and camptothecin (Chen *et al.*, 2012; Lu *et al.*, 2012). The vinca alkaloids (Figure 2.7) inhibit cell multiplication by binding to microtubules, prompting a mitotic block and the initiation of apoptosis (Mooberry, 2011).

Vinca alkaloids are also reported to induce protein p53 and cyclin-dependent kinase inhibitor 1A (p21) (Jordan and Wilson, 2004). These protein kinases specifically phosphorylate Bcl-2 resulting in its inhibition and failure to form heterodimers with Bax and with an increase in activities of p53 and p21 lead to apoptosis (Wang *et al.*, 1999; Fan *et al.*, 2000).

2.4.2.3. Plant phenols

Phenols are generally defined as compounds possessing aromatic rings with one or more hydroxyl substituents including functional derivatives such as glycosides, esters and methyl. It describes a group of diverse compounds from the shikimate-phenyl- propanoids-flavonoid pathways (Harborne, 1983; Bennett and Wallsgrove, 1994). The products of this pathway are diverse and includes hydroxybenzoic acids, phenylpropanoids, lignin precursors, cinnamic acids, catechols and coumarins derived from the modification of aromatic amino acids. They also includes dihydroxy-phenols and flavonoids polymerised by the action of peroxidases and polyphenoloxidases such as tanins, flavonoids and isoflavone (Harborne, 1989; Bennett and Wallsgrove, 1994)

Phenols may be classified into flavonoids and non-flavonoids, according to the number of aromatic rings and elements that joins them (Stalikas, 2007; Laura *et al.*, 2009). Flavonoid is made up of compounds with a C6-C3-C6 structure like flavanones, flavones, dihydroflavonols, flavonols, flavan-3-ols, isoflavones, anthocyanidins, proanthocyanidins and chalcones

(Khadem and Marles, 2010).
Based on the carbon skeleton, the non-flavonoids may also be classified into simple phenols, benzoic acids, tannins, acetophenones, phenylacetic acids, cinnamic acids, lignans, coumarins and coumarin acid derivatives such as hydroxycinnamic acid (Khadem and Marles, 2010).

2.4.2.3.1. Flavonoids

Flavonoids are aqua soluble phenolics with 15 carbon atoms, yellow in colour and ubiquitous in nature. Collectively, they are known as vitamin P or citrin. They are characterised by flavan nucleus (Figure 2.8) (Heim *et al.*, 2002). Flavonoids and their derivatives with the strongest antioxidant potential include kaempferol, luteolin, epicatechin, quercetin and delflina.

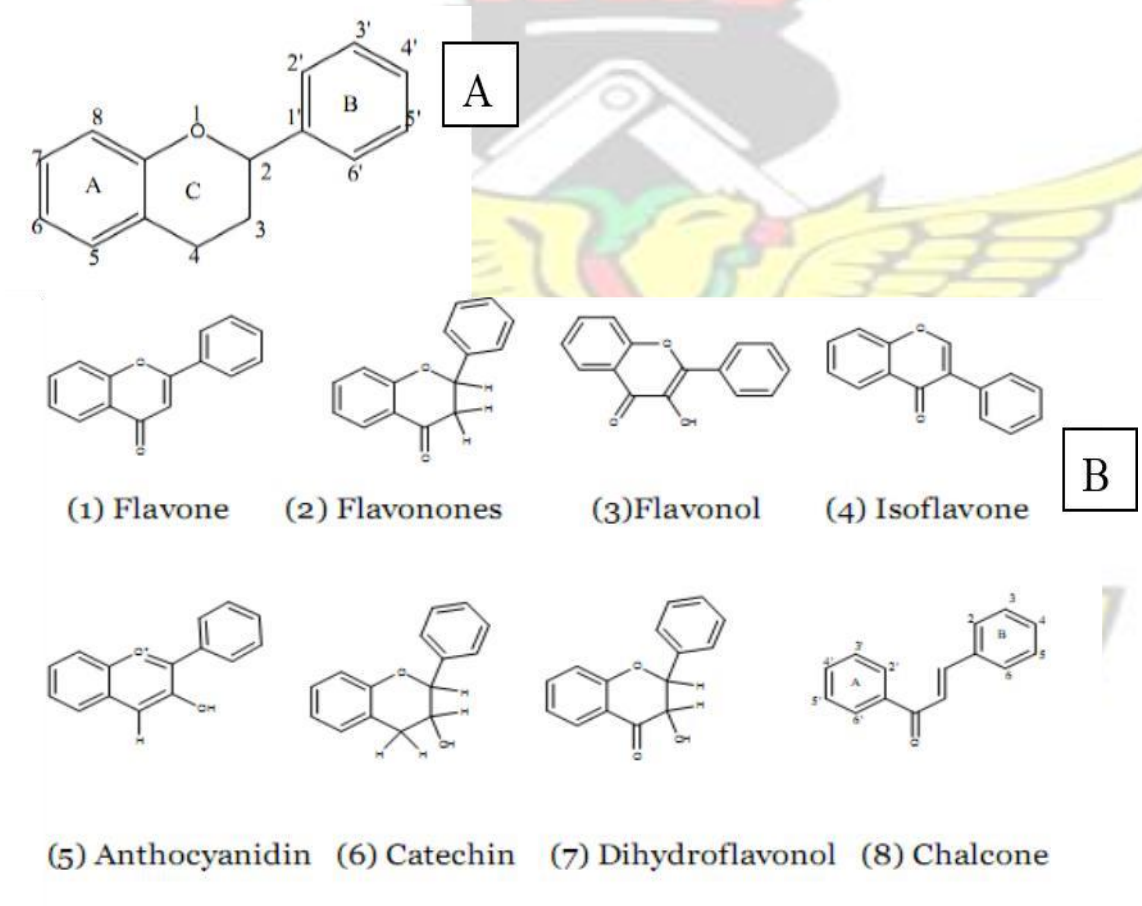


Figure 2.8: Basic structure of flavonoids (A) and chemical structures of different types of flavonoids (B)

(Source: Kumar *et al.* (2011)

Flavones (Figure 2.8) have a planar structure due to a double bond in the central aromatic ring. An example is quercetin, which is abundant in berries and apples. Flavonoid (Figure 2.8) as a subgroup, has been suggested to be a powerful hypolipidemic agent in some studies (Narender *et al.*, 2006). Medicinal plants are reported to exhibit a high antioxidant potential as a result of their hydroxyl groups, which also enables them protect more proficiently against free radical related illnesses like arteriosclerosis (Vaya *et al.*, 2003).

This finding supports the idea of flavonoids involvement in oxygen-derived free radical scavenging (Nijveldt *et al.*, 2001). The inhibitory effect of black tea polyphenols on aromatase activities has also been suggested. The polyphenols present in black tea inhibited the proliferation of MCF-7 cells in *in vivo* models (Way *et al.*, 2004).

2.4.2.3.2. Tannins

These are astringent and bitter tasting polyphenolic compounds of plant origin that bind and precipitate proteins and other organic compounds like alkaloids (Roberts, 1990). There are two different classes namely, hydrolysable tannins (polyesters of gallic acid) and condensed tannins (flavone) (McSweeney *et al.*, 2001).

The hydrolysable tannins (Figure 2.9) are esters of gallic and ellagic acids that comprise polyols, for example sugars and phenolics. These compoundds are vulnerable to enzyme and non-enzymatic hydrolysis than proanthocyanidins, and more soluble in water. The classification of hydrolysable tannins is based on the products of hydrolysis as gallotannins yield gallic acid, whereas ellagitannins produce ellagic acid and glucose (Roberts, 1990). Proanthocyanidins, commonly alluded to as condensed tannins (Figure 2.9), are polymers of flavan-3-ols linked through an interflavan carbon bond that is not vulnerable to hydrolysis (Reed, 1995).

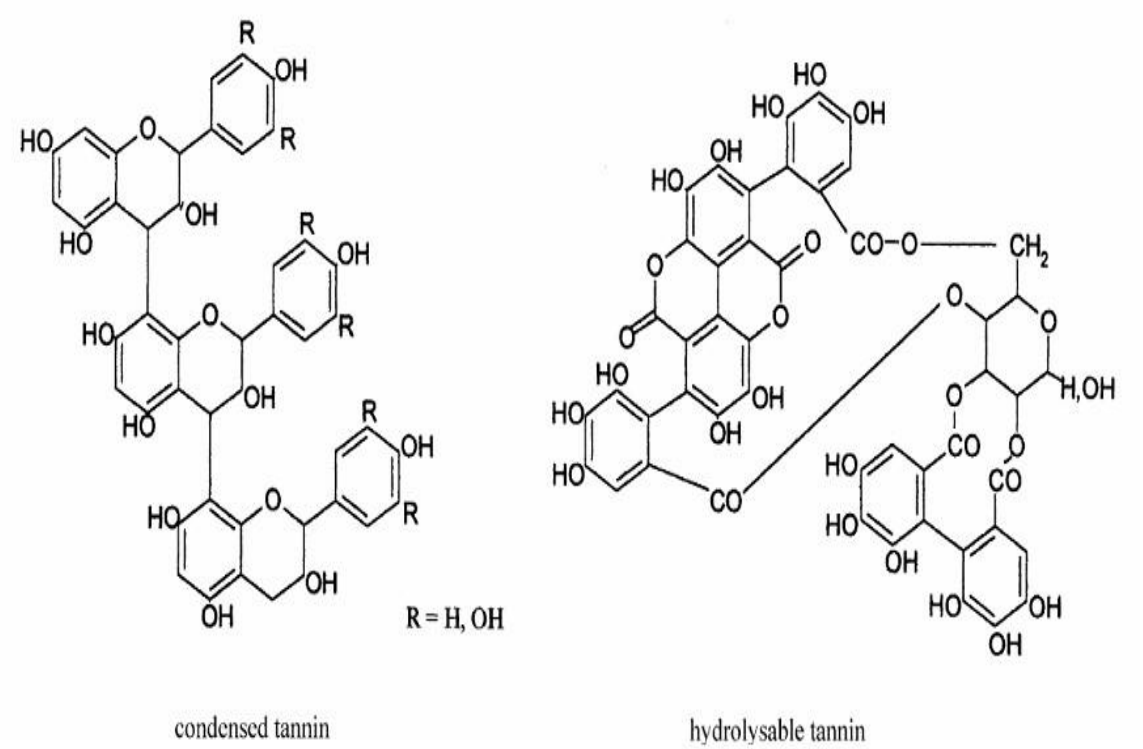


Figure 2.9: Chemical structure of condensed and hydrolysable tannins

(Source: McSweeney *et al*., 2001).

From the respective proanthocyanidins, procyanidin, and prodelphinidin, the corresponding most common anthocyanidins produced are cyanidin and delphinidin (Reed, 1995).

The anti-nutritive effects of tannins are related to their capacity to bind to dietary proteins, polymers (cellulose, hemicellulose and pectin) and minerals thus hindering their digestion (McSweeney *et al.*, 2001). The tannins can likewise impede digestive processes by complexing with enzymes and endogenous proteins (McSweeney *et al.*, 2001).

2.5. OIL PALM

Oil palms belong to the genus *Elaeis*, and consist of two species; *E. guineensis* native to West and Central Africa, and *E. oleifera* native to South and Central America. The Elaeis spp belong to the family: Arecaceae, Order: Arecales, Class: Monocotyledonae, subphylum: Angiospermae and phylum: Spermatophyta.

2.5.1. Morphology of Oil Palm

The oil palm is a perennial and relatively long-living plant. It can grow to heights of 24 metres in it natural environment but as a fruit crop, it hardly reaches more than 14 metres. It is a vast pinnated plume with singular column stem with short internodes. The oil palm, like most palm species, have a solitary vegetative shoot apical meristem localised at the focal point of the leaf crown. This meristem is constantly actively producing a new leaf primordium every two weeks (Hélène Adam *et al.*, 2005).

The palm is ordinarily monoecious with male or female inflorescence‘s, however sometimes hermaphrodite inflorescences occur. Inflorescence develops all-year round in an acropetal fashion (Hélène Adam *et al.*, 2005). The male and female inflorescence are produced in alternating cycles at variable length of time contingent upon age, and ecological conditions. Male infloresence are particularly favoured during water stressed conditions (Corley and Tinker, 2008; Hélène Adam *et al.*, 2005).

The oil palm is cross-pollinated by both wind and insects (Corley and Tinker, 2003a). Wind was initially thought to be the main pollinator but Syed (1979), and other reports suggested that insects were the main pollinators. Numerous species of insects are found involved in oil palm pollination, however the main pollinators are *Derelomininae*, of which *Elaeidobius* predominates (Corley and Tinker, 2003a). These weevils visit the flowers to lay their eggs and feed on the soft parts of these flowers (Corley and Tinker, 2003a).

Harvesting of fresh fruit bunches from an oil palm tree averagely commences between 24 to 30 months in the wake of planting and every palm can produce 8-15 fresh fruit bunches per year depending on the planted material and age of the palm (Razali *et al.*, 2012).

Figure 2.10: Plantation of oil palm trees (A) *Elaeis guineensis* (B) *Elaeis oleifera*

The fruit is a sessile drupe borne on a bunch. It has varying shapes from nearly spherical to ovoid or elongated and bulging somewhat at the top. In length it varies from about 2 cm to more than 5 cm and weight of about 3 g to over 30 g (Corley and Tinker, 2003a).

2.5.2. Cultivars and Species of Oil Palm

Cultivar and/or varieties of oil palm are differentiated by the colour and characteristics of their fruits. The most common varieties based on fruit character like size of shell and mesocarp are Dura, Tenera and Pisifera. Dura have 2-8 mm thick shell and 35-55% by fruit weight being mesocarp. Tenera have 0.5-3 mm thick shell and 60-95% of fruit weight being mesocarp.

Pisifera palms most often have no shell and about 95% mesocarp. Based on fruit character, palms may also be grouped as nigrescens, virescens or albescens depending on the colour of the immature fruit.

The major cultivar/species of Oil palm; *E. guineensis* of African origin and *E. oleifera* of American origin, are morphologically similar in many respects but are different in few features. *E. oleifera* is relatively shorter, with an annual height increment of only 5-10 cm, less than onefifth of *E. guineensis* (Corley and Tinker, 2003a). The leaves of *E. oleifera* are generally larger, 1.9 m long to 12 cm wide, all lying in one plane with no basal swelling and the spines on the petiole are thick and short. The varieties based on fruit colour exist but no evidence of Dura,

Tenera and Pisifera varieties have been reported in *E. oleifera* populations (Corley and Tinker, 2003b)

The most important difference between these two species is the fruit and bunch formation. *E. oleifera* has been reported to have a higher percentage of parthenocarpic fruits due to its pollination difficulties. However, *E. oleifera* remains an important gene source for oil palm development due to some distinctive characteristics. *E. oleifera* thrives in ecosystems that are normally marginal for *E. guineensis* and it has slow height growth (5-10 cm per year) permitting possible commercial harvesting for relatively longer periods. Its tolerance of pest and diseases common to *E. guineensis* coupled with its relative higher content of unsaturated fatty acids, carotenes, vitamin E and its ability to produce hybrids with *E. guineensis* are of interest (Mendez *et al*., 2012; Cadena *et al*., 2013, Rivera *et al*., 2013) .

2.5.3. Medicinal Applications of *E.guineensis*

E. guineensis has been ascribed varied ethnopharmacological relevance. The distinctive parts of the plants have been utilized in various folkloric medicines. For example, the fresh sap

extracted from the crown of the tree or stem is administered as a potent purgative and the partially-fermented palm wine is believed to help first-time mothers enhance lactation

(Sasidharan *et al.*, 2011). The husk of fruit is utilized in preparing remedies for skin infections. The roots are the main ingredients in the preparations of decoctions for treatment of gonorrhoea, menorrhagia and bronchitis. (Gill, 1992; Sasidharan *et al.*, 2011; Yin *et al.*, 2013) Crude red palm oil has been traditionally revered for it medicinal applications. The oil is applied as an antidote for ingested poison. It is also generous applied on wounds and is a component of herbal concoctions for wound and skin disease treatment. Palm kernel oil is applied to convulsive children to mitigate their body temperature (Gill, 1992; Syahmi *et al.*, 2010; Sasidharan *et al.*, 2011; Yin *et al.*, 2013; Imoisi *et al.*, 2015).

Carotenoids from crude red palm oil are considered as vital antioxidants protecting cells and tissues from reactive oxygen species and other free radicals (Supta, 2009; Mukherjee and Mitra, 2009). The crude red palm oil is also proven to contain substantial quantities of vitamin E existing in four isomers of tocopherols and tocotrienols that possess antioxidant and anticancer activities.

Oil palm leafs are ignored in the palm oil industry but domestically utilised as ruminant feed. Due to its medicinal properties and no report of toxicity, it is also used in traditional herbal preparations (Anyanji *et al.*, 2013b). The leaf is noted for its wound healing properties in Africa particularly Nigeria, where it‘s juice is squeezed onto wounds. Oil palm leaf is not used as a main ingredient in food for humans due to its high insoluble fibre content but its extract has had numerous beneficial health applications.

Wound healing and antimicrobial properties of oil palm leaf have been reported. Oil palm leaf extract of *E. guineensis* is indicated to possess remarkable wound closure , re-epithelization and accelerated tissue generation at injury site (Sasidharan *et al.*, 2011). A significant repressive growth effect of the *E. guineensis* leaf extract on *C. albicans,* broad spectrum of

bacteria and fungus have been reported. It was observed that *E. guineensis* extract effectively inhibited *C. albicans* growth (Vijayarathna *et al.*, 2012).

The immunoregulatory property was exhibited in aged rats. Oil palm leaf extracts also exhibited biphasic effects enhancing inflammation at minute dose with remarkable late phase oedema inhibitive properties and also effectively suppressing inflammation at larger doses (Anyanji *et al.*, 2013b). The oil palm leaf extracts demonstrate *in-vivo* immunoregulatory properties that have potential applications in cell mediated immune responses. At doses of 300 mg/kg, paw oedema was suppressed during intial 6 hours of observation and at 150 mg/kg, a decrease in lymphocyte count was observed (Prasain *et al.*, 2010; Anyanji *et al.*, 2013b; Mohamed, 2014).

Therapeutic and preventive property, as well as organ and tissue regeneration protection has been shown in a number of studies. An example is the study to evaluate the potential antidiabetic effects of oil palm leaves ethanol extract on normal and streptozotocin-induced hyperglycaemic rats. Oil palm leaves were administered orally at 50, 100 and 200 mg/kg body weight per day) to Sprague Dawley rats and monitored for its glycaemic, lipidemic and antioxidant modulating effects. Treatment with oil palm ethanol leaf extracts produced a reduction in blood glucose in a dose-dependent fashion, as well as oxidation in the streptozotocin -induced rats restoring antioxidants enzymes levels. An optimum dose of 100 mg/kg was found to reduce kidney and liver damage to the level of normal rats (Tan *et al.*, 2011). This study indicated the ability of oil palm leaf extracts to modulate physiological responses and show organ protective effects against tissue damage.

When oil palm leaf extracts were administered to breast tumour-bearing rats, it exhibited preventive and therapeutic tumour suppressive effects (Anyanji *et al.*, 2013b). Oxidative status, hormonal levels, tumour size and tumour incidence rate monitoring indicated that oil palm leaf extracts mitigated tumour incidence and tumour size in a dose and time dependent manner. The

phytoestrogenic oil palm leaf extracts enhanced apoptosis, serum estradiol levels and antioxidative status of the tumour-bearing rats (Anyanji *et al.*, 2013b).

2.6. MEASUREMENT OF ANTIOXIDANT ACTIVITY AND CYTOTOXICITY

2.6.1 Antioxidants

All living aerobic organisms use oxygen for the production of energy. The use of oxygen is, however, associated with the risk of producing free and bound reactive radicals. These free and bound reactive radicals are commonly referred to as reactive oxygen species (ROS) (Shivprasad *et al.*, 2005). These radicals such as hydroxyl, hydrogen peroxide, singlet oxygen and super oxide anion, tend to react rapidly with` nucleic acids, proteins, free amino acids and lipids predisposing the cell to lipid peroxidation, protein and DNA damage. Reactive oxygen species have been associated with diseased conditions such as neurodegenerative disorders, diabetes mellitus and cancer (Ajitha and Rajnarayana, 2001).

Although the human body possesses defensive mechanisms, such as glutathione (GSH) and superoxide dismutase (SOD), and antioxidant nutrients to balance its oxidative and antioxidative process. The continuous exposure to environmental contaminants and diseased states causes overburdening and insufficiency of the anti-oxidative system may lead to a disruption of the vital balance of oxidant-antioxidant required. An imbalance that causes an increase and rapid generation of reactive oxygen species results in oxidative stress.

Oxidative stress has been implicated as a factor in various diseases and injury states such as inflammatory diseases like hepatitis, diseases of central nervous system like epilepsy, diseases of the pulmonary organs like asthma and psoriasis.

Antioxidants are therefore basically compounds that prevent or delay the oxidation of biomolecules by mitigating the initiation or propagation of oxidizing chain reactions (Velioglu *et al.*, 1998).

2.6.1.1 Estimation of antioxidant activity.

Antioxidant activity of plant extracts emanates from the occurrence of its constituting compounds acting as radical scavengers, reducing agents, metal chelators, singlet quenchers and hydrogen donors (Osawa, 1994; Rice-evans *et al.*, 1995; Kähkönen *et al.*, 1999). For most of the plant products, the compounds responsible for the antioxidant effects are phenolic due to the redox properties of polyphenols and the report of positive correlation between phenolic content and antioxidant activity (Velioglu *et al.*, 1998; Ismail *et al.*, 2004; Pantelidis *et al.*, 2007). A preliminary assessment of antioxidant activity begins with total phenol and flavonoid assay. The free radical scavenging activity of specific radicals, such as superoxide or hydrogen peroxide, greatly affecting oxidative stress may be measured by 1,1-diphenyl-2-picrylhydazyl (DPPH) assay. Another measurement is the capability of the antioxidant to reduce Ferric ions, thus test such as Reducing Power Assay is very necessary.

2.6.1.2. Total phenolic content

The measurement of polyphenols in extracts requires a method that is not specific for any type or group of phenol but detects and cumulatively quantifies all phenolic group present in the plant extract, including those found in extractable proteins. A typical less expensive means of estimating total phenols is the spectroscopic method based on the Folin-Ciocalteau reagent (Pelozo *et al.*, 2008). Phenolic compounds are oxidized by Folin-Ciocalteu reagent to form a mixture of phosphotungstic acid, $H_3PW_{12}O_{40}$, and phosphomolybdic acid, $H_3PMo_{12}O_{40}$. They are then reduced to a mixture of blue oxides of tungstate, (W_8O_{23}), and molybdate, (Mo_8O_{23}), A blue coloration is produced with a maximum absorption of 765nm, and is proportional to the total quantity of phenolic compounds originally present (Muchuweti *et al.*, 2007).

2.6.1.3 Total flavonoid content

Aluminuim chloride ($AlCl_3$) form stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of flavones and flavonols. These Acid labile complexes are also formed with the ortho-dihydroxyl groups in the A- or B- ring (Fig. 2.11) of flavonoids. These formed complexes have maximum absorption at 415 nm

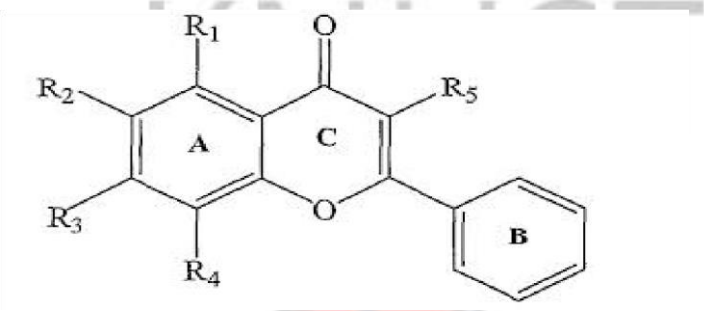


Figure 2.11: Basic structure of flavonoid

(Source: (Bag *et al.*, 2015)

2.6.1.4. 1,1-diphenyl-2-picrylhydazyl (DPPH) Assay

A free radical compound, 1,1-diphenyl-2-picrylhydazyl (DPPH) is characterised by the delocalisation of a spare electron, hence its inability to dimerise like most free radicals. The delocalisation results in a deep purple colour that characterises an absorption band around 520 nm (Molyneux, 2004). An antioxidant compound on reacting with DPPH donates a hydrogen atom to the DPPH molecule reducing its purple colour to yellow due to the picryl group still present (Molyneux, 2004).

2.6.1.5. Reducing Power assay

Transition metals such as copper and iron are important factors in biological activities and biochemical reactions, but its bioavailability must be `generally limited because pathological accumulation of such metals within tissues promotes the generation of reactive oxygen species and elicits toxic effects , which are mainly related to oxidative stress (Galaris and Pantopoulos, 2008). Therefore, an important line of defence against oxidative stress lies in the limitation of

available catalytically active transition metals either by reduction to a less active form or chelation.

In reducing power assay, a direct measurement of the potential of an antioxidant to reduce ferric to their ferrous complexes at low pH, are estimated. Substances which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}) which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700 nm (Jayanthi and Lalitha, 2011).

2.6.2. Cytotoxicity

Cytotoxicity refers to the ability of an agent being toxic to cells. Toxicity may influence antiproliferation, decreased cell viability and initiate cell necrosis or apoptosis.

The measurement and assessment of cytotoxicity as influenced by an agent, whether physical or chemical is a valuable tool in the identification of situations that might pose health risks. It is especially indispensable in the process of developing and assessing of therapeutic and cancer drugs, as chemotherapy relies heavily on the ability of anticancer drug to preferentially target and kill rapidly proliferating cancer cells (Chabner and Longo, 2011).

Cytotoxicity assessments are based on the premise that cells with compromised membrane integrity allow the export of cytoplasmic components such as proteases and Lactate dehydrogenase (LDH). LDH on the outside reduces NAD to NADH eliciting a colour change with specific probes (Decker and Lohmann-Matthes, 1988; Yang *et al.*, 2009b). Some protease biomarkers are active only in cells with membrane integrity intact. These proteases lose activity when exposed outside the cell and dead-cell protease cannot cross cell membrane and hence can be only measured in culture media after cell membrane integrity has been compromised

(Darzynkiewicz, 1997; Lossi *et al.*, 2009). Compromised cell membrane integrity also allows the import of florescent dyes such as trypan blue and propidium iodide within the cell structure to stain intracellular components.

While cytotoxicity and cell viability are assessed in a number of different ways, the most common methods used are vital dyes such as formazan dyes, protease biomarkers or by measuring ATP content (Riss and Moravec, 2004). The formazan dyes are chromogenic products formed by the reduction of tetrazolium salts by dehydrogenases such as lactate dehydrogenase (LDH) and reductases released at cell death (Aula *et al.*, 2015). Common tetrazolium salts are 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT), and 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT).

2.6.2.1. MTT assay

The assay is based on the ability of the cellular mitochondrial reductase enzyme in living cells to reduce water-soluble substrates such as the yellow 3-(4, 5-dimethylthiazol- 2yl)-2, 5diphenyl tetrazolium bromide (MTT) into a purple formazan crystals soluble in acidified isopropanol. MTT reduction can only occur in metabolically active cells; hence, the level of activity is a measure of the viability of the cells. The colour change from yellow to purple is measured at 570 nm using a spectrophotometer.

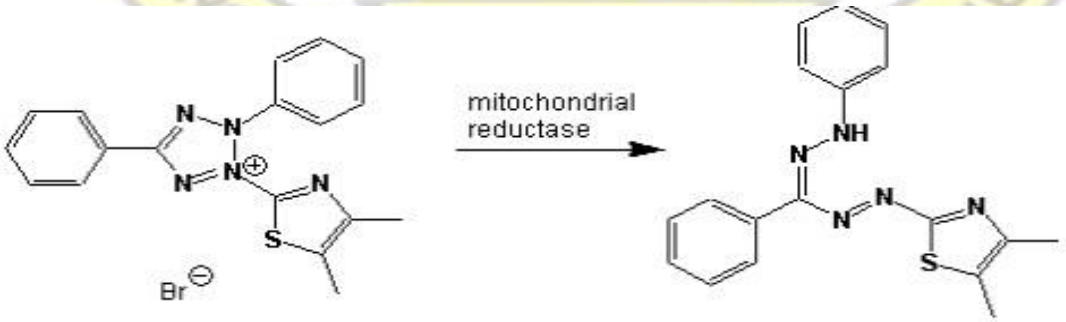


Figure. 2.12: Reduction of 3-(4, 5-dimethylthiazol- 2yl)-2, 5-diphenyl tetrazolium bromide (MTT)

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. REAGENTS AND CANCER CELL LINES

All reagents used for the extraction such as ethanol, petroleum ether, chloroform and ethylacetate were of analytical grade (Sigma-Aldrich, Illnois, USA). Rose Park Memorial Institute (RPMI)-1640 and Dulbecco modified Eagle's culture media, foetal bovine serum (FBS), penicillin streptomycin L-glutamine (PSG), curcumin, 2,2- diphenyl-1-picryl hydrazyl (DPPH) free radical reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye, dimethyl sulphoxide (DMSO), butylated hydroxyl toluene (BHT), glutathione (GSH), glutathione standard and buffer (pH 8.0), O-phthaldialdehyde (OPT), gallic acid, FolinCiocalteau reagent, sodium carbonate solution, phosphate buffered saline (PBS) and trypan blue were all purchased from Sigma-Aldrich (Illnois, USA).

Human leukaemia-immortalized T lymphocyte (Jurkat), human hormone-responsive breast carcinoma (MCF7) and Chang liver cell lines were obtained from the Clinical Pathology Department, Noguchi Memorial Institute for Medical Research (NMIMR) Cell Bank where the *in vitro* work was performed.

3.2. PLANT MATERIALS

Palm trees of *E. guineensis* and *E. oleifera* were randomly selected from research fields of the Oil Palm Research Institute of the Council for Scientific and Industrial Research (CSIR-OPRI), Ghana. Leaflets from each frond were detached, cleaned with cotton wool soaked in distilled water and midrib removed.

Leaflets were then air-dried in a well-ventilated room for 2 weeks at room temperature. The dried leaflets were then milled and powdered using a hammer mill (Christy Lab Mill, England) at the Department of Pharmaceutics, College of Health Sciences, KNUST, Kumasi

3.2.1. Crude extract preparation

3.2.1.1. Aqueous

A 100 g sample of each powdered plant material was extracted with 1000 ml of distilled water by heating on a hot plate at 80 ºC for 1 h. The extracts were centrifuged (Homef LC-30 centrifuge, LH Wagneningen-04065) for 20 minutes at a speed of 1106 ×g at room temperature in 50 mL eppendorf tubes. The supernatants were taken and the pellets were subsequently resuspended in the same volume of distilled water and the extraction process repeated. All the supernatants totalling a volume of 2 L were pooled together and frozen at -20 ºC. Frozen samples were then freeze dried with a vacuum freeze dryer (YK-118, Taiwan) at the Zip Unit, Crop Research Institute (CSIR-CRI), Fumesua.

3.2.1.2. Hydroethanolic

A 100 g sample of each powdered plant material was extracted with 1000 ml of hydroethanol while shaking on an Orbital shaker (Gallenkamp, England) for 24 h at room temperature. The supernatants were filtered by centrifugation (Homef LC-30 centrifuge, LH Wagneningen04065) for 20 minutes at a speed of 1106 ×g in 50 mL centrifuge tubes at room temperature. A volume of 1000 mL of 50% ethanol was subsequently used to resuspend the pellets and the extraction repeated. Supernatants were pooled together and concentrated using a rotary evaporator (Buchi Rotavapor R-205, Switzerland) at 50 ºC. About 50 mL of the concentrate which remained out of a total volume of one liter (1 L), was frozen at -20 ºC. The frozen samples were lyophilized using a vacuum freeze dryer (YK-118, Taiwan).

3.3 PHYTOCHEMICAL QUALITATIVE SCREENING

Screening of the various phytochemicals present in test extracts was carried out according to the methods described by Sofowara and Harborne with modifications as presented below (Sofowara, 1993; Harborne, 1998).

3.3.1. Test for tannins

About 0.5 g of the dried samples was boiled in 20 ml of water and filtered. About 3 ml of 0.1% ferric chloride was added and observed for caramel green or blue-black colouration indicating the presence of tannins.

3.3.2. Test for saponin

Two grams (2 g) of the sample was boiled in 20 ml of distilled water and filtered. About 5 ml of distilled water was added to10 ml of the filtrate and shaken vigorously for a stable steady foam. The foaming was blended with 3 drops of olive oil and shaken vigorously, then watched for the development of emulsion, which is indicative of the presence of saponin.

3.3.3. Test for flavonoids

Five millilitres (5 ml) of dilute ammonia solution was added to portions of the aqueous filtrate of each plant extract and three drops of concentrated H_2S0_4 added. The development of a yellow colouration, which disappears on standing, in each extract is indicative of the presence of flavonoids.

3.3.4. Test for alkaloids

About 10 mg of crude plant extracts were dissolved in 2 ml of acid alcohol. Afterwards, the solution was boiled for 3 min and centrifuged to obtain the supernatant. About 1 ml of dilute

ammonia was added to the supernatant. Thereafter, 2 ml of chloroform was added and shaken to extract alkaloidal base. The chloroform fraction was removed with 2 ml of acetic acid. The development of a reddish brown precipitate upon the addition of four drops of Draggendorf's reagent is indicative of the presence of alkaloids.

3.3.5. Test for terpenoid and sterol

About 0.5 of test extract was dissolved in 5 ml of chloroform. The chloroform extract was divided into two namely, A and B. To A, 3 ml of concentrated sulfuric acid was carefully let down the side of the tube to form a lower layer. The occurrence of a reddish brown coloration at interface of the two layers was indicative of the presence of triterpenoid nucleus.

To B, 3 ml each of acetic anhydride and concentrated sulfuric acid was carefully let down the side of the tube to form a lower layer. The occurrence of a bluish coloration at interface of the two layers was indicative of the presence of steroidal ring.

3.4 *IN VITRO* ASSESSMENT OF THE ANTIOXIDANT ACTIVITIES OF CRUDE EXTRACTS OF *ELAEIS GUINEENSIS AND ELAEIS OLEIFERA*

3.4.1. Total antioxidant activity (2, 2- diphenyl-1-picryl hydrazyl-dpph) assay procedure

Stock solutions of the aqueous and ethanol extracts were prepared by dissolving 10 mg of each of the freeze-dried samples in 1 ml of distilled water and 50% ethanol solvents respectively. Stock solutions of 10 mM of the standard butylated hydroxyl toluene (BHT) and 0.5 mM of DPPH were also prepared by dissolving 2.2 mg of BHT and 3 mg of DPPH in 1ml and 15 ml absolute methanol, respectively. The solutions were then vortexed until complete dissolution was achieved. The DPPH solution was immediately kept in the dark as it is light sensitive. The effects of DPPH antioxidant activity of the extracts was determined according to the procedures described by (Brand-Williams *et al.*, 1995) with slight modifications on a 96 well plate, the

extracts were serially diluted in distilled water (for aqueous samples) and 50% ethanol (for ethanolic extracts) to obtain a concentration range of 0.156–10 mg/ml. The reaction mixture was made up of 100 ml of 0.5 mM DPPH, and 100 ml of each concentration of the test sample. The concentration range of BHT used was 0.156–10 mM. The solvents, methanol, distilled water and 50% ethanol were used as blanks. Triplicate experiments were performed. The plates were covered with aluminum foil, shaken gently and kept in the dark for 20 min after which the absorbance was read on a Tecan-PC infinite M200 Pro Plate reader (Austria), at the absorbance wavelength of 517 nm. The percentage scavenging activity was determined as follows:

$$\%\ scavenging\ activity = \frac{[Absorbance\ of\ blank(OD) - Absorbance\ of\ Extract(OD1)]}{Absorbance\ of\ blank(OD)}\ x\ 100$$

The mean percentage antioxidant activity for the triplicate experiment was plotted for the standard and samples. The effective concentration at 50% (EC_{50}) values, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, were deduced with graphpad prism 5.0 software.

3.4.2. Ferric reducing power assay

Procedure

Stock solutions of the aqueous and hydroethanolic extracts were prepared by dissolving 10 mg of each of the freeze-dried samples in 1 ml of distilled water and 50% ethanol solvents, respectively. Two-fold serial dilutions were made of sample stock solutions to obtain seven (7) concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 mg/ml) of the extracts. Blanks of absolute ethanol, 50% ethanol and distilled water were prepared.

Stock solutions of reference standard, ascorbic acid, was prepared by dissolving 10 mg in 1 ml of distilled water. Two-fold serial dilutions of ascorbic acid stock solution were made to obtain ten different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.0390625, 0.01953 mg/ml) of standard solution.

A 200 µL volume of each sample concentration and ascorbic acid dilutions were aliquoted into respective test tubes with 200 ul aliquots of potassium ferrocyanide. These mixtures were incubated at 50^0C for 20 min. Aliquots of 200ul Trichloroacetic acid (10% w/v) were added and centrifuged at 3000 rpm for 10 min. The upper layer was collected and 200 ul dispensed into designated wells in triplicate on a 96-well plate. Subsequently, 200 ul of distilled water and 40 ul of freshly prepared ferric chloride (0.1% w/v) solution were added. The mixture was incubated for 30 min. The absorbance was read at 700 nm using microplate spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria).

A graph of absorbance against concentration was plotted for the ascorbic acid standard. The concentration of reductones in each sample was determined using the ascorbic acid standard plot and the ascorbic acid equivalence for each extract also calculated.

3.4.3. Total Phenol Content (TPC)

Procedure

The method described by Ghasemi *et al* (2009) was adopted for the determination of the total phenolic content of the extracts, with slight modification. Gallic acid stock solution was prepared by dissolving 5 mg of gallic acid in 1 mL absolute ethanol. Stock solutions of aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera*, were prepared by weighing and dissolving 10 mg of extract in 1ml of distilled water and 50% ethanol

respectively.

A two-fold serial dilution was carried out on the gallic acid standard to obtain six different concentrations: 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mM. Two-fold serial dilutions of sample stock solutions were done to obtain three concentrations (10, 5, and 2.5 mg/ml) of sample extracts. Blanks of absolute ethanol, 50% ethanol and distilled water were prepared.

A 10 µl volume of each sample and gallic acid dilutions were put into designated wells in triplicates on a 24-well plate. Aliquots of 790 µl of distilled water and 50 µl of Folin-Ciocalteau reagent were then added to each well and incubated in darkness at room temperature for 8 min. Aliquots of 150 µl of sodium carbonate solution was added to each well, and incubated in the dark at room temperature for 2 h.

After incubation, absorbance was read at 750 nm using microplate spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria). A graph of absorbance against concentration was plotted for the gallic acid standard. The concentration of phenolics in each sample was determined using the gallic acid standard plot and the gallic acid equivalence for each extract also calculated.

3.4.4. Total Flavonoid Content (TFC)

Procedure

The method described by Ghasemi et (2009) was adopted for the determination of the total flavonoid content of the extracts with slight modification. Quercetin stock solution was prepared by dissolving 1 mg of quercetin in 1 ml of methanol. A ten-fold followed by nine twofold serial dilution by methanol on quercetin stock solution to obtain 10 different concentrations (0.01, 0.005, 0.0025, 12.5 x 10^{-4}, 6.25 x10^{-4}, 3.125 x10^{-4}, 1.563 x10^{-4}, 0.781 x10^{4}, 0.391 x10^{-4}, 0.195 x 10^{-4} mg/mL).

Stock solutions of the aqueous and hydroethanol extracts of *E. guineensis* and *E. oleifera*, were prepared by dissolving 10 mg of extracts in 1 ml of distilled water and 50% ethanol, respectively.

Two-fold serial dilutions of stock solutions were done to obtain three concentrations (10, 5, and 2.5 mg/mL) of extracts. Blanks of absolute ethanol, 50% ethanol and distilled water were also prepared.

A 100 µl volume of each sample and quercetin as standard dilutions were put into designated wells in triplicates on a 96-well plate. Aliquots of 100 µl of 2% aluminium chloride were then added to each well and incubated in darkness at room temperature for 20 min.

After incubation, the absorbance was read at 415 nm using microplate spectrophotometer (Tecan Infinite M200 Pro plate reader, Austria). A graph of absorbance against concentration was plotted for quercetin as standard. The concentration of flavonoids in each sample was determined using the quercetin standard plot and the quercetin equivalence for each extract was also calculated.

3.5 *IN-VITRO* ASSESSMENT OF CYTOTOXICITY OF *E. GUINEENSIS* AND *E. OLEIFERA* CRUDE EXTRACTS.

3.5.1. Cell culture

Cells were cultured as described by Ham *et al*. (2012), with slight modifications. Jurkat and Chang liver cells were cultured in RPMI 1640 medium while MCF-7 cells were cultured in DMEM medium. All cultured media were supplemented with 1% PSG and 10% FBS. The cells were maintained in an incubator at 5% CO_2 concentration and 37 °C and passed on reaching about 80% confluence.

3.5.2. Cell Viability Assay

Cytotoxicity of hydroethanolic and aqueous extracts of *E. guineensis* and *E. oleifera* on the cancer and normal cell lines were determined by tetrazolium-based Colorimetric Assay (MTT) (Ayisi *et al*. 2011).

Procedure

Stock solutions of aqueous and 50% ethanolic extracts of *E. guineensis* and *E. oleifera* were prepared by dissolving 50 mg of each of the freeze-dried samples in 1 ml of distilled water and 1 ml of 50% ethanol, respectively. The solutions were vortexed and filter sterilized into cryotubes in a biosafety cabinet through 0.22 µm pore filters before storage at -20 °C until use. Dilutions of the 50 mg/ml stock of each plant extract were made in distilled water for the aqueous extracts and in 50% ethanol for the ethanolic extracts, to obtain a final concentration of 1 mg/ml. Subsequently, two-fold serial dilutions were prepared from each extract to obtain four concentrations (0.5, 0.25, 0.125 and 0.0625 mg/mL).

Jurkat cells were then transferred from a culture flask into 50 ml centrifuge tubes, spun down and the resulting pellets resuspended. A haemocytometer was used to count the viable cells and the cell suspension adjusted with medium containing 10 % FBS in order to obtain final density of $1x10^5$ cells/mL. A volume of 100 µL (1×10^5 cells/mL) of cells was seeded into each well of 96-well plate. The cells in the plates were immediately treated with 10 µL of each extract dilutions in triplicates and incubated in an incubator with 5% CO_2 concentration at 37 °C for 72 h (3 days). The final concentrations were in the range of 62.5-1000 µg/mL.

Monolayer cells (MCF7 and Chang liver cells) were detached with trypsin and single cell suspensions were made using culture media. A haemocytometer was used to count the viable cells and the cell suspension was diluted with a media containing 10 % FBS in order to obtain final density of $1x10^5$ cells/mL. The 96-well plates at plating density of 10,000 cells/well were seeded with 100 µL per well of cell suspension and incubated for cell attachment at 37°C, with 5% CO_2 in a humidified incubator for 24 h. After the attachment, 10 µL of each extract dilution (concentration range of 625-10,000 µg/mL) were added to the cells in triplicate. The final concentrations were in the range of 62.5-1000 µg/ml (at 1% ethanol). This was also followed by 72 h incubation.

A 20 µl volume of 2.5 mg/ml MTT solution was then added to each well on the 96-well plate and incubated further in a humidified 5% CO_2 incubator at 37 °C for 4 h. Curcumin in 1% DMSO (concentration range of 10-100 µM) was used as positive control in all assays. Acidified isopropanol (150 µl) was added to each well to stop the reaction and the plates were incubated in the dark at room temperature overnight, before reading the absorbance at 570 nm using the microplate spectrophotometer. A colour control plate was also setup for each extract including the positive control, curcumin. Contents of wells in this plate were made up of 10 µl of sample in 100 µl of media. The plate was incubated as describe above and absorbance read at 570 nm using the microplate spectrophotometer. The percentage cell viability was determined as follows:

$$\% \text{ Cell Viability} = \frac{Ao - A1}{Ao}$$

Where A_o = Mean absorbance of wells with untreated cells

A1 = Mean absorbance of wells treated with extracts

The mean percentage cell viability obtained from triplicate determinations at each concentration was plotted as a dose response curve using Microsoft Excel.

The selectivity index (SI) was also determined by the ratio between IC_{50} of each extract (and standard drug) on normal Chang liver cell line and IC_{50} of the extract (and standard drug) on cancer cell lines. SI value indicates selectivity of the sample to the cell lines tested. Samples with an SI greater than 2 were considered to have a high selectivity towards cancerous cells (Badisa *et al.*, 2009).

Selectivity index (SI) = $\underline{IC_{50} \text{ of the extract/standard drug on Chang liver cell}}$

IC_{50} of the extract/standard drug on cancer cell lines

3.6 STATISTICAL ANALYSIS

Microsoft Excel Version 2.4.0.0 was used for the calculation and plotting of mean and standard deviation (S.D) estimates in the graph. Mean EC_{50} and IC_{50} values were compared by one way ANOVA using GraphPad Version 6.1 and values with $p < 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0. RESULTS

4.1. PRELIMINARY PHYTOCONSTITUENTS OF TEST EXTRACTS

The phytochemicals namely terpenoids, alkaloids, flavonoids, tannins and saponins, were found in both aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera*. Comparing the aqueous and hydroethanolic extracts of both *E. guineensis* and *E. oleifera*, the presence of the phytochemicals did not differ from each other qualitatively (Table 4.1).

Table 4.1: Phytoconstituents in different extracts of *E. guineensis* and *E. oleifera*

	Aqueous extract		**Hydroethanolic extract**	
	Elaeis guineensis	***Elaeis oleifera***	***Elaeis guineensis***	***Elaeis oleifera***
Alkaloids (Quinidine)	+	+	+	+
Terpenoid (Ursolic acid)	+	+	+	+
Saponins	++	+++	++	++
Flavonoids (Quercetin)	++	+++	+++	+++
Tannins (Gallic acid)	++	+++	+++	+++

+++ high concentration; ++ moderate concentration; + low concentration; - Absent. Compounds in parenthesis were standards used for the tests.

4.2 *IN-VITRO* ASSESSMENT OF THE BIOLOGICAL ACTIVITIES OF THE DIFFERENT CRUDE EXTRACTS OF *ELAEIS GUINEENSIS* AND *ELAEIS OLEIFERA.*

4.2.1 DPPH scavenging activity

The DPPH radical scavenging activities of crude aqueous and hydroethanolic extracts of both

E. guineensis and *E. oleifera* as well as the reference antioxidant, Butylated hydroxytoluene (BHT) are presented in Figure 4.1 below.

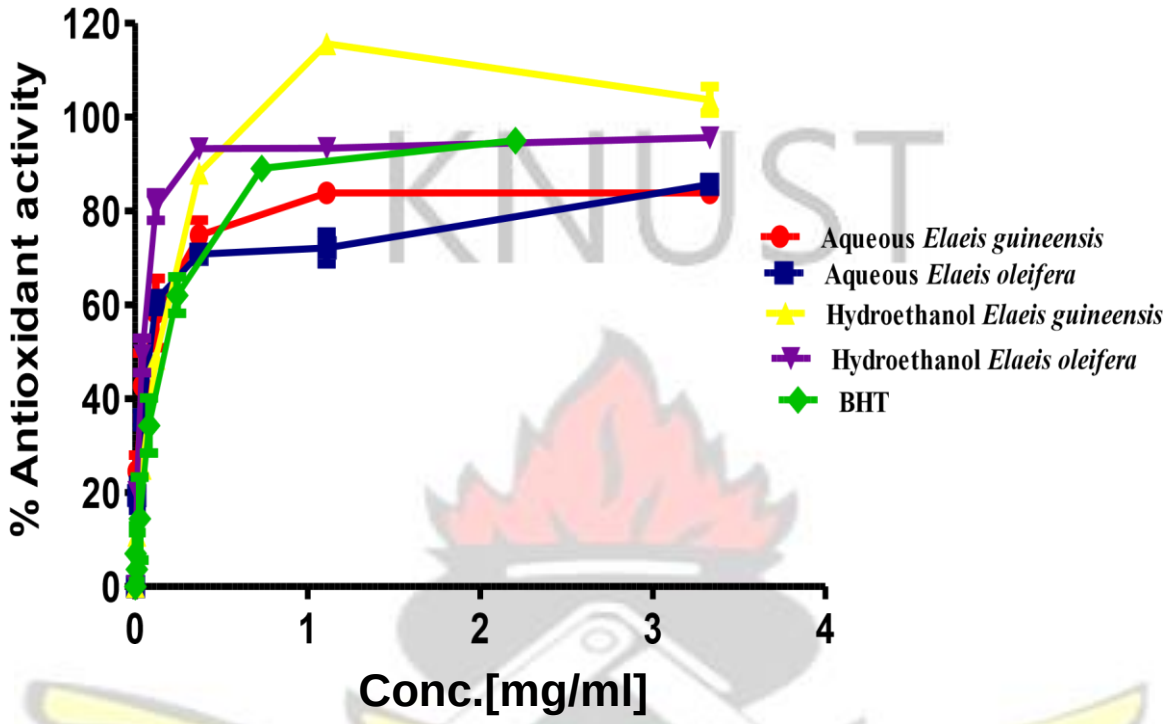


Figure 4.1: DPPH scavenging activity of different extracts of *E. guineensis* and *E. oleifera* and butylated hydroxytoluene

The DPPH scavenging activity of all extracts and BHT followed a dose-dependent pattern. EC_{50} values represent the effective concentration for 50% scavenging of total DPPH radicals. The EC_{50} values for hydroethanolic and aqueous extracts of *E. guineensis* and *E. oleifera* as well as the reference antioxidant BHT are presented in Table 4.2 below.

Table 4.2: EC_{50} values (mg/ml) of DPPH scavenging activities of the different extracts of *E. guineensis, E. oleifera*.

	E. guineensis	***E. oleifera***
Sample Extract	n= 3	n= 3

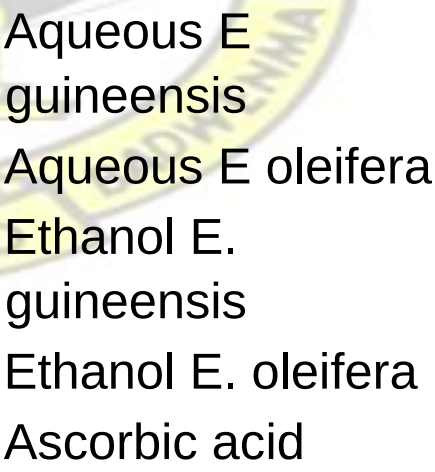
Aqueous	0.09 ± 0.03*	0.07 ± 0.01 *
Hydroethanolic	0.13 ± 0.01	0.05 ±0.01 *
BHT (Standard)	0.17 ± 0.02	

Values are mean ± standard error of mean (SEM) of three replicates. Means with superscript asterix (*) are significantly different from standard (BHT) at p < 0.05

The extracts had higher ($p < 0.05$) scavenging potential than BHT, except for hydroethanolic extract of *E. guineensis* which also indicated the weakest scavenging activity compared with the other extracts. The DPPH scavenging capability of *E. oleifera* appears to be slightly stronger than *Elaeis guineensis* while the hydroethanolic extract of *E. oleifera* gave the strongest scavenging potential.

4.2.2 Reducing power assay

The reducing power of ascorbic acid, aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera* are presented in Figure 4.2.



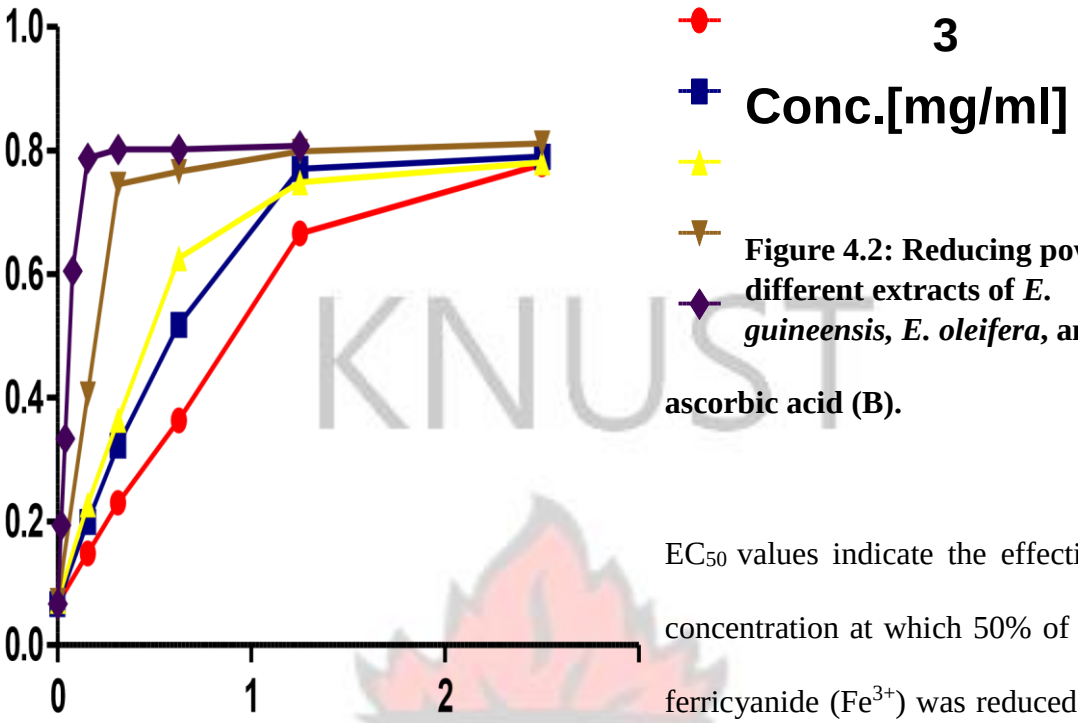


Figure 4.2: Reducing power of different extracts of *E. guineensis, E. oleifera,* and ascorbic acid (B).

The EC_{50} values indicate the effective concentration at which 50% of all ferricyanide (Fe^{3+}) was reduced to ferrocyanide (Fe^{2+}) and provides a means of comparing the reducing potential of compounds and extracts. The EC_{50} values of ascorbic acid, aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera* are presented in Table 4.3. The reducing potential of all test extracts were significantly ($p < 0.05$) lower than ascorbic acid. Hydroethanolic extracts of both *E. guineensis* and *E. oleifera* exhibited greater reducing potential than respective aqueous extracts. The reducing potential of aqueous and hydroethanolic extracts of *E. oleifera* are observed to be higher than respective extracts of *E. guineensis*.

Table 4.3: EC_{50} values (mg/mL) of Reducing power of different extracts of *E. guineensis, E. oleifera* and Ascorbic acid

Sample Extract	*E. guineensis* n= 3	*E. oleifera* n= 3
Aqueous	$(0.91 \pm 0.01)^{*d}$	$(0.60 \pm 0.01)^{*c}$

Ethanolic	$(0.47 \pm 0.01)^{*b}$	$(0.20 \pm 0.01)^{*a}$
Ascorbic acid	(0.06 ± 0.01)	

Values are mean ± standard error of mean (SEM) of three replicates. Means with asterix are significantly different ($p < 0.05$) from standard (ascorbic acid) such that a<b<c<d.

4.2.3 Total Phenol Content

The content of total phenols in extracts was measured by Folin-Ciocalteau's reagent at a wavelength of 750 nm, quantified with reference to gallic acid per 100 g of extract (Figure 4.3). The total phenol content of aqueous and hydroethanolic extracts of *E. guineensis* were significantly lower than that of the respective extracts of *E. oleifera*.

Hydroethanolic extract of *E. oleifera* showed the highest total phenol content and the aqueous extracts of *E. guineensis* extract, the lowest phenol content. No significant difference ($p>0.05$) in phenol content was observed between the aqueous extract of *E. oleifera* and hydroethanolic extract of *E. guineensis*.

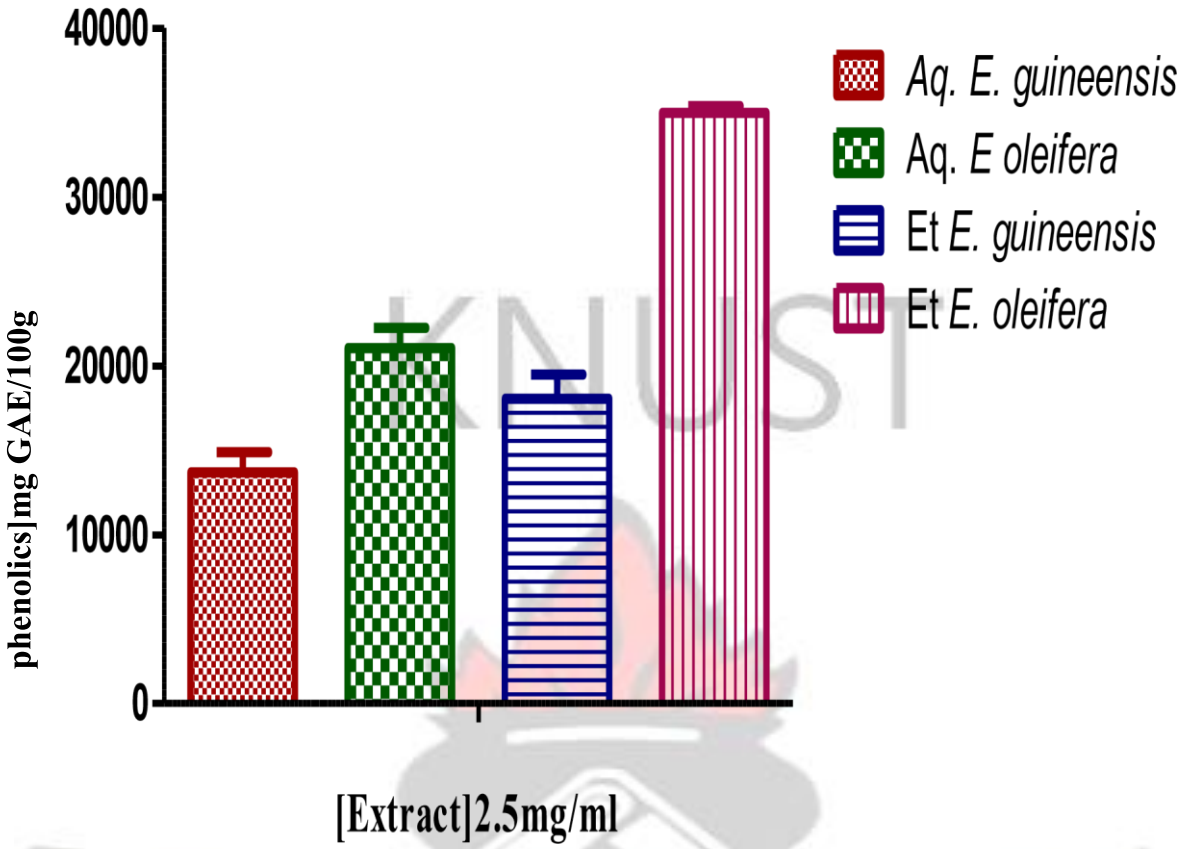


Figure 4.3: Total phenolic content of aqueous extracts of *E. guineensis* and *E. oleifera* and hydroethanol extract of *E. guineensis* and *E. oleifera.*

4.2.4 Total flavonoid content.

The flavonoid content of test extracts was measured by the aluminium chloride method. Absorbance was read at 415 nm and flavonoid content was quantified with reference to quercetin per 100g of test extract (Figure 4-4).

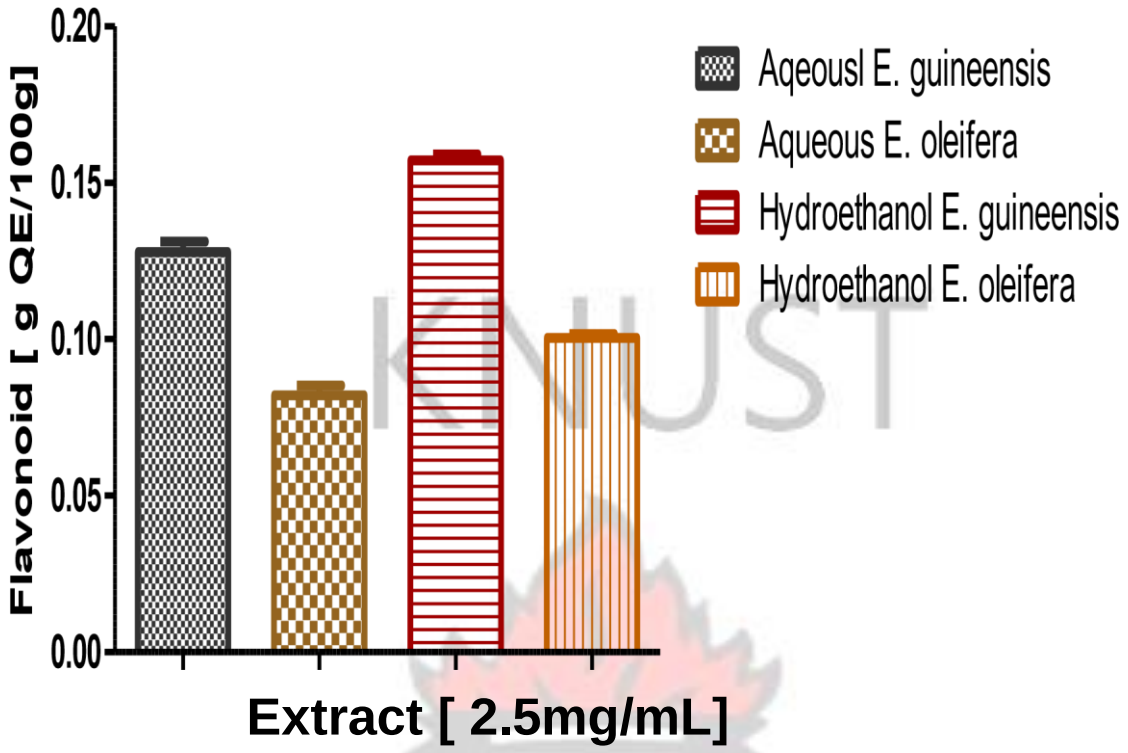


Figure 4.4: Total Flavonoid content of different extracts of *E. guineensis* and *E. oleifera*.

The hydroethanolic extracts of *E. guineensis* and *E. oleifera* total flavonoid content is significantly higher ($p<0.05$) than their respective aqueous extracts. The total flavonoid contents *of E guineensis* for either aqueous or hydroethanolic extracts are also significantly higher ($p < 0.05$) than their respective *E. oleifera* extracts.

4.3. *IN -VITRO* ASSESSMENT OF ANTICANCER ACTIVITY OF *E. GUINEENSIS* AND *E. OLEIFERA* ON JURKAT AND MCF-7 CELLS

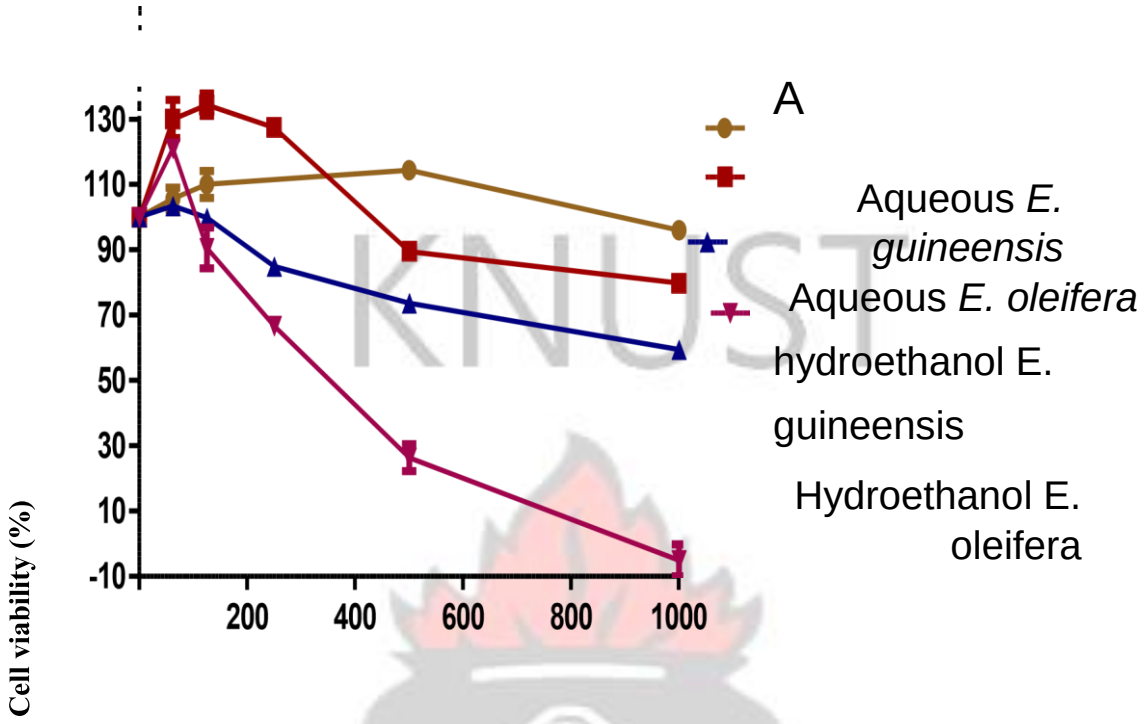
4.3.1. *In-vitro* cytotoxicity assessment of crude extracts of *E. guineensis* and *E. oleifera*

The cytotoxic effect of the aqueous and hydroethanolic crude extracts of *E. guineensis and E. oleifera* were ascertained on human leukemia (Jurkat) cells, human breast cancer (MCF-7) cells and normal human liver (Chang) Cells. Curcumin, a cytotoxic compound was used as positive control. The dose-response curves for the extracts and reference compound are presented in

figures 4.5 to 4.7. The IC_{50} values for the dose-response curves and selectivity indices are in Tables 4.4 and 4.5.

Viability of normal chang liver cells, as illustrated in Figure 4.5 was significantly lower after treatment with aqueous than hydroethanol extracts of *E. guineensis* than *E. oleifera* extracts than with the curcumin standard. Proliferation of Chang liver cells treated with hydroethanolic extracts of *E. guineensis* and *E. oleifera* decreased for concentrations higher than 62.5 µg/ml in a concentration-dependent manner over the concentration range of 62.5-1000 µg/ml.

Jurkat cells were sensitive to each of the four crude extracts as illustrated in Figure 4.6. Incubation of Jurkat cells with hydroethanolic and aqueous extracts of *E. oleifera* and *E. guineensis* inhibited proliferation over the concentration range of 62.5-1000 µg/ml. Cell viability decreased with concentration of test extracts greater than 62.5 µg/ml to viabilities equal or less than 1% for 1000 µg/ml concentrations. Relatively, hydroethanolic extracts exerted significantly greater cytotoxicity and lesser selectivity for Jurkat cells than aqueous extracts, as observed from the IC_{50} values presented in tables 4.4 and 4.5.


A
Aqueous E. guineensis
Aqueous E. oleifera
hydroethanol E. guineensis
Hydroethanol E. oleifera
Cell viability (%)
130
110
90
70
50
30
10
-10
200
400
600
800
1000
0

!

[extract] µg/ml

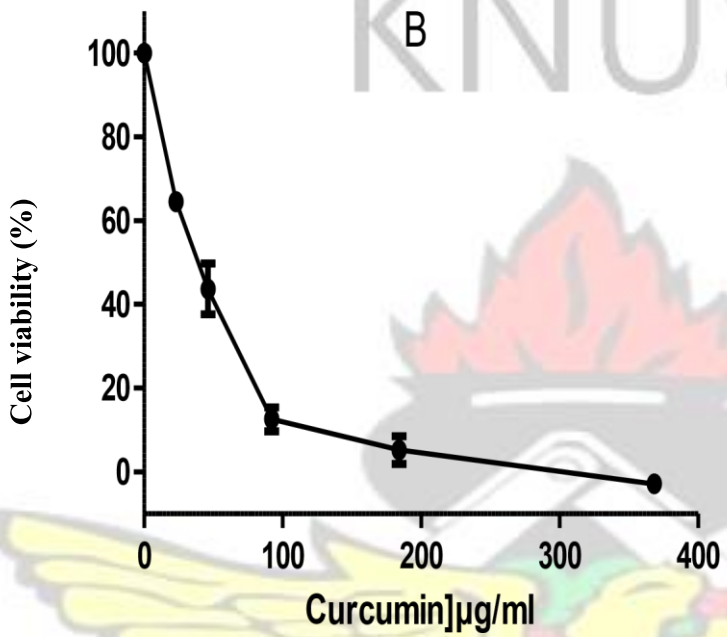
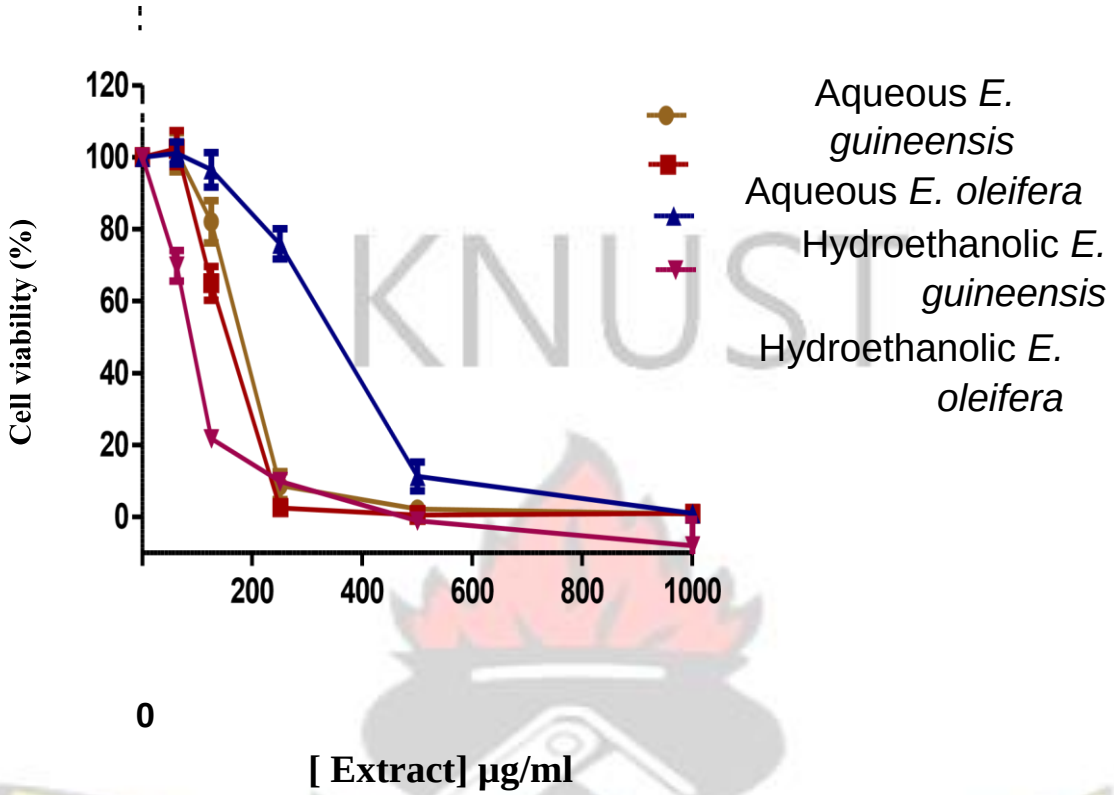
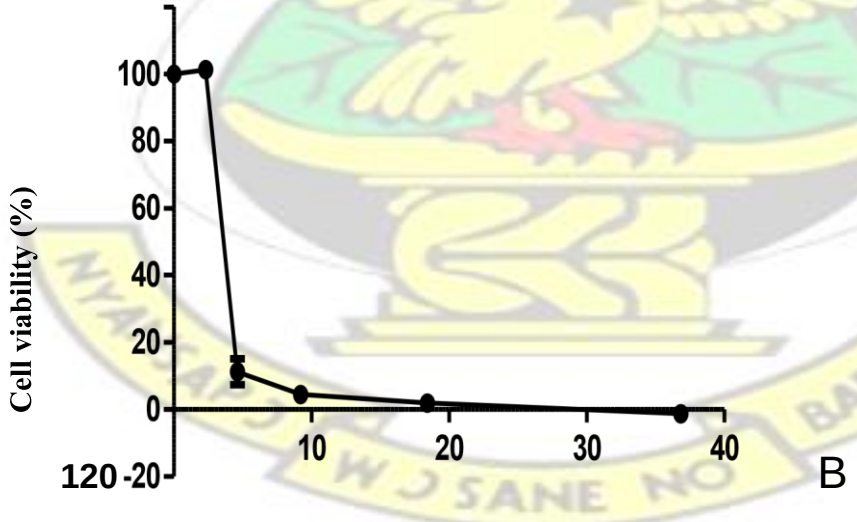


Figure 4.5: Cytotoxicity of (A) aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera* and (B) Curcumin on Chang Liver cells

A


Cell viability (%)
120
100
80
60
40
20
0
200
400
600
800
1000
0
[Extract] µg/ml
Aqueous E. guineensis
Aqueous E. oleifera
Hydroethanolic E. guineensis
Hydroethanolic E. oleifera

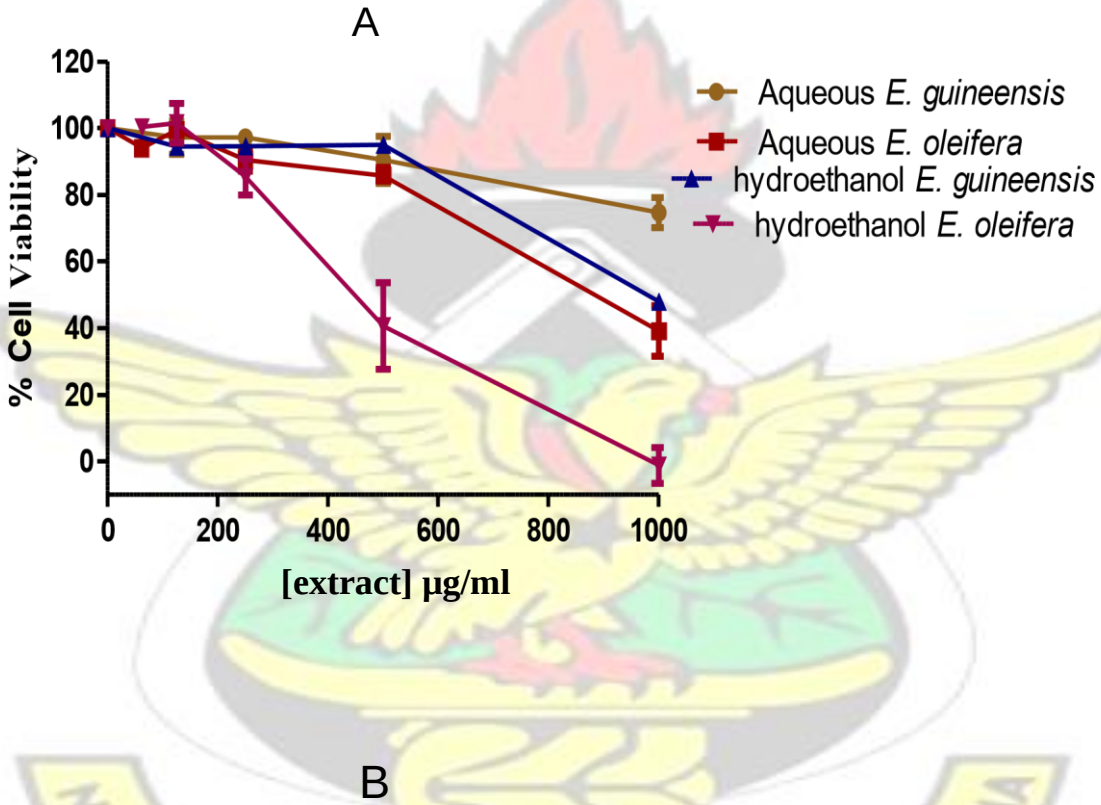

Cell viability (%)
100
80
60
40
20
0
120
-20
10
20
30
40

B

!

[Curcumin]µg/ml

Figure 4.6: Cytotoxicity of aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera* (graph A) and Curcumin (graph B) on leukaemia cancer cells (Jurkat)



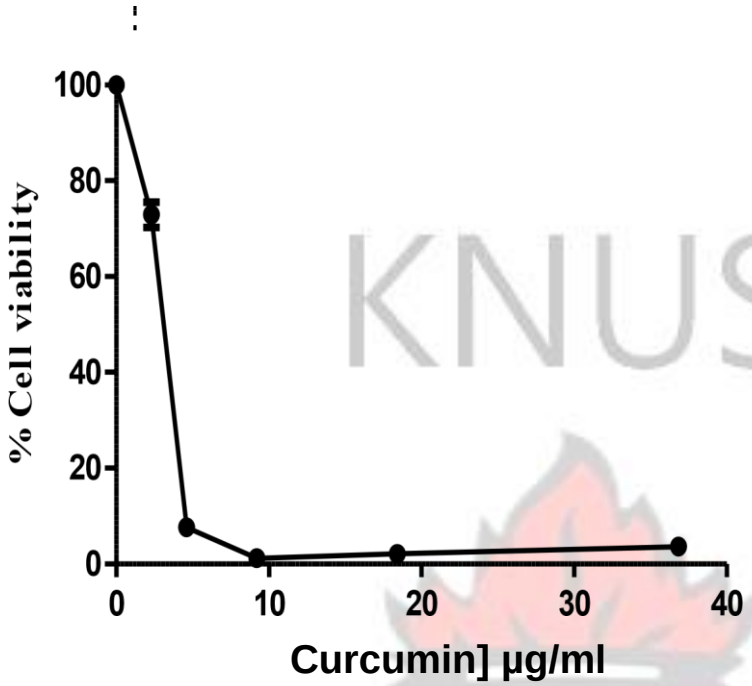


Figure 4.7: Cytotoxicity of aqueous and hydroethanol extracts of *E. guineensis* and *E. oleifera* (graph A), and Curcumin (graph B) on Breast cancer cells (MCF-7).

Table 4.4: Cytotoxicity (IC_{50} values) of Aqueous and hydroethanolic extracts of *E.guineensis* and *E. oleifera* on MCF-7 and Jurkat cells and Chang liver.

Cell Line	Extract	IC50, µg/mL		
		E. guineensis	***E. oleifera***	**Curcumin**
Normal Chang liver	Aqueous	> 1000 ^{*b}	> 1000 ^{*b}	57.12 ± 13.33
	Ethanol	> 1000 ^{*b}	354.97 ± 21.10^{*a}	
Jurkat leukemia	Aqueous	178.91 ± 7.14 ^{*c}	153.63 ± 6.98 ^{*b}	3.37 ± 0.35
	Ethanol	349.93 ± 12.57 ^{*d}	87.54 ± 4.27 ^{*a}	
MCF-7 Breast Cancer	Aqueous	>1000 ^{*b}	902.97 ± 58.87 ^{*b}	3.14 ± 0.06
	Ethanol	981.56 ± 23.40 ^{*b}	476.32 ± 82.74 ^{*a}	

Values are mean ± standard error of mean (SEM) of three replicates

Means across rows with asterix (*) are significantly different at $p < 0.05$ from standard (curcumin) such that $a < b < c < d$

Table 4.5: Selectivity of cytotoxicity of Aqueous and ethanol extracts of *E. guineensis* and *E. oleifera* to Jurkat and MCF-7 cell lines.

Cell Line	Plant extract	Selectivity Index		
		E. guineensis	***E. oleifera***	**Curcumin**
Jurkat leukemia	Aqueous	5.61 ± 0.22 ^{*b}	8.7 ± 0.11^{*c}	17.06 ± 3.37
	Hydroethanol	6.54 ± 0.30 ^{*b}	4.08 ± 0.41^{*a}	
MCF-7 Breast Cancer	Aqueous	1^{*b}	1.02 ± 0.07 ^{*b}	18.13 ± 4.12
	Hydroethanol	1.02 ± 0.03^{*b}	0.77 ± 0.08 ^{*a}	

Values are mean ± standard error of mean (SEM) of three replicates

Means with asterix (*) across rows are significantly different at $p < 0.05$ from standard such that a<b<c

4.3.2. *In-vitro* cytotoxicity assessment of petroleum ether, chloroform and ethyl acetate fractions of *E. guineensis* and *E. oleifera* aqueous and ethanol crude extracts.

The cytotoxic effect of the petroleum ether, chloroform and ethyl acetate fractions of *E. guineensis and E. oleifera* of crude aqueous and hydroethanolic extracts were ascertained on Jurkat, MCF-7 and normal Chang liver cells. Curcumin, a known cytotoxic compound was used as positive control. The dose-response curves for the extracts and reference compound are presented in figures 4.8 to 4.10 and IC_{50} values for the curves and selectivity indices are on Tables 4.5-4.5.

Viability of Chang liver cells, as illustrated in Figures 4.8, 4.9, 4.10 and Table 4.6 were least affected by increasing concentrations of the fractions. Ethyl acetate and chloroform fractions of hydroethanolic *E. oleifera* extract exhibited the highest ($p<0.05$) cytotoxicity, respectively to Chang liver cells. The least cytotoxic fraction to Chang liver cells were ethyl acetate and chloroform fractions of the aqueous extract of *E. guineensis* and the chloroform and petroleum ether fractions of the hydroethanolic extract of *E. oleifera* .

The Petroleum ether fractions exhibits varying cytotoxicities to Chang liver, Jurkat and MCF-7 cells, as illustrated by viability curve presented in Figure 4.8 and cytotoxicities expressed as IC_{50} values and selectivity indices in Table 4.6. Petroleum ether fractions were more cytotoxic to jurkat cells than MCF-7 cells and also with higher selective indices. No significant differences were observed for sensitivity by jurkat and MCF-7 to petroleum ether fractions of either *E. guineensis* or *E. oleifera*; aqueous or hydroethanolic crude extracts.

Chloroform fractions of test extracts were more cytotoxic to jurkat cells than MCF-7 cells except for hydroethanolic *E. guineensis* extract. Chloroform fractions of hydroethanolic *E. guineensis* were most cytotoxic and selective for MCF-7; whiles chloroform fraction of

aqueous *E. oleifera* was the least cytotoxic and selective. Sensitivity and selectivity for jurkat cells by chloroform fractions was in the order of aqueous extracts of *E. guineensis* and *E. oleifera* being greater than hydroethanolic extracts.

Ethyl acetate fractions of both aqueous and hydroethanolic extracts of *E. guineensis and E. oleifera* were observed to exhibit varying cytotoxicity and selectivity to MCF-7 and jurkat cells as illustrated in Figure 4.10 and Table 4.6. Significant variances in cytotoxicity and selectivity for MCF-7 cells was observed between *E. guineensis* and *E. oleifera*; as ethyl acetate fractions of hydroethanolic extract of *E. oleifera* exerted highest ($p<0.05$) toxicity and aqueous extract of *E. guineensis* exerted the least toxicity to MCF-7.

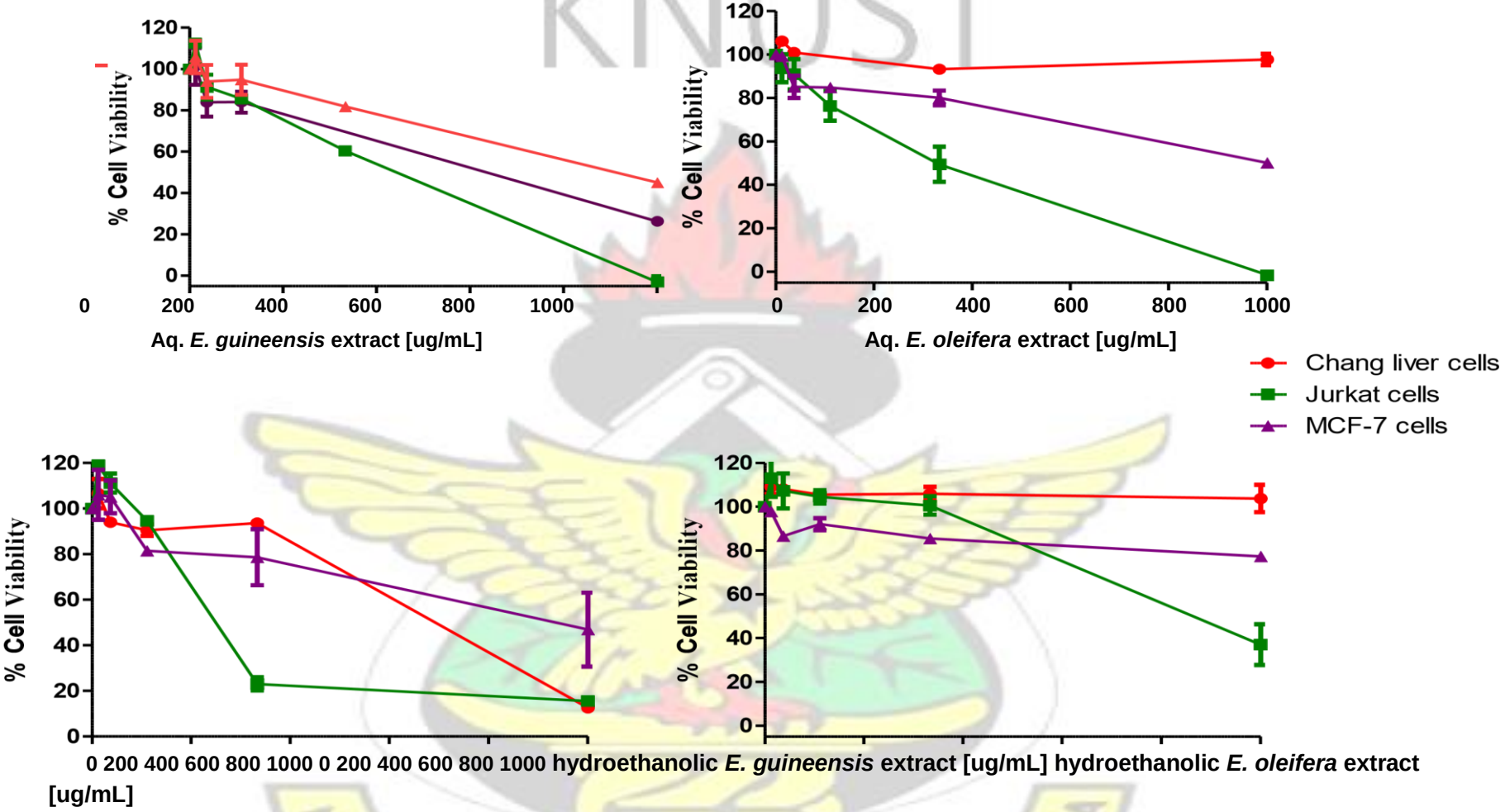
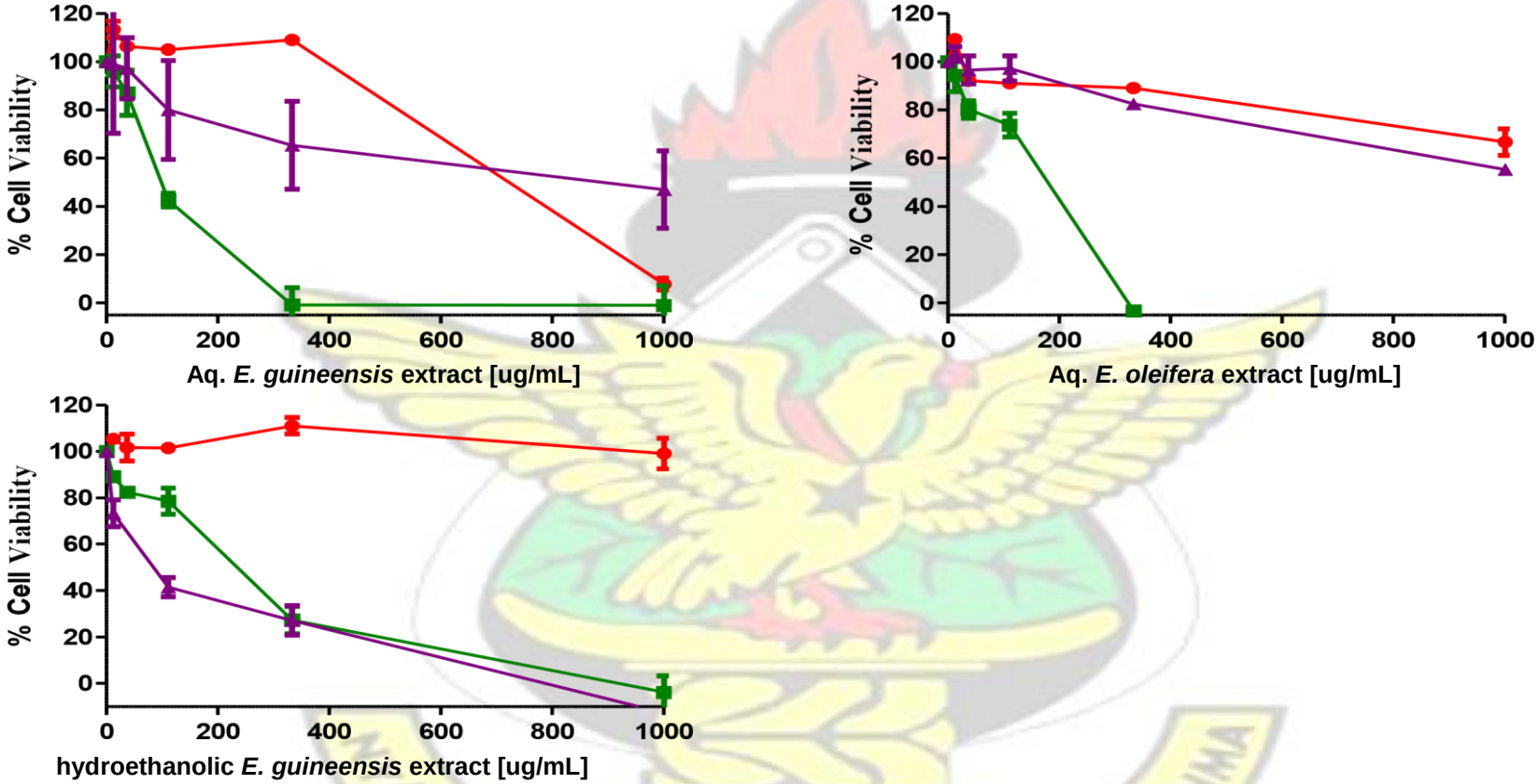

% Cell Viability
Aq. E. guineensis extract [ug/mL]
Aq. E. oleifera extract [ug/mL]
Chang liver cells
Jurkat cells
MCF-7 cells
hydroethanolic E. guineensis extract [ug/mL]
hydroethanolic E. oleifera extract [ug/mL]

Figure 4.8: Cytotoxicity of petroleum ether fractions of *E. guineensis* and *E. oleifera* on normal Chang liver, Jurkat and MCF-7 cells.



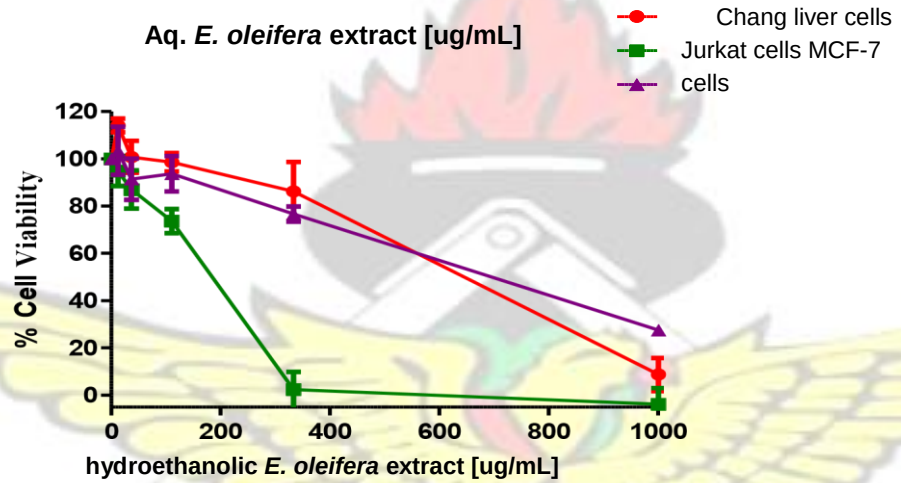


Figure 4.9: Cytotoxicity of chloroform fractions of *E. guineensis* and *E. oleifera* on normal Chang liver, Jurkat and MCF-7 cells.

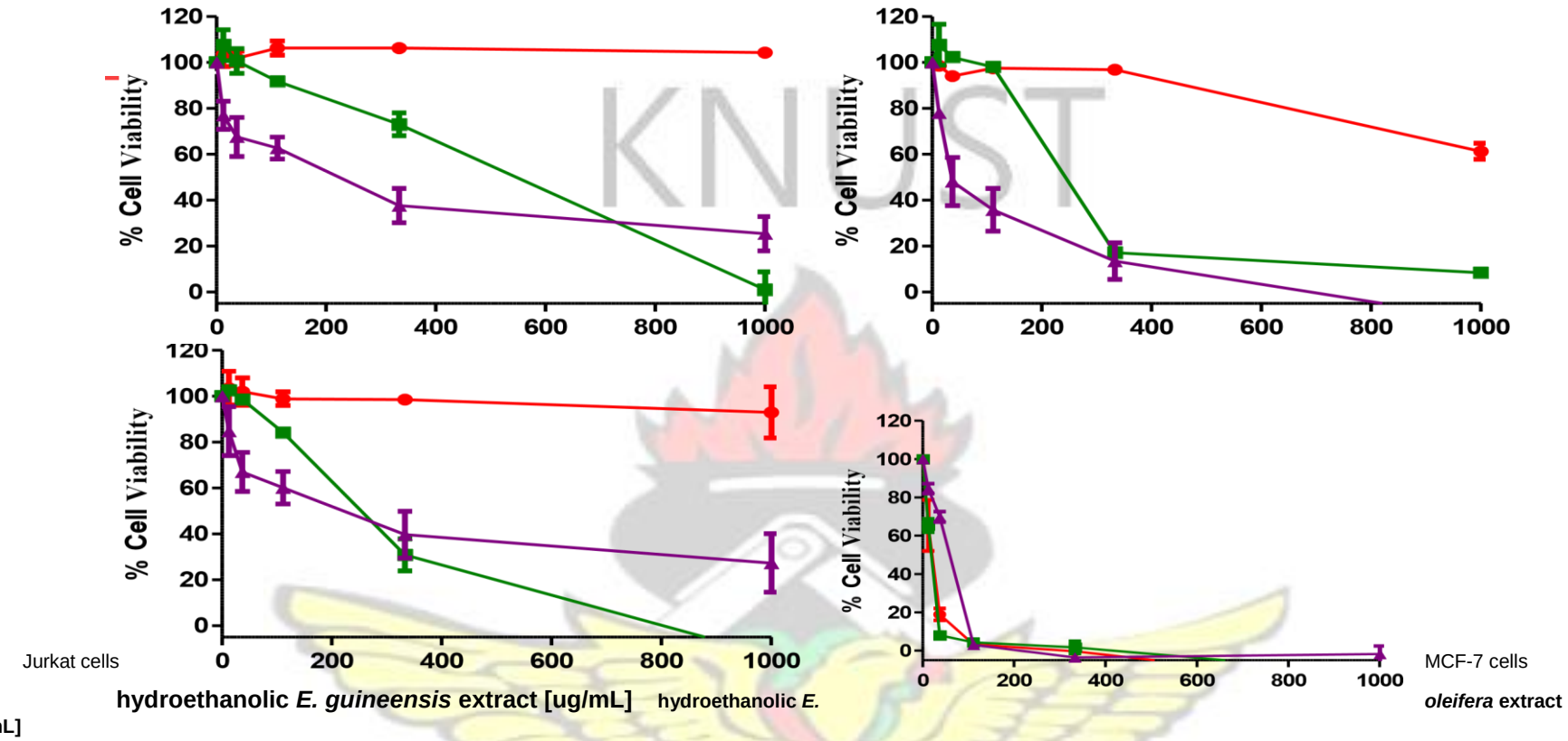


Figure 4.10: Cytotoxicity of ethyl acetate fractions of *E. guineensis* and *E. oleifera* on normal Chang liver, Jurkat and MCF-7 cells.

Table 4-5: IC_{50} values and selective indices of petroleum ether, chloroform, and ethyl acetate fractions of crudes extracts.

		Chang liver Cells	MCF-7 Cells		Jurkat Cells	
Fraction	**Extract**	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**	**Selctive index**	**IC_{50}**	**Selctive index**
Pet. Ether	Aq. *E. guinensis*	817.807 ± 52.50 ^{c}	910.863 ± 8.99 ^{d}	0.898 ± 0.60 ^{a}	443.100 ± 26.54 ^{e}	1.864 ± 0.19 ^{a}
	Aq. *E. oleifera*	>1000 ^{d}	997.71 ± 2.10 ^{d}	>1.002 ^{a}	288.567 ± 34.17 ^{d}	3.579 ± 0.48 ^{b}
	EtOH *E.guineensis*	691.543 ± 12.20 ^{bc}	741.81 ± 111.45 ^{cd}	0.976 ± 0.14 ^{a}	249.96 ± 7.27 ^{cd}	2.774 ± 0.13 ^{ab}
	EtOH *E. oleifera*	>1000 ^{d}	>1000 ^{d}	1.00 ^{a}	827.14 ± 50.01 ^{f}	1.218 ± 0.08 ^{a}
Chloroform	Aq. *E . guinensis*	721.85 ± 10.79 ^{bc}	535.473 ± 217.41 ^{bc}	2.405 ±1.38 ^{a}	93.887 ± 3.18 ^{ab}	7.714 ± 0.38 ^{d}
	Aq. *E. oleifera*	>1000 ^{d}	>1000 ^{d}	1.000 ^{a}	174.06 ± 3.92 ^{bc}	5.751 ± 0.13 ^{c}
	EtOH *E.guineensis*	>1000 ^{d}	41.967 ± 11.61 ^{a}	27.445 ± 6.71 ^{b}	235.37 ± 24.77 ^{cd}	4.356 ± 0.51 ^{bc}
	EtOH *E. oleifera*	677.337 ± 73.53 ^{b}	682.943 ± 6.49 ^{cd}	1.464 ± 0.12 ^{a}	180.057 ± 13.83 ^{bcd}	3.838 ± 0.63 ^{b}
Ethyl ether	Aq. *E . guinensis*	>1000 ^{d}	225.023 ± 7.75 ^{ab}	4.455 ± 0.15 ^{b}	512.997 ± 17.02 ^{e}	1.954 ± 0.06 ^{a}
	Aq. *E. oleifera*	>1000 ^{d}	27.26 ± 3.59 ^{a}	36.15 ± 5.61 ^{b}	242.09 ± 0.70 ^{cd}	4.131 ± 0.01 ^{b}
	EtOH *E.guineensis*	>1000 ^{d}	164.78 ± 34.73 ^{a}	6.726 ± 1.59 ^{a}	257.747 ± 18.29 ^{cd}	3.920 ± 0.28 ^{b}
	EtOH *E. oleifera*	35.177 ± 1.98 ^{a}	58.84 ± 1.74 ^{a}	0.598 ± 0.03 ^{a}	18.88 ± 1.26 ^{a}	1.874 ± 0.12 ^{a}

Aq=Aqueous and EtOH=Hydroethanolic

Each value is expressed as Mean ± Standard error of mean(S.E.M).

Means with different superscripts within same column are significantly different ($P<0.05$) such that a < b < c < d

CHAPTER FIVE

5.0. DISCUSSION

Phytochemicals act in many ways to assist the body in fighting against diseases and health problems. They are thought to augment the intrinsic defence mechanisms and provide additional protection such as the scavenging of free radicals before they can cause damage from within the body (Okaka and Okaka, 2001).

The phytochemical analysis of the extracts revealed the presence of plant bioactive constituents in both aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera*. A strong presence of flavonoids, tannins, terpenoids, saponins and alkaloids relative to their respective standards were detected (Table 4.1). This observation was consistent with previous studies in which flavonoids were the most predominant bioactive compounds in oil palm leaf extracts (Yin et al, 2013) .

Antioxidant functions are reportedly ascribed to a wide range of phytochemicals including polyphenols (Masella *et al.*, 2005). These polyphenols, such as flavonoids, terpenoids and carotenoids amongs others, exert their antioxidant activities by scavenging various oxidizing species such as hydroxyl , peroxy, and superoxide radicals ; Hence, counteracting conditions of oxidative stress (Szeto and Benzie, 2002; Ramasamy and Agarwal, 2008).

DPPH assay is an indirect method of measuring the free radical scavenging potential of a substance based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors from test antioxidants. The DPPH, an N centered radical, has a characteristic absorbance at 517 nm, which decreases with the scavenging of the proton radical (Chia_Ling and Wen_Ching, 2002)

The effectiveness or strength of DPPH free radical scavenging activity of aqueous and hydroethanolic leaf extracts of *Elaeis guineensis* and *Elaeis oleifera* were assessed by determining and comparing respective EC_{50} values. EC_{50} values are the concentration of test

extract or compound at which 50% of its maximal effect is exhibited. In this instance, it measures the ability of the extracts to mop free radicals of DPPH. The EC_{50} values of extracts and butylated hydroxytoluene (BHT), a well known antioxidant compound were deduced and compared (Table 4.2). Ferric reducing power assay estimates the ferric reducers in the extracts and compounds. In this study, the reducing power of aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera* were assessed and compared to a known ferric reducer, ascorbic acid. The results of DPPH free radical scavenging activity and reducing power of the extracts supports claims by Ibraheem *et al.* (2012) and Sasidharan *et al.* (2009) that palm oil leaf extract is an effective antioxidant comparable to vitamin C and BHT. In addition, Lee *et al.* (2012) reported that EC_{50} values less than 10 mg/ml indicated that extracts were effective antioxidants. Since all the extracts had EC_{50} values lower than 10 mg/mL, the extracts could be considered as having good antioxidant properties; however *E. oleifera* extracts generally exhibited better DPPH antioxidant properties than *E. oleifera*.

Total phenolic and flavonoid contents are commonly used in conjunction with DPPH and ferric reducing power assays to evaluate antioxidant activity. The antioxidant activity of polyphenols including flavonoids emanates from their hydroxyl groups conferring scavenging ability (Hughes *et al.*, 2013). Earlier studies (NgMei and Choo Yuen, 2010) reported that *E. guineensis* leaf extracts contain substantial quantities of polyphenols such as flavonoids as well as tannins, and suggested that, these contributed to the antioxidant activity of oil palm leaf extracts. In this study, the total phenolic content (TPC) and flavonoid content of aqueous and hydroethanolic extracts of *E. guineensis* and *E. oleifera* were ascertained. Hydroethanolic extracts of both *E. guineensis* and *E. oleifera* contained significantly higher quantities of phenols and flavonoids than the aqueous extracts. The total phenol content of *E. guineensis* was significantly lower than that of *E. oleifera* and the inverse is true for the flavonoid content, probably due to fact that flavonoid are more aqua-soluble.

Lu and Foo (2000), and Siriwardhana *et al.* (2003) suggested a relationship between free radical scavenging activity, total phenols and flavonoid content in various plant products. Oki *et al.* (2002), also suggested that the radical scavenging activity increases with increasing polyphenolic content and that the varying concentrations of different polyphenols *in vitro* influence pharmacological actions modulating several cell activities as suggested by Masella *et al.* (2005). The significantly higher polyphenolic content of *E. oleifera* may account for the significantly higher oxidative potential of *E. oleifera* observed. Similarly, the significantly higher antioxidant activity of hydroethanolic extracts compared to the aqueous extracts was probably due to the ability of hydroethanol to extract more hydrogen donating components.

Numerous studies have demonstrated that some phytochemicals are excellent sources of various anticancer agents (Yang *et al.*, 2009; Kaur *et al.*, 2009). Ethnobotanical studies revealed the folklore medicinal claim of oil palm being used to treat cancer, headaches and rheumatism and also considered as an aphrodisiac, diuretic and liniment (Irvin, 1985). A number of researchers have conducted studies and suggested the anticancer potential of *E. guineensis* leaf extracts on various cancer cell lines. For example, Vijayarathna and Sasidharan (2012) suggested the anticancer property of *E. guineensis* by observing cytotoxicity of methanol leaf extracts to breast cancer cell line, *MCF-7* and its non-toxicity to Vero cell lines. This study indicated that oil palm leaf extracts of *E. guineensis* and *E. oleifera* exhibit varying degrees of cytotoxicity to MCF-7 and Jurkat leukemia cell lines in a concentration dependent fashion.

With the exception of hydroethanolic *E. oleifera* crude extract, which caused cytotoxicity with an IC_{50} value of 87.54 µg/mL, all IC_{50} values of crude test extracts on MCF-7, Jurkat and normal Chang cell lines were higher than 100 µg/mL. This implies that hydroethanolic *E. oleifera* crude extract was the most cytotoxic to MCF-7 cells. The IC_{50} values of test extracts on normal Chang liver cells were all greater than 300 µg/ml, whiched indicated weak cytotoxicity of the leaf extracts from both varieties of oil palm as suggested by Vijayarathna

and Sasidharan (2012). Sensitivity of cell lines to both varieties of oil palm, as indicated by IC_{50} values, were in the increasing order; normal Chang liver, MCF-7 and Jurkat cell lines. *E. oleifera* extracts exerted greater cytotoxicity on test cell lines than *E. guineensis* indicating that *E. oleifera* may have either stronger concentration or a specific compound influencing the observed anticancer activity.

The hydroethanolic extracts of both varieties of oil palm leaf extract exhibited greater toxicity to cancer cell lines than respective aqueous extract. This indicated that, the hydroethanol extracts contained greater amounts of compounds influencing anticancer property. The differences in extraction procedure or the solvents may also account for the IC_{50} values realised in this study being higher than the IC_{50} value of 15 µg/ml reported by Vijayarathna and Sasidharan (2012) for methanol extracts of *E. guineensis*.

The selectivity of an extract or compound for cancer cells is very important. A selective index greater than two (2) is required for an extract or compound to be considered therapeutic. Therefore both aqueous and hydroethanol extracts of *E. guineensis* and *E. oleifera* may be considered as promising anticancer agent for Jurkat cells and not MCF-7 cells, because the selective index for MCF-7 was less than two (2) and greater than two (2) for Jurkat cells. Selective indices deduced for crude extracts of oil palm leaves were significantly lesser than that of curcumin, the positive control. The selective indices of crude extracts ranged from three (3) to four (4) folds lesser than curcumin for Jurkat cells (17.06) and about ten (10) folds for MCF-7 cells (18.13). It is, however, worth noting that curcumin is a pure compound extracted from turmeric plant. Therefore, bio-guided assay fractionation and purification of test extracts could possibly produce a cancer therapeutic agent of similar activity as curcumin.

Fractions of aqueous and hydroethanolic crude extracts of *E. guineensis* and *E. oleifera* resulting from successive fractionation with petroleum ether, chloroform and ethyl acetate

exhibited varying cytotoxicity and selective indices as illustrated in figures 18 - 20 and Table 8. With reference to the American National Cancer Institute guidelines, which set the limit for significantly cytotoxic activity of crude extracts at IC_{50} values less than 30 µg/ml after an exposure time of 72 hours (Suffness and Pezzuto, 1990) and those with IC_{50} values lower than 20 µg/ml as highly cytotoxic (Mahavorasirikul *et al.,* 2010). Ethyl acetate fractions of *E. oleifera* aqueous extract had the most appreciable activity against MCF-7 cell lines. It exhibited cytotoxicity to MCF-7 cells with an IC_{50} value of 27.26 µg/mL and a selective index of 36.15. On Jurkat cells, ethyl acetate fractions of hydroethanol *E. oleifera* was most cytotoxic, with IC_{50} value of 18.88 µg/mL with selective index of 1.87. It is therefore evident that further bioassay guided fractionations could produce a fraction with greater cytotoxicity.

The cytotoxicity, antioxidant activity and polyphenolic content of *E. guineensis* have been studied by other authors (Sasidharan *et al.*, 2009; Ibraheem *et al.*, 2012; Vijayarathna and Sasidharan, 2012). Different extraction methods and cell lines were used and have all concluded that *E. guineensis* leaves are a good source of chemotherapeutic agents, even though they reported different IC_{50} and EC_{50} values. The differences in IC_{50} and EC_{50} values reported in literature and those obtained in this study could be due to differences in extraction solvents, procedures and natural variability in plants.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Oil palm leaves of both *E. guineensis* and *E. oleifera* species possess some biological properties such as antioxidant and anticancer properties. Crude aqueous and hydroethanolic leaf extracts contains the active principles influencing biological properties exhibited by both species. These activities may be due to the presence of phytochemicals such as tannins, flavonoids, saponins and terpenoids.

Data from this study suggests that *E. oleifera* leaf extracts possesses more total phenols and flavonoids than *E. guineensis* possibly resulting in greater antioxidant activity. The anticancer effects of *E. oleifera* leaf extracts are significantly greater than that exerted by *E. guineensis* leaf extract and Jurkat leukaemia is more sentisitive to leaf extracts of *E. oleifera* and *E. guineensis* than mcf-7 and chang liver cells. Fractionation of the aqueous and hydroethanol leaf extracts with petroleum, chloroform and ethyl acetate produced some fractions with higher cytotoxicity and selectivity towards Jurkat and MCF-7 cells.

6.2 RECOMMENDATIONS FOR FURTHER WORK

The crude hydroethanol and aqueous extracts should be further fractionated with other solvent systems and the fractions screened for antioxidant and anticancer activity. The fraction with the greatest activity be selected for compound isolation. Since apoptosis is a preferred mechanism for anticancer activity, effect of the active components on mechanisms that characterize apoptosis must be investigated using the cell lines tested. These include molecular mechanisms such as DNA fragmentation, morphological changes (chromatin condensation), and cell cycle analysis.

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