Scientific Times Journal of Cancer

Thesis Open Access

Assessment Of Anti-Proliferative Activities Of Selected Medicinal Plant Extracts Used For Management Of Diseases Around Lake Victoria Basin

Tyrus Omondi Swaya[1*], Philip Aduma[2], Kiprotich Chelimo[3] and Obuya Were[4]

[1]School of Public Health and Community Development, Maseno University, Kenya
[2]School of Public Health and Community Development, Maseno University, Kenya
[3]School of Public Health and Community Development, Maseno University, Kenya
[4]School of Physical and Biological Sciences, Chemistry Department, Maseno University, Kenya

Corrsponding Author: Tyrus Omondi Swaya (Bsc), School of Public Health and Community Development, Maseno University, Kenya. Email: omondityrus18@gmail.com

Received Date: 22/02/2017, **Accepted Date:** 02/03/2017, **Published Date:** 15/03/2017

Abstract

The devastating effect of cancer is a worldwide concern. Unfortunately current chemotherapeutic and radio therapeutic modalities have been found to possess side effects coupled with the emergence of drug resistance. This has necessitated the search for novel therapeutic products with better efficacy, safety and affordability through identification of anti-tumor agents from natural products. In this study the antineoplastic activity and phytochemical screening of methanol extracts of four medicinal plants that are native to Lake Victoria Basin, including Piptadiniastrum africanum, Kigelia africana, Centella asciatica and Chaemocrista nigricans was investigated. Extraction and concentration of the collected and dried plant samples to obtain crude extracts as well as phytochemical screening of the crude extracts was done following standard procedures. Lung adenocarcinoma cell line, from American type cell culture (ATCC), was exposed to the extracts and antiproliferative analysis using 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2-tetrazolium bromide (MTT) colorimetric assay was performed. Phytochemical analysis of each of the four plants showed presence of terpenoids, alkaloids, saponins, tannins and steroids. Steroids were higher in the bark of P. africanum and the fruits of K. africana. Flavonoid was absent only in extracts of C. nigricans and coumarins present only in the extracts of K. africana. The methanolic extracts of the fruits of K. africana, bark of P. africanum, leaves of C. asciatica and leaves C. nigricans had inhibitory effects with IC50 28.86ug/ml, 26.57ug/ml, 15.69ug/ml and 8.07ug/ml (p values of 0.079, 0.069, and 0.042 and 0.055, ANOVA) respectively. Statistical differences among fractions of the extract were determined by one way ANOVA and considered significant at $P< 0.05$. The MTT assay results indicated that all of the extracts had the capacity to inhibit the proliferation of tumor cells. The Phytochemical compounds which were tested are antioxidant and shown to trigger morphological changes, such as blebbing pattern and cell shrinkage, associated with apoptosis in the lung adenocarcinoma cell lines. The varying inhibitory activities against tested human lung adenocarcinoma cell lines justify the traditional use of these plants in the management of cancers and other illnesses. These extracts have a potential to be further developed into anti-tumor agents. However, further phytochemical characterization of the extracts is necessary for more extensive biological evaluation of the active ingredients and to exhaustively describe the mechanisms of cancer cell apoptosis of these four plant extracts.

Acknowledgement

For the successful completion of this work, I am indebted to recognise and thank the Inter-University Council of East Africa and The Lake Victoria Research Initiative (IUCEA/VicRes) for providing the financial support for this work. I would like to thank Prof. Philip Aduma, team leader of the VicRes project, who has been my supervisor, academic mentor, and lecturer for availing the plants, providing the cell lines and reagents used in this study. I would like to thank Dr. Chelimo and Dr. Were for their supervision and guidance in the assays. I would also like to thank Mr. Dan, Statistician, for his input assistance in the statistical analysis of the data. In addition, I thank Evans Odhiambo and James Odhiambo who helped me in plant extraction and cell culture. I am also grateful to my course mate Margret Oteyo and Sammy Kinuthia for their cooperation in running the laboratory experiments and assays.

Finally, I am thankful to my mother Rose Akoth, father Nathaniel Swaya, and my wife Belieze Atieno for their emotional support and encouragement that made this work a success.

May God shower you with His blessings and give you good life.

Chapter One

1.0 Introduction

1.1 Background Information

Recent global trends show that cancer is considered the second cause of death worldwide after human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) (Anand et al., 2008). Cancer is an abnormal growth of cells. In the Lake Victoria basin various studies shows that incidences of cancer are on the increase some of which are of viral etiology such as Kaposi's sarcoma, Burkett lymphoma, and cervical cancer while others are as result of environmental pollution, and changes in lifestyle. High poverty rate, late detection of cancers, and lack of modern inexpensive medicines for needy population in the Lake Victoria basin has resulted into malnutrition and increased use of cheap, alternative medicine which is more affordable than the current therapeutics in the management of cancers and catastrophic illnesses (Lamorde et al., 2010). The high poverty level in these communities has made them unable to afford essential foods that provide adequate micronutrients, proteins, vitamins and carbohydrates to boost their immunity.

The application of ethnomedicines to manage cancers has recently gained public and scientific attention majorly due to the low selectivity of current chemotherapeutic agents, late detection of cancers, different levels of cytotoxicity and rapid emergence of drug resistance to known anti-cancer agents (Joshi et al., 2002). Plants have unlimited capacity to produce secondary metabolites which possess many biological activities since they serve as either protective agents against various pathogens (eg viruses, fungi, and bacteria) or growth regulatory molecules (eg hormone like substances) that stimulate or inhibit cell division and morphogenesis (Cragg and Newman, 2005).The physiological effects of these extracts make some of them sources of therapeutic agents and potentially anti-cancerous agents, due to either their direct cytotoxicity on cancer cells or modulation of tumor initiation, promotion, or inhibition.

Plants and other natural products present a large repertoire from which novel anti-proliferative compounds with low cytotoxicity effect can be obtained. Most of these promising naturally derived anti-cancer compounds are flavonoids, coumarins, diterpenes, triterpenes, biflavonoids, coumarins, caffeic acid tetramers, hypericin, gallotannins, galloylquinic acids, curcumins, saponins,and alkaloids. These are believed to inhibit various steps in the cell cycle and induce apoptosis. Some examples of natural anti-cancers products include the taxoid compounds (docetaxel and paclitaxel) obtained from Pacific Yew, Taxus brevifolia (Walker and Croteau, 2001), Camphtotecin derivatives (irinotecan and topotecan) obtained from Camptotheca acuminate (Mattos et al., 2001), Vinblastine and Vincristine obtained from Catharanthus roseus (Schnkel et al.,

1999). Antitumor area has the greatest impact of plant derived drugs where drugs like vinblastine, vincristine, taxol and camptothecin have improved the chemotherapy of some cancers (Newmann et al., 2002). Therefore further studies are needed to discover and develop more efficient drugs with acceptable safety levels. Several natural products, mostly of plant origin within the Lake Victoria basin have been shown to possess promising activities that could be used in the prevention and/or amelioration of the disease. Many of these have other medicinal values as well, which afford them further prospective as novel leads for the development of anti-tumors. The cell culture antiproliferative testing is a valuable and inexpensive approach for anticancer agents from plants.

Cytotoxicity of plant extract should be evaluated before their impact in drug discovery and development are taken into consideration (Lall and Mayer, 2000). Many plant extracts and isolated compounds have been tested in vitro for cytotoxicity and antiproliferative activities by using different cell lines (prostrate, stomach, liver, colon etc.) as well as animal cells such as monkey kidney cells (Don et al., 2006; Lamidi et al., 2005, Al-Fatimi et al.,2005, Jo et al 2005). Thus, cytotoxicity screening models provide important preliminary data to help select plant extracts with potential anti-tumor properties for future studies (Cardellina et al., 1999).

Assessing proliferation, cell cycle arrest and apoptotic end-points in established cell line could be first steps for anticancer drug discovery and development. This study aimed at screening for the preliminary phytochemical and anti-proliferative actions of methanolic extracts of four-ethnobotanically selected medicinal plants commonly used by the traditional practitioners within Lake Victoria basin against established lung adenocarcinoma cell lines.

1.2 Statement of the Problem

The urgent need for new anti - cancer drugs is a global concern. Currently over a hundred types of cancer are known, differentiated by etiology, history, pathology and metastasis. In spite of availability of therapies, cancer treatment still poses serious challenge especially in developing countries. In addition to obvious economical and commercial hurdles, cancer patients are faced with multifarious difficulties associated with the currently approved therapeutic strategies such as late detection, the emergence of drug resistance, bone marrow function inhibition, nausea, vomiting, and alopecia. The currently FDA approved anti-cancer drugs include alkylating agents (cisplatin), antimetabolites (Methotrexate, cytarabine), anti-microtubules (paclitaxel), topoisomerase inhibitors (irinotecan, mitoxantrone) and cytotoxic antibiotics (doxorubicin). This array of drugs has made the prognosis of cancers manageable. However, the use of these drugs have been relatively limited by their toxicity, drug resistance development, low bioavailability and as well as emergence of cancers associated with viral infections. These issues along with side effects and poor tolerability of these drugs make it apparent that new anti-cancer drugs with high selectivity against only malignant cells, ability to repress tumor metastasis, better efficacy, affordability, acceptable toxicity and resistance profiles and, more importantly, with novel mechanisms of action are required in search of alternative strategies of managing cancers. One of the strategies has been to identify anti- cancer compounds from natural sources by evaluating the anti-proliferative activities of ethno botanically selected medicinal plants.

1.3 Objectives

1.3.1 General objectives

To assess the in vitro anti- proliferative activities of selected medicinal plants extracts used by traditional health practitioners around the Lake Victoria basin of Kenya.

1.3.2 Specific objectives

1. To conduct qualitative phytochemical analyses of four selected medical plants used for the management of diseases in the Lake Victoria Basin of Kenya.
2. To determine anti-proliferative activities of selected medicinal plant extracts on Lung adenocarcinoma cell lines.

1.4 Research Question

The questions are;

1. What are the phytochemical components of the extracts from the selected medicinal plant?
2. What are the anti-proliferative activities of the selected medicinal plant extracts on cancer cells?

1.5 Significance of the Study

Cancer is one of the leading cause of death worldwide and more specifically in the Lake Victoria basin. Traditional healers are the major source of knowledge on the medicinal plants that are currently being used in the management of enlarged body tissues and cancers in Africa. The findings of this research shows the importance of natural plant products used by traditional healers in management of diseases/ illnesses in the Lake Victoria Basin. Moreover, it has addressed the question of whether methanolic extracts of the four selected medicinal plants have phytochemicals that possesses selective mediated suppression of cell viability. Hence knowledge obtained from the study may lead to further activity guided fractionation and spectroscopic analysis of extracts with the view of developing of an active chemotherapeutic analog.

1.6 Study Limitations

The study was limited to; ethnobotanical surveys, conducted by VicRes team, which are majorly, based on the traditional knowledge of plants medicinal values without differential diagnosis. The study also focused on phytochemical screening, which only gives gross ideas of the qualitative components of the extracts without activity guided purification and characterizing the lead compound. In addition, the antiproliferative cell culture based assays is a single cell system as compared to the multi-cellular and multi-organ system in the in vivo assays, hence limited to biotransformation activities and effects in the systemic circulation.

1.7 Study Justification

Morbidity and mortality that results from cancer is still unacceptably high and is becoming a growing public health problem in the 21st century. Most of the drugs in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects. Hence there has been need for alternative drugs that are endowed with fewer side effects and more potent in their mechanism of actions. Moreover, studies on anti-tumor inhibiting compounds originating from plants have yielded an impressive array of novel compounds. This is the case with the powerful anti-leukemial drugs vinblastin and vincristine isolated from the native Madagascar plant, Catharanthus roseus and the podophyllins isolated from the roots of the mayapple, Podophyllum peltatum. An anti-proliferative activity of plant extract against human cell lines is novel and work along this line is highly correlated with plants use against a wide variety of illnesses. However, the anti-proliferative action of the four medicinal plant extracts; Piptadiniastrum africanum, Kigelia africana, Centella asciatica and Chaemocrista nigricans used for the management of illnesses within the Lake Victoria basin (LVB) is not yet researched on. Similarly, despite the exceptional biodiversity of Africa, few scientific studies have been carried out in the continent regarding the anti-proliferative properties of medicinal plants in comparison to their antimicrobial effects.

The present study was designed to evaluate the in vitro anti-proliferative assays of the four ethno botanically selected medicinal plant extracts on the lung adenocarcinoma cell lines and to contribute to development of anti-cancer agents.

Chapter Two

2.0. Literature Review

2.1 Cancer and Neoplasm

Cancer is an abnormal growth of cells caused by multiple changes in gene expression leading to dysregulated balance of cellular proliferation and differentiation. This ultimately evolves into a population of cells that can invade tissues and metastases to distant sites causing significant morbidity and mortality if untreated (Hanahan et al., 2000). It is caused by the interaction between genetic and environmental factors (Hugdson, 2008) leading to accumulations of genetic mutations in oncogenes

(genes that promote cancer) and tumor suppressor genes (genes that help to prevent cancer), which gives cancer cells their malignant characteristics, such as uncontrolled growth (Parera, 1997).

Malignant tumor is characterized by abnormal cell growth with potential to invade or spread to other parts of the body (WHO, 2014). Not all tumors are cancerous; the characteristics that delineate a malignant cancer from a benign tumor are the ability to invade locally, spread to regional lymph nodes and to metastases to distant organs in the body. The possible signs include a new lump, abnormal bleeding, a prolonged cough, unexplained weight loss, and change in the bowel movement (NHS choices, 2014). As the cancerous growth progresses, genetic drift in the cell population produces cell heterogeneity in such characteristics as cell antigenicity, invasiveness, metastatic potential, rate of cell proliferation, differentiation state, and response to chemotherapeutic agents (WHO, 2014).

The major causes of cancer are tobacco use, obesity, poor diet, lack of physical activity, infections, alcohol drinking, exposure to ionizing radiation and environmental pollutants (WHO, 2014). Many cancers can be prevented by not smoking, eating more vegetables, fruits and whole grains, eating less meat and refined carbohydrates, maintaining a healthy weight, exercising, and being vaccinated against certain infectious diseases. (Anand et al., 2008 and Kushi et al., 2014). Most chemotherapeutic drugs work by impairing mitosis, effectively targeting fast dividing cells. They prevent mitosis by various mechanisms including damaging DNA, inhibition of cellular machinery involved in cell division (Kehe et al., 2009) and inducing apoptosis (Makin et al., 2000). Therefore, inhibitors to accumulation of genetic materials or conferment of resistance to the interaction between genetic susceptibility and environmental factors by agents obtained from plant extracts can results in cessation of tumourigenesis. Moreover plant has served as the basis of valuable materials for drug discovery and development in all facets of life.

2.2 Characteristics of Neoplastic Cell

Cancer cells show cellular and nuclear pleomorphism, abnormal arrangement of cells, develops changes in the cell membranes and organelles, exhibit abnormal mitoses and chromosomal abnormalities. There is increased motility of malignant cells which may be associated with increased amounts of contractile proteins in their microfilaments, with loss of contact inhibition (probably caused by alteration in calcium ion concentration in the malignant cell membrane). Interrupted cellular adhesiveness (for the solid surface) and contact inhibition (among cells) results from changes in the cell surface glycoproteins and the poorly developed tight junctions and desmosomes in malignant cells. Changes in motility, adhesiveness and contact inhibition promotes invasion and subsequent establishment of secondary malignant growth – metastasis.

Cancer cells exhibit differences in metabolism as compared to normal cells. The metabolism of malignant cells is usually more anaerobic than that of normal non-rapidly dividing cells and is greatly accelerated. Malignant cells may be able to withstand hypoxic conditions. They may have increased glucose and amino acid uptake. These cells have high levels of hexokinase increasing their glucose utilization. The cancer cells loss capabilities to synthesize specialized proteins typical for differentiated cells. Enzymes and other proteins produced by cancer cells are needed for the tumor growth (Mladosievicova, 2007). Agents that have potential of preventing metastasis of neoplasm and can inhibit angiogenesis form the basis of future chemotherapeutic agents.

2.3 Cancer Drugs in Clinical Use

2.3.1 Anti-cancer drugs

Chemotherapy is the use of drugs to inhibit or kill proliferating cancer cells. Chemotherapy may also harm cells that divide rapidly under normal circumstances such as cells in the bone marrow, digestive tract, and hair follicles. This results in the most common side-effects of chemotherapy. Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells. Apoptotic induction has been a target for innovative mechanism-based drug discovery. Resistance to programmed cell death (apoptosis) is an integral part of cancer cell

development, and reestablishment of control of apoptosis is a known target mechanism for anticancer drugs (Gibb et al., 1997; Joshi et al., 2002). Certain products from plants are known to induce apoptosis in neoplastic cells, but not in normal cells, which would be the ideal characteristic of a successful anticancer drug (Hirano et al., 1995). Plant derived compounds comprise a diverse group with different mechanisms of action, but ultimately seem to have the ability to induce apoptosis (Tharapadar et al., 2001). For example, alkylating agents, such as cisplatin (Gibb et al., 1997), act directly on the DNA by cross-linking the guanine nucleobases and thus hindering the strands of DNA to uncoil and separate and therefore making replication impossible. This in turn, triggers apoptosis of the cells. Hence there is need to explore the potential of new anticancer compounds from medicinal plants with low harm in anticancer drug discovery and development.

2.3.2 Mechanism of antineoplatic drugs

Most chemotherapeutic drugs prevent mitosis by various mechanisms including damaging DNA, inhibition of DNA duplication, inhibition of nucleic acid and protein synthesis, interfering with hormone balance and inhibition of the cellular machinery involved in cell division. The anticancer agents include;

Alkylating agents: These binds covalently to DNA via their alkyl group. This follows either cross linking of the two DNA strands preventing replication or DNA damage leading to apoptosis (Siddik, 2005 and Damia et al., 1998). They are phase nonspecific and kills rapidly proliferating cells and non-proliferating cells. Examples are Mechlorethamine used for the treatment of non-Hodgkin lymphoma and cyclophosphamide. However because this drugs damage DNA they can cause long term damage to the bone marrow, loss of hair and in rare cases leads to the acute leukemia.

Anti-metabolites: are a group of molecules that inhibit DNA and RNA synthesis. Anti-metabolites resemble either nucleobases or nucleosides (a nucleotide without the phosphate group), but have altered chemical groups (Parker, 2008). These drugs exert their effect by either blocking the enzymes required for DNA synthesis or becoming incorporated into DNA or RNA. By inhibiting the enzymes involved in DNA synthesis, they prevent mitosis because the DNA cannot duplicate itself. This leads to DNA damage inducing apoptosis. Subtypes of the anti-metabolites are the anti-folates, fluoropyrimidines, deoxynucleoside analogues and thiopurines. Example is 5-flourouracil which is metabolized to its deoxynucleotide form, 5- Fluorodeoxyuridine, to inhibit the enzyme, thymidilate synthatase which is involved in the methylation of deoxyuridycilic acid to thymidylic acid; arabinosylcytosine blocks the reduction of cytidylic to deoxycytiylic acid inhibiting DNA replication. 6-mercaptopurine and 6-thioguanine prevent purine biosynthesis and interconversion of the purine bases (David A. and Karnofsky M, 1968).

Topoisomerase inhibitors: These affect the activity of two enzymes: topoisomerase I and topoisomerase II hence interfering with the DNA replication and transcription. Inhibition of these enzymes causes DNA strand breaks, and leads to apoptosis. These agents include etoposide, doxorubicin, mitoxantrone and teniposide (Lodish et al., 2000). The second groups, catalytic inhibitors, are drugs that block the activity of topoisomerase II, and therefore prevent DNA synthesis and translation because the DNA cannot unwind properly. This group includes novobiocin, merbarone, and aclarubicin, which also have other significant mechanisms of action. Treatment with topoisomerase inhibitor increases the risk of second cancer, acute myelogenous leukemia (Nitiss, 2009).

Natural products: Anti-microtubule agents are plant-derived chemicals that block cell division by preventing microtubule function. Vinca alkaloids, notably vincristine and vinblastine, derived from Catharanthus roseus (formerly known as Vinca rosea) bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules. The original vinca alkaloids are completely natural chemicals that include vincristine and vinblastine. Following the success of these drugs, semi-synthetic vinca alkaloids were produced: vinorelbine, vindesine, and vinflunine (Yue et al., 2010). The vinca alkaloids prevent the formation of the microtubules, whereas the taxanes prevent the microtubule disassembly. By doing so, they prevent the cancer cells from

completing mitosis. Following this, cell cycle arrest occurs, which induces apoptosis (Lind, 2008). Further studies are needed to unveil potential inhibitors of proto-oncogene mutations from plant sources to help curb the challenges posed by cancer treatments.

2.3.3. Challenges in use of current anti-cancer drugs

The major problem in cancer chemotherapy is the development of multi drug resistance (MDR) against anti-cancer drugs. It has been discovered that drug resistance in cancer cells results from elevated expression of certain proteins, such as cell membrane ATP-binding cassette transporters (Choi, 2005), which can result in an increased efflux of the cytotoxic drugs from the cancer cells (Ambudkar et al., 1999; Thomas & Coley, 2003). The problems of acquired drug resistance may be circumvented by targeting endothelial cells associated to the tumor instead of the tumor cells themselves (Boehm et al., 1997). Endothelial cells are genetically more stable and thus are less likely to develop MDR compared to cancer cells, hence targeted. The process of angiogenesis is an attractive target of cancer therapy since tumors are dependent on a functioning vascular system, and metastasis is dependent on the formation of new vessels around the cell foci (Griggs et al., 2001). Also already established tumor vasculature is a good target for anticancer therapy since it differs from normal vasculature in its permeability, the absence of vascular smooth muscle cells and lymphatic drainage (Matsumura & Maeda, 1986). There are a number of promising anti-angiogenic and antivascular agents, some of them originating from higher plants, and are undergoing clinical trials. Among them the stilbene combretastatin-A4, originally isolated from the South African tree Combretum caffrum (Pettit et al., 1987, 1988, 1989 and 1999).

Resistance is a major cause of treatment failure in chemotherapeutic drugs. There are a few possible causes of resistance in cancer, one of which is the presence of small pumps, p-glycoprotein, on the surface of cancer cells that actively move chemotherapy from inside the cell to the outside (Goldman, 2003; Crowley et al., 2009). Cancer cells can also cause defects in the cellular pathways of apoptosis (Luqmani , 2005) thereby resulting to resistance.

The blood brain barrier poses a difficult obstacle to passage of chemotherapeutic drug to the brain. Only small lipophilic alkylating agents such as lomustine or temozolomide are able to cross this blood brain barrier (Deeken et al., 2007 and Gerstner et al., 2007). Therefore, plants which contain alkylating agents as their major phytochemical components can be sourced to overcome blood brain barrier obstructions.

Chemotherapeutic techniques have a range of side-effects that depend on the type of medications used. This includes immune suppression, nausea, alopecia, teratogenicity and infertility (Chen and Clerck , 2009).

These challenges coupled with high mortality rate of cancer necessitate the exploration of new agents to succumb tumourigenesis.

2.4. The Importance of Traditional Medicine in Different African Countries

Traditional medicine is an important part of the health-care system in most of the African countries. About 80 – 90 % of the populations in African countries are dependent on traditional medicine for their primary health care (Hostettman et al., 2000). The rising cost of Western medicine means that the people in African countries are increasingly turning to traditional medicine as an affordable alternative. In Nigeria, traditional medicine is well acknowledged and established as a viable profession (Kafaru, 1994), and almost all plants seem to have some kind of application in traditional medicine (Babayi et al., 2004). In many countries in Africa medicinal plants are now being used for the treatment and alleviation of the symptoms of various diseases including HIV infection (Morris, 2002) and malaria (Njoroge & Bussman, 2006). These traditional methods are sometimes claimed to give fewer side effects than conventional antiretroviral therapy (Morris, 2002). Gradually, traditional medicinal practitioners (TMP) are being officially accepted as part of African health services and their medical knowledge is finding its place in hospitals and clinics (Neuwinger, 2000). The abundance of information on the traditional medicinal

uses of plants in Africa is in danger of disappearing since the knowledge of how to use medicinal plants is mostly passed down orally and even to date is poorly documented (Gurib-Fakim, 2006).

It is rare to find healers around Lake Victoria basin with written documents, apart from minor memory aids as to plant characteristics, which help to find medicinal plants similar in appearance but different in healing effects. This oral transfer of knowledge is vulnerable to disruption and interference and may result in the loss and distortion of valuable ethno medical information. Therefore there is need for identification and documentation of medicinal plants for future research and referencing

2.5 Traditional Medicines with Anti-Cancer Properties.

Natural products are important templates for chemotherapeutic agents. Since 1961, nine plant-derived compounds have been approved for use as anticancer drugs in the United States; vinblastine (Velban), vincristine (Oncovin), navelbine (vinorelbine), etoposide (VP-16), teniposide (VM-26), taxol (paclitaxel), taxotere (docetaxel), topotecan (Hycamtin) and irinotecan (Camptosar) (Lee, 1999).

The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among Food and Drug Administration (FDA) approved anticancer and anti-infectious preparations, drugs of natural origin have a share of 60% and 75% respectively. It is worthy to mention the vivid current interest in discovery of natural drugs for cancer treatment and chemoprevention (Balunas et al., 2005). Medicinal plants either through systematic screening programs or by serendipity possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures. Hence, despite the advantages of the synthetic and combinatorial chemistry as well as molecular modeling, medicinal plants remain an important source of new drugs, new drug leads and new chemical entities (Newman et al., 2000). According to Gilani and Atta-ur-Rahman (2005),"the use of plants, plants extracts or plant derived pure chemicals to treat diseases is a therapeutic modality, which has withstood the test of time." The search for biological active agents from plants is part of a wider resurgence of scientific interest to produce new chemotherapeutics. Plants synthesize very complex molecules with specific stereochemistry and can show biological activity with novel mode of action (Houghton, 2005). These complex materials are called secondary metabolites or phytochemicals (Akinmoladun et al., 2007). It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidants effect (Farombi et al., 1988). Antioxidants protect other molecules (in vivo) from oxidation when they are exposed to free radicals and oxygen species which have been implicated in the etiology of many diseases and in food deterioration and spoilage species (Kasaikina, 1997; Koleva et al., 2000).

Large populations in L. Victoria basin rely on herbal medicine to cub illness. Herbal medicine have greatly been used and accepted by the pharmacological bodies in the treatment of various ailments (Mander, 1998). Despite the rich L. Victoria repertoire from which to select medicinal plants, the cytotoxicity and anti-proliferative activity of traditional herbal medicines are still not well-researched, and African knowledge of herbal remedies used to manage cancer is scanty and not well documented. Natural products can be selected for biological screening based on ethnomedical use, random collection and a chemotaxonomic approach i.e. screening of species of the same botanical family for similar compounds (WHO, 1989).

Numerous plants are available in nature which has anticancer properties and majority of them are still to be examined through screening against established cell lines. Compounds from medicinal plants may not serve as final drug but they comprise of lead compounds for the development of potential anticancer agents.

2.6 The Selected Medicinal Plants

2.6.1 Piptadeniastrum africanum

Piptadeniastrum africanum is a large buttress tree called variously as:" Kiryar Kurmi" in Hausa, "Ofie" in Igbo and "agboin or agboyin" in Yoruba

(Hutchinson and Daiziel, 1963). The leguminosae are mostly tropical and subtropical trees and shrub comprising about 40 general and 2000 species. The tree sprouts freeing from the stump. The sapwood when fresh is pale reddish – yellow or pinkish – white and comparatively wide (Tatiana, 2008). It is a strong, stiff wood, of moderate toughness and hardness of good appearance when polished and varnished. People use the timber for strong canoes, planks and building timbers, carpentry and furniture. In Nigeria, the root and the bark are prepared for use as enema, the bark chipped off for antimalarial use and an infusion of the bark is used to relieve toothache (Hutchinson and Dalziel, 1963). The root of this plant has been reported by (Mengome et al., 2009) to be used against human colonic cancer cell. Recent phytochemical studies on the root of Piptadeniastrum africanum revealed the presence of tannis, flavones, alkaloids, steroids, terpenoids, saponins and glycosides among others (Mengome et al., 2009). The results are encouraging enough to pursue bioactivity guided fractionation of this extracts and structural elucidation of the phytoconstituents from the extract of the plant species as possible antibacterial agents. Further work is necessary to ascertain the clinical safety of extract from plants (Effraim et al., 2001) and to determine appropriate concentration for the therapy so as to safeguard the health of the teeming mass of user who more often than not does not take these factors into consideration. No data shows the anticancer property of the methanolic bark extract of this plant within the L. Victoria basin.

2.6.2 Kigelia africana

Kigelia africana belongs to the family Bignoniaceae. The plant is widely distributed throughout the African tropics. It is also found in China, South East Asia, Australia, Hawahii and South and Central America (Grace and Davies, 2002). Kigelia africana is traditionally used in African folk medicine for treatment of various clinical conditions which include; malaria, dysentery, constipation, cough and kidney disorders. Analgesic and anti-inflammatory activities of orally administered extracts of the stem bark of K. Africana in mice and guinea pigs have been reported (Owalabi and Omogabi, 2007). The powdered fruit is sprinkled on wounds and sores as topical dressings for their antibacterial and antifungal properties (Grace and Davies, 2002). Verminoside and verbascoside are the major constituents of K. africana, plant used in African folk medicine for its emollient, anti-eczema and anti-psoriasis properties. Remedies from root bark are also used for the treatment of venereal diseases and naphtoquinones extracted from K. africana showed cytotoxic activity against melanoma and renal carcinoma cells (Houghton et al., 1994). No data as well shows the anticancer property of the methanolic fruit extract this plant within the L. Victoria basin.

2.6.3 Centella asciatica

Centella asciatica or “pegaga” is well known for its ability in promoting wound healing. Centella asciatica belongs to the Umbelliferae family. In India it is also known as Gotu kola or pennywort in English. It originates from Asia (India, Sri Lanka) and East Africa widespread to South America, West Indies and South East Asia like Malaysia, Pakistan, Japan, China, and Australia. It is commonly found in swampy areas of India, as a weed crop in fields and other waste places throughout India up to an altitude of 600 m (Dastur, 1962). Centella contains several active constituents, of which the most important are the triterpenoid saponins, including asiaticoside, centelloside, madecassoside, and asiatic acid. Centella asciatica has been reported to possess antiulcer (Asakawa et al., 1982; Gohil et al., 2010), anti-inflammatory (Guo et al., 2004; George et al., 2009; Huang et al., 2011), immunomodulating (Punturee et al., 2005), antitumor (Babu et al., 1995), antibacterial (Zaidan et al., 2005), antioxidant (Gupta & Flora, 2006), and antigenotoxic (Siddique et al., 2007) properties. Besides, C. asciatica could act as cardio protective agent that can enhance myocardial antioxidants and thus prevents the extent of cardiac damage (Gnanapragasam et al., 2004).

Wound and ulcer healing are enhanced by the promotion of fibroblast proliferation and collagen synthesis in response to topical treatment with extracts of C. asciatica herb (Maquart et al., 1990; Maquart et al., 1999). Centella asciatica increases the production of basic fibroblast growth factor (bFGF), induce angiogenesis and cell proliferation, therefore it promotes wound healing activity (Shukla

et al., 1999a, 1999b; Cheng et al., 2004; Gohil et al., 2005). Asiaticoside enhance the burn wound healing at low doses by promoting angiogenesis during skin wound repair (Kimura et al., 2008). It is used for the treatment of psoriasis and found to be effective in destroying cultured cancer cells (Maquart et al., 1990). Likewise, Yu et al (2006) also found that the extract of the whole plant has a strong anti-cancer activity. In Brazil, C. asciatica is used to treat the uterine cancer (Yoshida et al., 2005). The presence of several bioactive components in the extract may possess anticancer activity. Previous studies reported that C. asciatica extract possess antipsoriatic effect due to an inhibition of keratinocyte proliferation by its constituent triterpenoid glycosides (Sampson et al., 2001). Coldren et al. (2003) found the anti-proliferation effect of C. asciatica extract on fibroblast cells. Recent study by Babykutty et al. (2009) shows that C. asciatica extracts induced apoptosis on human breast cancer cells. The C. asciatica aqueous extract demonstrated an inhibitory effect towards the proliferation activities of human respiratory epithelial cells (Mutua et al., 2013). No data as well shows the anticancer property of methanolic leaf extract this plant within the L. Victoria basin.

2.6.4 Chaemacrista nigricans

Chaemacrista nigricans belongs to family Fabaceae-ceasalpinioideae. Chaemacrista nigricans grows wild in tropical Africa, western Asia, and India. Its leaves are used by traditional healers in treatment of measles, inflammation, haemorrhoids, peptic ulcers, headache, meningitis, cough, itching, stomachache, diarrhea, worms, and malaria. The in vitro test on the leaves extracts of Chamaecrista nigricans showed significant action against Herpes simplex (Schmelzer and Gurib-Fakim, 2008). Chamaecrista nigricans leaves, and roots show interesting pharmacological actions, hence they warrant further research on the medicinal actions (Schmelzer and Gurib-Fakim, 2008).

However, there is no information on the anticancer properties of these plants against lung adenocarcinoma cell lines within Lake Victoria basin. The present study therefore attempted to assess the antineoplastic properties and phytochemical analysis of these plant extracts' on established lung adenocarcinoma cell line.

2.7 Anti-Proliferative Activity

Antiproliferative activities is monitored using the 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) or MTS assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTS reagent to a colored formazan product. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In the US National Cancer Institute plant screening program, a crude extract is generally considered to have in vitro cytotoxic activity if the IC_{50} value (concentration that causes a 50% cell kill) in carcinoma cells, following incubation between 48 and 72 hours, is less than 20ug/ml, while it is less than 4 ug/ml for pure compounds (Boik J, 2001). Screenings of medicinal plants used as anticancer drugs have provided modern medicine with effective cytotoxic pharmaceuticals. The diversity of the biosynthetic pathways in plants has provided a variety of lead structures that have been used in drug discovery and development. In this last decade, investigations on natural compounds have been particularly successful in the field of anticancer drug research. Early examples of anticancer agents developed from higher plants are the antileukemic alkaloids (vinblastine and vincristine), which were both obtained from the Madagascar periwinkle (Catharanthus roseus).

2.8 Cytotoxicity

Most anti-cancer drugs are designed to eliminate rapidly proliferating cancerous cells, and therefore, they typically show cytotoxicity and induce apoptosis in cancer cells (Kaufmann & Earnshaw, 2000). Apoptosis is a highly organized cell death process characterized by loss of plasma membrane phospholipid asymmetry, enzymatic cleavage of the DNA into oligonucleosomal fragments, and segmentation of the cells into membrane-bound apoptotic bodies. The main action of anticancer agents is by triggering the apoptotic pathway. However, intrinsic alterations in the apoptotic pathway are a hallmark of cancer cells and are considered to be a major cause of drug efficacy (Waxman and Schwartz, 2003; Shkreta et al., 2008). Cells undergoing necrosis

typically exhibit rapid swelling, lose membrane integrity, shut down metabolism and release their contents into the environment. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components (Riss et al., 2004).

Chapter Three

3.0 Materials And Methods

3.1. Study Area

The priotized medicinal plants for this study were selected from an ethnobotanical and ethnopharmacological excursion conducted by Aduma and Tabuti within the Lake Victoria Basin region under the VicRes project. Lake Victoria occupies a shallow depression in Africa and has a maximum depth of 84 m (276 ft) and an average depth of 40 m, 130 ft (UN, 1999). Its catchment area covers 184,000 square kilometers (71,040 sq mi). The lake has a shoreline of 4,828 km (3,000 mi), with islands constituting 3.7% of this length and is divided among three countries: Kenya (6% or 4,100 km^2 or 1,600 sq mi), Uganda (45% or 31,000 km^2 or 12,000 sq mi) and Tanzania (49% or 33,700 km^2 or 13,000 sq mi) (Prado et al., 1991). This region has high prevalence of HIV and AIDS defining cancers as well as availability of many traditional healers who use medicinal plants in management of HIV, cancers and other infections. The Lake Victoria basin covers several districts and counties in the five partner states of the East African Community. These states include Burundi, Kenya Rwanda, Tanzania and Uganda.

3.2. Study Design

The selection of medicinal plants was a cross-sectional study of key informant of the people in the region which use medicinal plants in management of cancer associated with HIV and AIDS infection and other illnesses. The participants were made to recall and recap the plants used, part of the plant used, and preparation of the plant and the correct quantity of the concoction administered to the patient. This knowledge was used to collect the plants for evaluation of phytochemical profiles and anti-proliferative activities of the plant extracts in the laboratory.

3.3 Plant Material, Extract Preparation, Fractionation and Formulation

Four plants; Piptadiniastrum africanum, Kigelia africana, Centella asciatica and Chaemocrista nigricans, materials were collected from different parts in the Lake Victoria basin following ethnobotanical survey and taxonomy. These plants had been ethnopharmacologically and ethnobotanically selected in project under the Lake Victoria Research initiative (VicRes) which focused on value chain analysis and development of plant derived medicinal products for the management of HIV/AIDS in the face of climate change in the Lake Victoria basin.

3.3.1 Extraction Using Methanol

About 2Kg of the bark of Piptadiniastrum africanum, 1Kg leaves of Centella asciatica and Chaemocrista nigricans, and 2kg of the fruit of Kigelia africana, was dried and extracted with 200 mL of 95% methanol using warring blender for eight successions. Each of the extracts was filtered and concentrated by rotary evaporator at room temperature.

3.3.2 Phytochemical screening for the crude extracts

The qualitative determination methods of phytochemical constituents as described by Trease and Evans (1983); Harbourne (1983) and Sofowora (1993) to test for alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates, proteins, anthraquinones, coumarins, glycosides and saponins was used for the phytochemical screening of the four selected medicinal plants in this study.

1. Test for proteins (**Millon's test**): 0.5g of crude extract was mixed with 2ml of Millon's reagent, and the appearance of a white precipitate which turns red upon gentle heating confirmed the presence of proteins.

Test for proteins (**Biuret test**): 0.5g of extract was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution, and observed for the formation of violet/pink colour as a positive result for the presence of proteins

2. Four test methods for carbohydrates were used;

Molisch's test: Crude extract was mixed with 2ml of Molisch's reagent and the mixture shaken properly. After that, 2ml of concentrated sulfuric acid (H_2SO_4) was then poured carefully along the side of the test tube. Appearance of a violet ring at the interphase showed the presence of carbohydrate.

Fehling's test: Equal volume of Fehling A and Fehling B reagents was mixed and 2ml of it is added to crude extract and gently boiled. A brick red precipitate that appeared at the bottom of the test tube indicated the presence of reducing sugars. (Fehling's "A" uses 7 g $CuSO_4.5H_2O$ dissolved in distilled water containing 2 drops of dilute sulfuric acid. Fehling's "B" uses 35g of potassium tartrate and 12g of NaOH in 100 ml of distilled water).

Benedict's test: Crude extract was mixed with 2ml of Benedict's reagent and boiled; a reddish brown precipitate observed indicated the presence of carbohydrates.

Iodine test: Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of carbohydrate.

3. Test for phenols and tannins: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

4. Test for flavonoids (Ferric chloride test): 0.5g of the extract was treated with few drops of ferric chloride solution. Formation for blackish red colour indicates the presence of flavonoids

5. Alkaline reagent test: 0.5 g of each selected plant extract was treated with 2ml of 2% sodium hydroxide solution. Increase in the intensity of yellow color which becomes colorless on addition of few drops of dilute hydrochloric acid, indicated the presence of flavonoid in each extract.

6. Test for saponins (Foam Test): The extract (0.5 g portions) was shaken with 2 ml of water. Foam produced which persisted for ten minutes indicated the presence of saponins.

7. Test for glycosides;

Salkowski's test for glycosides: Crude extract when mixed with 2ml of chloroform and then 2ml of concentrated H_2SO_4 is added carefully and shaken gently, reddish brown colour indicated the presence of steroidal ring, i.e.,glycone portion of the glycoside.

Keller-kilani test for glycosides: Crude extract when mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$, and the mixture is then poured into another test tube containing 2ml of concentrated H_2SO_4, a brown ring at the interphase indicates the presence of cardiac glycosides.

8. **Test for steroid**: Crude extract when mixed with 2ml of chloroform and concentrated H_2SO_4 is added sidewise a red colour produced in the lower chloroform layer indicates the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid are poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

9. **Test for terpenoids**: Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids. In another test, a small amount of 0.8 g of selected plant sample was taken in a test tube, then poured 10 ml of methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform was mixed in the extract of selected plant sample and 3 ml of sulphuric acid were added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

10. **Test for alkaloids**: The Crude extract was

mixed with 2ml of 1% HCl and heated gently and Mayer's and Wagner's reagents are then added to the mixture, turbidity of the resulting precipitate was an evidence for the presence of alkaloids.

11. **Test for Coumarins**: In a test tube, 1 g of each of the extracts was placed and covered with filter paper moistened with dilute sodium hydroxide (NaOH), then heated on water bath for a few minutes. The filter paper was examined under UV light, yellow fluorescence indicated the presence of coumarins.

12. **Test for anthraquinones**: 0.5 g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for bright pink or red coloration.

3.4 General Experimental Conditions and Storage of Samples

The extracted crude extracts was stored and kept free from any contamination in a restricted lockable cabinet at room temperature in the laboratory. Each of the extracts was dissolved in Dimethyl sulfoxide (DMSO) to form the preparation used for bioassays. The cell lines was stored in liquid nitrogen, and slowly thawed before being treated with the crude extracts of each of the plants. The bioassays was conducted in level II biohazard biosafety cabinet (Telster BIO II A, en-12469-200) in an ATCC certified and accredited cell culture laboratory contained with a hyper filter Air conditioner and only restricted to the principal investigators.

3.5 Cell Culture

3.5.1 Cell media

The culture cells was suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, 1% antibiotic-antimycotic mixture (10.000 U/ml K-penicillin, 10.000 µg/ml streptomycin sulphate 25 µg/ml amphotericin B) and 1% L-glutamine

3.5.2 Cell culture plate set-up

Approximately 5×10^4 cells were cultured per well in a 96-well plate. In cell culture, sterility is of importance and was maintained throughout the culturing period. All the benches and working solutions were decontaminated using 10% sodium hypochlorite prior to use. Complete culture media, comprised of 88% RPMI 1640, 5% AB+ serum, 5% Fetal Bovine Serum (FBS), 1% Penicillin-Streptomycin and 1% L-glutamine. The complete media was filter sterilized using 0.2µl filter unit and stored at 4^0C. After counting the cells, they were diluted up to 5×10^4 cells/ml in a 96-well plate and cultured in complete medium at 37^0C in 5% CO_2, in a water-jacketed incubator and examined after 24hours.

3.6 Antiproliferative Assays

3.6.1 Determination of cell number and viability.

Morphological features of the cells were examined every day under a light microscope (Olympus, Shinjuku-ku, Tokyo) and their photomicrograph recorded at every passage. The viability of cells was determined by staining the cells with trypan blue. Exponentially growing cells were harvested and counted by using a haemocytometer.

3.6.2 Assay of Anti-proliferative activity on tumor cell lines

A MTT colorimetric assay using ELISA reader spectrophotometer was utilized to determine the antiproliferative activities of the plant extracts. The activities were compared to 5-fluorouracil as anticancer positive controls. Qualitative characterization of the bioactive constituents responsible for the activity was also done through preliminary phytochemical screening.

The tumor cell line, lung adenocarcinoma, acquired from American Type Culture Collection (ATCC), was kept in Roswell Park Memorial Institute media-1640 (RPMI 1640) containing 10% fetal bovine serum (FBS), 100 mg/L streptomycin and 100 IU/ml penicillin at 37^0C in a humidified atmosphere of 5% CO_2. Cell suspensions (100 µL) were dispensed in triplicate in 96-well culture plates at optimized concentrations of ~1.0×10^5 cells/mL per cell line. After 24-hr incubation at 37^0C, 100 µL

culture medium was removed from the wells and 100 µL fresh medium containing the extracts (1000, 500, 250, 125, 50, 25, 0 µg/mL) was added to each well and incubated for another 48 hrs. Wells containing RPMI 1640 were used as the negative controls. At the end of the treatment period, the medium in each well was aspirated and replaced with 20 µL of 5 mg MTT working solution (MTT stock solution mixed with medium to attain a final concentration of 0.5 mg/mL). Briefly, MTT powder was dissolved in PBS to form an MTT stock solution (5 mg/mL). The stock solution was filter-sterilized through a 0.22 µm filter and stored at −20 ^{0}C until used. The cells were incubated at 37 ^{0}C for 4 hrs, and then the medium was aspirated and replaced with 100 µL DMSO to dissolve the formazan crystals formed. The culture plates were shaken for 5 min and the absorbance (OD) of each well was read using an enzyme-linked immunosorbent assay (ELISA) reader at 450 nm. Each test was done in triplicate and the values reported as mean values of three experiments. Growth inhibition of 50% and above was considered the criteria of activity.

3.7 Data Management and Analyses

The results were presented as means ± SEM of three independent experiments. Statistical differences among fractions of the extract were determined by one way ANOVA using Graph Pad Prism 5 (GraphPad Software Inc., San Diego, USA) to get IC_{50}. Pearson's correlation coefficient test was used to assess correlations between means. Differences were considered statistically significant at $p < 0.05$. The data was stored safely in a restricted and password computer excel document.

3.9 Ethical Considerations

Approval to carry out this study was provided by the school of graduate studies of Maseno University. Ethical approval was obtained from Maseno university ethical review committee (See Appendix 2).

Chapter Four

4.0. Results

Plants Phytochemicals	P. africanum bark	C. asciatica leaves	C. nigricans leaves	K. africana fruit
Steroids	++	+	+	++
Terpenoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Carbohydrates				
Molich's test	+	-	+	-
Fehlings test	+	+	-	-
Benedict's test	+	+	+	-
Iodine test	++	+	+	-
Tannins	++	++	++	++
Flavonoids				
Ferric chloride	+	+	-	++
Alkaline reagent	-	+	-	+
Proteins	+	+	+	-
Glycosides				
Salkowiski's test	+	+	+	+
Keller-kalani test	++	+	+	++
Anthraquinones	+	-	-	+
coumarins	-	-	-	++

Figure-1: Qualitative Phytochemical Assays. The above figure is a picture that shows the laboratory experimental assays for qualitative phytochemical analysis of the methanol extracts as was carried out in the Chemistry laboratory, Maseno.

Plants extract	IC50 ±SEM	Sig
P. africana	28. 86 ±0.143	0.079
K. africanum	26.57 ±0.127	0.069
C. asciatica	15.69 ±0.135	**0.042**
C. nigricans	8.07 ±0.125	0.055

Table-2: Antiproliferative activities of the four selected medicinal plant extract on Lung adenocarcinoma cell line determined by MTT assay

Table-2: MTT antiproliferative assays of the four selected medicinal plant extract on Lung adenocarcinoma cell line. The absorbance was read using an ELISA microplate reader at 450nm. The data indicates that P. africanum had the highest half inhibitory (IC50) attributed to its rich phytochemical constituents. C. nigricans had the lowest IC50. The differences obtained in comparison to the positive control are not statistically significant except for C.asciatica.

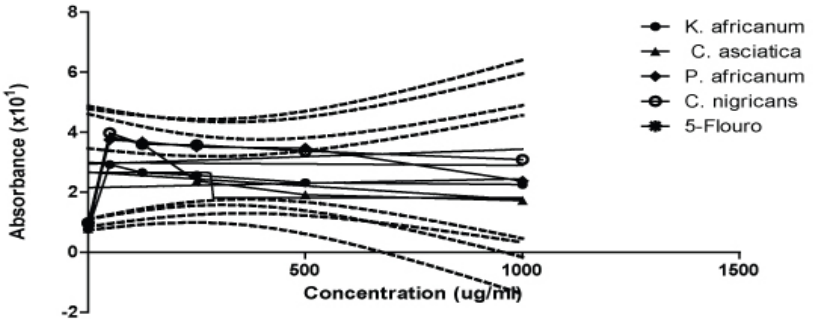


Figure 2: Non-Linear regresion curves for the antiproliferative assays of the plant extracts. The absorbance of plants extract and the control, 5- Flourouracil, were within the significant area of the curve as indicated by the dotted lines.

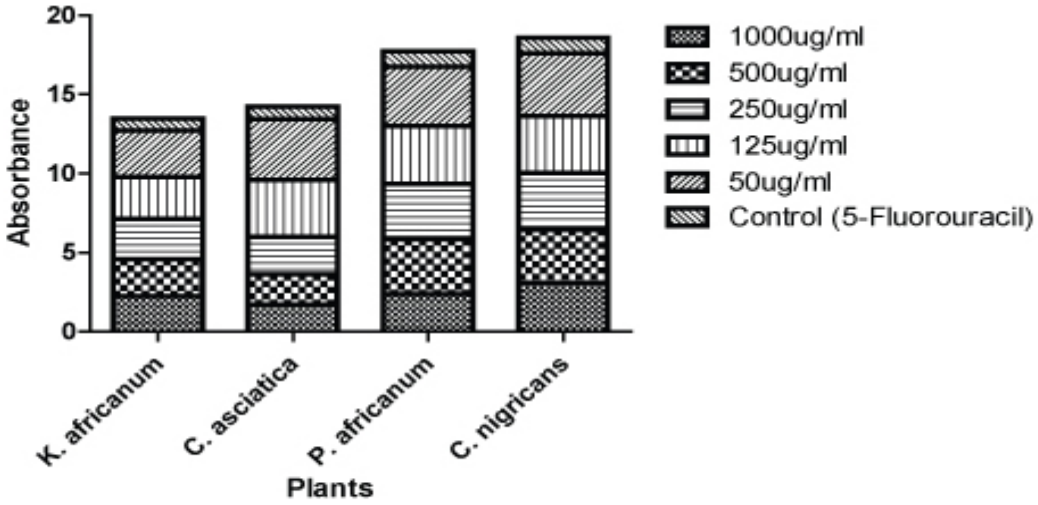
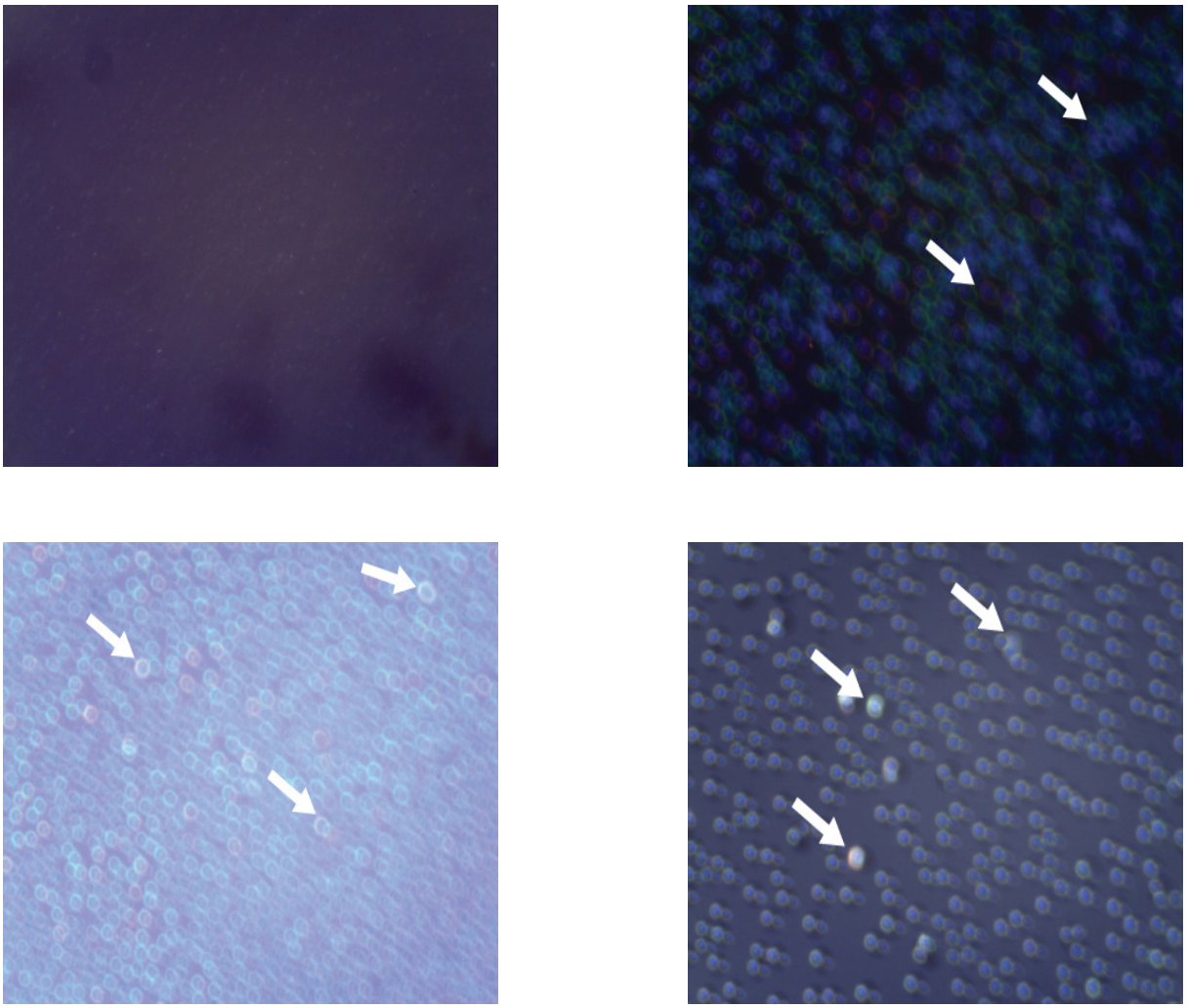


Figure 3: Graphical representation of the antiproliferative effects of the plants extracts against Lung adenocarcinoma cell line. The absorbance of each plants extract was carried out in serial dilution of the crude extract concentration. Highest absorbance was at 125ug/ml in *C. asciatica* and *P. africunum* whereas in *C. nigricans* the highest absorbance was 500ug/ml of the crude extract. This show its low activities compared to other extracts.



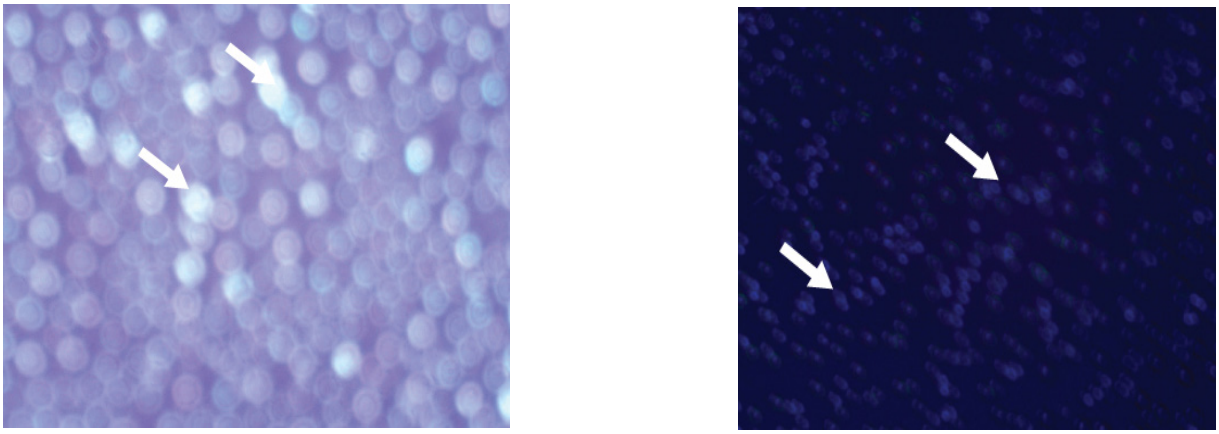


Figure-4: Morphological changes in Lung adenocarcinoma cells assayed to assess cell apoptosis. (A) Negative control; (B) Cells treated with 125µg/ml of C. nigricans methanol extract; (C) Cells treated with 125µg/ml of C. asciatica (Leaves) methanol extract. (D) Cells treated with 125µg/ml K. africana (fruits) methanol extract; (E) Cells treated with 125µg/ml of P. africanum (F) Positive control as observed under the inverted light microscope. Dark stained nuclei which indicate nuclear fragmentation, cytoplasmic blebbing and nuclear condensation (Arrows)

4.1. Phytochemical Testing.

Secondary metabolites include phenolic compounds, alkaloids, terpenoids, steroids, quinones, saponins, flavonoids, with complex structures that are known to support bioactive activities in medicinal plants extracts (Krishnaiah et al., 2009).

The screening of the selected four different plant species namely Piptadiniastrum africanum, Kigelia africana, Centella asciatica and Chaemocrista nigricans for phytochemical constituent was performed using generally accepted laboratory technique for qualitative determinations. Qualitative Phytochemical Screening, based on tests of coloration and precipitation, was carried out on methanol extract of the four selected plants as described in the methods above.

Keys: (-) Negative (Absence of turbidity, flocculation, and precipitation); (+) Weak positive (If the reactant has slight opacity); (++) test strongly positive (If the test produces a precipitate or heavy color change). Each of the extracts was tested for eleven phytochemical components as tabulated in bold above. Four comparative assays were done in testing for carbohydrates whereas two comparative tests were used for testing both Flavonoids and Glycosides.

4.2 Antiproliferative activities of the plant extracts

The antiproliferative activity of the extracts against the human lung adenocarcinoma cell line was tested using the microtitration colorimetric method of MTT reduction.

Chapter Five

5.0 Discussion

5.1 Phytochemical analysis

The phytochemical analysis was done to detect qualitatively the presence of eleven secondary metabolites in the four plants, namely Kigelia africana, Piptadiniastrum africanum, Centella asciatica, and Chaemocrista nigricans. Overally, tannins, steroids, alkaloids, glycosides, saponins and terpenoids were detected in all the four plant extracts. Flavonoids were present in C. asciatica and K. africana whereas coumarins were only present in K. africana. Anthraquinones were detected in P. africanum and K. africana. Previous studies show that the presence of these compounds contributes to their antiproliferative activity through antioxidant and free radical scavenging effects (Zakaria et al., 2011). It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidants effect (Farombi et al., 1988). The antitumor potential of plant extracts and compounds

could be attributed to their ability to induce changes in the regulation of target molecules in oncogenic signal transduction pathways implicated in cell growth, replication, apoptosis, as well as in angiogenesis, invasion and metastasis of cancer cells (Amin, et al., 2009 and Mehta, et al., 2010). The phytochemical analysis revealed the presence of many chemical groups in the extracts that could be responsible of their antioxidant, anti-cancer activity and other bioactivities.

Tannins and polyphenolic compounds are useful are useful compounds that have remarkable cancer prevention and anti-cancer activities (Aiyegoro et al., 2010). Laboratory rodent studies have shown that polyphenols have cancer-preventing properties and considered to be potential chemopreventive agents (Ramos, 2008; Lambert, et al., 2005; Fresco, et al., 2006). They can influence important cellular and molecular mechanisms associated with multiple carcinogenic steps, such as expression of key proteins in signal transduction pathways (e.g., mitogen activated protein kinases (MAPKs) or activator protein (AP)-1), the transcription factor nuclear factor-kappa B (NF-κB) and its its downstream gene products, modulation of cell-cycle regulation and induction of apoptosis (Fresco, et al., 2006), which affect cell differentiation, proliferation and apoptosis, immune responses and metabolism of carcinogens (Nichenametla, et al., 2006). Many chemopreventive agents are able to block or delay the promotion and/or progression of premalignant or malignant cells by modulating cell proliferation and/or differentiation (Sun, et al., 2004; Hail, 2005)

Several plant species rich in flavonoids are reported having disease preventive and therapeutic properties. This observation is of particular importance since flavonoids are ingredients of many vegetables and fruits and the association of vegetable and fruit consumption with reduced cancer risk has been reported (Ferguson et al., 2004 and Kinadaswami et al., 2005). Antiproliferative activity recorded in the present study is in accordance with this finding, since the phytochemical evaluation indicated the presence of flavonoids in three plant species with promising activity except in C. nigricans. Flavonoids, also among the extract constituents, have a wide range of biological activities that include antimicrobial, anti-inflammatory, anti-angionic, analgesic, and anti-allergic effects, and cytostatic and antioxidant properties. The ability of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals, and lipid peroxyl radicals, which is important for preventing diseases associated with oxidative damage of membranes, proteins, and DNA (Aiyegoro et al., 2009), highlights many of their health-promoting functions in living organisms. In the human diet, flavonoids may reduce the risk of various cancers as well as prevent menopausal symptoms. The results of this study are in accordance with this finding since the phytochemical screening showed the presence of flavonoids in all active extracts (Table 1). While the presence of alkaloids with flavonoids in P. africanum, C. asciatica and K. africana extracts may explain higher activity compared with C. nigricans extract studied in this work. Park et al., 2008 claimed that flavonoids would induce apoptosis in cancer cells. Reed and Pellecchia, (2005) work in this direction stressed on inducing apoptosis as a desired strategy of controlling cancers. Antiproliferative activities of these plants may also be ascribed to the presence of sequisterpenoids in all the four plant extracts. It is Probable that these antioxidant compounds trigger intracellular signaling pathways which induce cell death through apoptosis.

The extract of C. asciatica consists of bioactive terpene acids such as Asiatic acids, madecassic acid and their respective glycoside, asiaticoside and madecassoside which are considered as antitumor (Babu et al., 1995, Inamdar et al., 1996). Previous studies reported that C. asciatica extract possess antipsoriatic effect due to an inhibition of keratinocyte proliferation by its constituent triterpenoid glycosides (Sampson et al., 2001). It is believed that the antioxidation activity of the phenolic compound from C. asciatica can prevent certain diseases like arteriosclerosis, cancer, diabetic and arthritis (Zainol et al., 2003).

More effective anticancer drugs with high selectivity against only malignant cells and with ability to repress tumor metastasis are desired. As candidates for such drugs, cytotoxic, antitumor or anticancer natural products have been often sought, and plant components such as Vinca alkaloids, taxoids, etoposide and irinotecan are now used in

clinical treatments (Montvale, 1998).

Furthermore, the extract contained saponins, anthraquinones and coumarins which have an inhibitory effect on inflammation, hypocholesterolemia, and diabetes (Kusuma et al., 2011). Coumarins compounds might inhibit cell proliferation by interfering with mitotic spindle microtubule function (Irene, 2005. However, the leaf of C. nigricans extract did not contain anthraquinones and coumarins hence could be the reason for its low activity.

The phytochemical results obtained from these plants corroborates to the results from other previously described studies. It could also be interesting to speculate that the anticancer activities of our extracts could be due to a synergy between their various chemical constituents. This would be in agreement with the results of Rui Hai Liu in 2004, revealing that the combination of phytochemical agents exerts most of the total antioxidant activity.

5.2 Determination of Antiproliferative Activity

Cancer is a pathologic condition where the normal mechanisms of cell cycle regulation are dysfunctional either due to excessive cell proliferation, insufficient apoptosis or both (Saikumar, et al., 1999; Wyllie, et al., 1999 and Reed, 1999)

The recent aim of cancer treatment is to reverse, suppress, or prevent carcinogenic progression through the use of natural dietary agents (Oyagbemi et al., 2009; Kundu and Chun, 2014; Thumvijit et al., 2014). Due to the high mortality rates of cancer and the absence of effective chemotherapy, there is a continued need for new alternatives for treatment and prevention of cancer, and natural products play a dominant role in the discovery of such new drugs (Cragg et al., 1997). Traditional medical practices in Lake Victoria basin (LVB) use various plants for the treatment of various diseases. Though these plants and their extracts have been used in the folk medicine, information from organized published literature does not provide the evidence for antitumor activities of the four selected medicinal plants used in this study. Also the increase in the use of these medicinal plants and their phytoconstituents in the recent past as well as the scarcity of scientific studies on their safety and efficacy have raised concerns in the scientific community and there is need to assess the potential effects of these plants.

With this perspective, the present study was designed to assess the anticancer potential of methanolic extracts of four medicinal plants, K. africana, P. africanum, C. asciatica and C. nigricans, from the Lake Victoria basin which had been previously selected based on their ethno botanical and ethno pharmacological uses by the Lake Victoria research initiative (VicRes) on Value chain analysis and development of plant derived medicinal products for the management of HIV and AIDS in the face of climate change in the Lake Victoria basin. These plants native to Lake Victoria basin are used in folk medicine for treating a variety of ailments, including cancer. The antiproliferative activity of the extracts against the human lung adenocarcinoma cell line was tested using the microtitration colorimetric method of MTT reduction. MTT is used to determine cell viability in cell proliferation and cytotoxicity assays. The activity of methanol extract of K. africana, P. africanum, and C. asciatica are thought to be as a result of the induction of cell death by apoptosis as indicate by the current findings.

Apoptosis is the common mode of cell death by therapeutic drugs used in cancer treatment and based on this fact, the cellular and nuclear morphological changes induced by the extracts treatment in lung adenocarcinoma cells were observed in this study. Subsequent MTT cell viability tests on the cell lines revealed that the plant extracts were able to suppress proliferation at different concentrations suggesting that proliferation suppression is dose dependent. While all four extracts showed activity against lung adenocarcinoma cells at low concentrations (< 50 µg/mL), only K. africana continued to exhibit low activity at elevated concentrations. All the extracts were found to significantly reduce lung adenocarcinoma cell proliferation. However, future studies should be designed to identify the possible signaling cascade of cell deaths.

According to the American National Cancer Institute (NCI), the criteria of cytotoxic activity for the crude extracts is at IC50 < 30 µg/ml (Itharat et

al, 2003 and Oskoueian et al, 2011). The methanol fraction of P. africanum, K. africana and C. asciatica which exhibited the highest antiproliferative potential among the four active plants with IC50 values of 28.86µg/ml, 26.57µg/ml and 15.69µg/ml respectively (Figure 1) fall within the NCI criteria, thus are considered as of promising anticancer potential. However, no studies have reported the antiproliferative activity of these extracts in lung adenocarcinoma cells. The pharmacological activity of the C. nigricans leaves has been investigated only in rats, rabbits and mice (Chidume et al., 2001) but not in cancer cell lines. Absence of flavonoids and other phytochemicals in C. nigricans is attributed to its low inhibitory activities.

5.3 Microscopic Examination of the Treated Cells

The process of programmed cell death is characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptotic cells show morphological changes such as cell shrinkage, pyknosis and extensive plasma membrane blebbing leading to the formation of apoptotic bodies, which are subsequently phagocytosed by macrophages parenchymal or neoplastic cells. Suppression/inhibition of apoptosis during carcinogenesis is known to play a role in the development and progression of cancers (Kurosaka, et al., 2003). Apoptosis is conceivably the most potent defense against cancer development since it is the mechanism used by metazoans to eliminate deleterious cells. Many chemo- preventive agents have been shown to induce apoptosis in transformed cells both in vitro and in vivo. Induction of apoptosis appears to be associated with their effectiveness in modulating carcinogenic processes (Sun et al., 2004). Since apoptosis provides a physiologic mechanism for eliminating initiated or abnormal cells, dietary factors affecting apoptosis can influence carcinogenesis. In fact, activation of apoptosis in pre-cancerous cells is one of the most important mechanisms of cancer chemoprevention by dietary factors (Martin, et al., 2006).

Morphological examinations of lung adenocarcinoma cells treated with four different plant extracts were visualized against the untreated controls using an inverted microscope. In comparison to untreated, the treated cells showed typical features of cell death at the morphological level such as rounding off of cells, cell shrinkage and detachment from the substrate which accumulated in a dose and time-dependent manner, thus supporting indication that these extracts induces cell death by apoptosis. Untreated cells showed large and prominent nuclei indicating no significant characteristics of apoptosis. The microscopic analysis showed the blebbing pattern and cell shrinkage at concentrations of 125.0µg/ml, an indication of cell apoptosis. This suggested that cytotoxicity of the extracts might involve the apoptosis pathway. This is in agreement with how commercially available chemotherapeutic agents and folk medicinal plants exert their anticancer activity by inducing cell apoptosis (Yu et al., 2007). Our apoptotic test results would support the previous studies especially nuclear fragmentation, nuclear condensation, and cell shrinkage which were clearly observed (Figure 4). This is not an exception since many commercially available chemotherapeutic agents and folk medicinal plants exert their anticancer effect by inducing cell (Yan-Wei et al., 2009).

Previous studies have shown that preparations of these plants possess anti-microbial properties, in addition to speeding the wound-healing process. However, there are currently no reports on the activities of the four medicinal plant extracts used in this study against lung adenocarcinoma cells. This represents the first instance in which the therapeutic potential of the four plants in cancer treatment has been demonstrated. Moreover, subsequent studies including the quantitative analysis of the chemicals from the studied plants should be made before any conclusion is made. It could also be possible to speculate that the anticancer activities of the four plant extracts could be due to a synergy between their various chemical constituents.

Chapter Six

6.0. Summary Of The Findings, Conclusions And Recommendations

6.1. Summary Of The Findings

The phytochemical analysis and antiproliferative activities of methanol extracts of four medicinal

plants that are native to Lake Victoria Basin, including Piptadiniastrum africanum, Kigelia africana, Centella asciatica and Chaemocrista nigricans which had been identified through ethno botanical surveys and taxonomy was investigated. Overally; tannins, steroids, alkaloids, glycosides, saponins and terpenoids were detected as the major phytochemical components in all the four plant extracts. Flavonoids were present only in C. asciatica and K. africana whereas coumarins were only present in K. africana. Anthraquinones were detected in P. africanum and K. africana only. The phytochemical analysis revealed the presence of many chemical groups in the plant extracts that are responsible of their antioxidant, anti-cancer activity and other bioactivities. The antiproliferative data indicates that all the plants extracts showed inhibitory activities. Among the Methanolic extracts, K. africana, P. africanum and C. asciatica had the highest inhibitory effects with IC50 28.86ug/ml, 26.57ug/ml and 15.69ug/ml respectively. The lowest IC50 value was obtained with the leaf extract of C. nigricans against lung adenocarcinoma cells. Piptadiniastrum africanum had the highest half inhibitory attributed to its rich phytochemical constituents. C. nigricans had the lowest half inhibition probably due to its low phytochemical contents.

6.2 Conclusion

In conclusion, these results demonstrate that:

1. Phytochemical analysis of the four methanol plant extracts showed that tannins, steroids, alkaloids, glycosides, saponins and terpenoids were present in all the four plant extracts. Flavoinoids was present in C. asciatica and K. africana whereas coumarins were only present in K. africana. Anthraquinone was detected only in P. africanum and K. africana. These findings suggested the potential therapeutic capability of the traditional preparations obtained from these plants.
2. Dose-dependent effect of the plants extracts shows that all of the extracts had the capacity to inhibit the proliferation of lung adenocarcinoma cultured cell lines hence the potential use of these traditional plants from Lake Victoria Basin as antineoplastic agents.

6.3 Recommendations

1. To exhaustively describe the mechanisms of antiproliferative activities of the these four plant extract used in the current study, further flow cytometric studies and phytochemical characterization of the extracts are necessary for more extensive biological evaluation of the most active ingredients as well as studies in other human cancer cell lines.
2. There is also need to promote conservation of these plant species and to encourage local farmers in the region to consider agroforestry.

References

1. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. Scientific Research and Essays. 2007 May 31;2(5):163-6.
2. Al-Fatimi M, Friedrich U, Jenett-Siems K. Cytotoxicity of plants used in traditional medicine in Yemen. Fitoterapia. 2005 Jun 30;76(3):355-8.
3. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter 1. Annual review of pharmacology and toxicology. 1999 Apr;39(1):361-98.
4. Amin AR, Kucuk O, Khuri FR, Shin DM. Perspectives for cancer prevention with natural compounds. Journal of Clinical Oncology. 2009 May 4;27(16):2712-25.
5. Amutha M, Lalitha P. Evaluation of Wound Healing Activity of Leaf and Stem Extract of Kedrostis Foetidissima (Jacq.) Cogn. Res J Pharm. Biol Chem Sci. 2013;4(2):445.
6. Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochemical pharmacology. 2008 Dec 1;76(11):1590-611.

7. Anand P, Kunnumakkara AB, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (September 2008). "Cancer is a preventable disease that requires major lifestyle changes". Pharm. Res.25 (9): 2097–2116.
8. Asakawa Y, Matsuda R, Takemoto T. Mono- and sesquiterpenoids from Hydrocotyle and Centella species. Phytochemistry. 1982 Jan 1;21(10):2590-2.
9. Aiyegoro OA, Okoh AI. Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of Helichrysum pedunculatum. International journal of molecular sciences. 2009 Nov 13;10(11):4990-5001.
10. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC complementary and alternative medicine. 2010 May 14;10(1):21.
11. Babu TD, Kuttan G, Padikkala J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to Centella asiatica (L.) Urban. Journal of ethnopharmacology. 1995 Aug 11;48(1):53-7.
12. Babayi H, Kolo I, Okogun JI, Ijah UJ. The antimicrobial activities of methanolic extracts of Eucalyptus camaldulensis and Terminalia catappa against some pathogenic microorganisms. 16 (2): 106-111.
13. Babykutty S, Padikkala J, Sathiadevan P, Vijayakurup V, Azis T, Srinivas P, Gopala S. Apoptosis induction of Centella asiatica on human breast cancer cells. African Journal of Traditional, Complementary and Alternative Medicines. 2009;6(1).
14. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life sciences. 2005 Dec 22;78(5):431-41.
15. Boehm T, Folkman J, Browder T, O'reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature. 1997 Nov 27;390(6658):404-7.
16. Boik J. Natural Compounds in Cancer Therapy. Oregon Medical Press, Minnesota, USA, p. 25 (2001). Stevigny. 2005
17. Cancer - Signs and symptoms.NHS Choices. Retrieved 10 June 2014
18. David A and Karnofsky M., (1968). Cancer journal for clinicians, vol. 18 No. 4 232-234, 1968).
19. Cardellina II JH, Fuller RW, Gamble WR, Westergaard C, Boswell J, Munro MH, Currens M, Boyd MR. Evolving strategies for the selection, dereplication and prioritization of antitumor and HIV-inhibitory natural products extracts. InBioassay Methods in Natural Product Research and Drug Development 1999 (pp. 25-35). Springer Netherlands.
20. Chen HX, Cleck JN. Adverse effects of anticancer agents that target the VEGF pathway. Nature reviews Clinical oncology. 2009 Aug 1;6(8):465-77.
21. Cheng CL, Guo JS, Luk J, Koo MW. The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. Life sciences. 2004 Mar 19;74(18):2237-49.
22. Chidume FC, Gamaniel K, Amos S, Akah P, Obodozie O, Wambebe C. Pharmacological activity of the methanolic extract of Cassia nigricans leaves. Indian journal of pharmacology. 2001 Sep 1;33(5):350-6.
23. Choi ES, Cho SD, Jeon JG, Cho NP. The apoptotic effect of the hexane extract of Rheum undulatum L. in oral cancer cells through the down-regulation of specificity protein 1 and survivin. Laboratory animal research. 2011 Mar 1;27(1):19-24.
24. Choi CH. ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal. Cancer cell international. 2005 Oct 4;5(1):30.
25. Comín-Anduix B, Agell N, Bachs O, Ovádi J, Cascante M. A new bis-indole, KARs, induces selective M arrest with specific spindle aberration in neuroblastoma cell line SH-SY5Y. Molecular pharmacology. 2001 Dec 1;60(6):1235-42.
26. Coldren, C. D.; Hashim, P.; Ali, J. M.; Oh, S. K.; Sinskey, A. J. & Rha, C. Gene expression changes in the human fibroblast induced by Centella asciatica triterpenoids. Planta Med., 69(8):725-32, 2003.
27. Cragg GM, Newman DJ. Plants as source of anticancer agents. J Ethnopharmacol.2005; 100: 72-79.

28. Crowley E, McDevitt CA, Callaghan R. (2009). Multidrug Resistance in Cancer. Generating Inhibitors of P-Glycoprotein: Where to, Now? Springer Protocols. pp. 405–432.
29. Damia G, D'Incalci M; d'Incalci (September 1998)."Mechanisms of resistance to alkylating agents".Cytotechnology27 (1–3): 165–73.
30. Dastur, J. F. Medicinal Plants of India and Pakistan. Bombay, D. B. Taraporevala Sons and Co., 1962.
31. Deeken JF, Löscher W; Löscher (March 2007). "The blood-brain barrier and cancer: transporters, treatment, and Trojan horses". Clin. Cancer Res. 13 (6): 1663–74.
32. Defining Cancer".National Cancer Institute. Retrieved 10 June 2014.
33. Dessisa D, A preliminary economic evaluation of medicinal plants in Ethiopia: trade, volume and price In: Medhin Z, Abebe D, editors. Proceedings of the National Workshop on Biodiversity Conservation and Sustainable use of Medicinal Plants in Ethiopia. Addis Ababa, Ethiopia: 28 th April-May1 st , 2001; 176-188.
34. Don M, Shen C, Syu W, Ding Y, Sun C (2006). Cytotoxic and aromatic constituents from Salvia Miltiorrhiza. Phytochemistry 67: 497-503.
35. Dymock W, Warden C. J. H and David H: Pharmacogeaphia Indica 1891; 97:90-92.
36. Effrain KD, Jack TW, Sodipo OA (2001).).Histopathological studies on the toxicity of o. gratissimum leaves extract on some organs of rabbit. Afr. J. Biomed 6: 21-25.
37. Farnsworth NR. Ethnobotany and the Search for New Drugs, Wiley Willett WC. Diet and health: what should we eat.Science. 1994;264(5158):532–7.
38. Farombi EO, Nwamkwo JO, Emerole GO (1998). Effect of methanolic extract of browned yamflourdieton7,12-Dimethylbenzanthracene (DMBA) and 3-methylcholanthrene (3-MC) induced toxicity in the rat. Proc. Fed. Afr. Soc. Biochem. Biol. 1: 5-10.
39. Ferguson PJ, Kurowska E, Freeman DJ, Chambers AF, Koropatnick DJ. A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines J. Nutr., 2004; 134(6):1529-1535.
40. Fidler I.J and Kripke M.L. (1977). Metastasis results from preexisting variant cells within a malignant tumor. Science 197, 893-895.
41. Fresco, P., Borges, F., Diniz, C. and Marques, M.P. (2006) New insights on the anticancer properties of dietary poly- phenols. Medical Research Reviews, 26, 747-766.
42. Friis I, Gilbert MG, Chenopodiaceae. In: Edwards S, Mesfin T, Sebsebe D, Hedberg I, editors. Flora of ethiopia and eritrea; Magnoliaceae to flacourtiaceae. Vol. 2, Published by National Herbarium of Addis Ababa University and Uppsala University, Uppsala. Sweden: 2000; 277.
43. Gett, A.V., Hodgkin, P.D., 1998. Cell division regulates the T cell cytokine repertoire, revealing a mechanism underlying immune class regulation. Proc. Natl. Acad. Sci. USA 95, 9488.
44. George, M.; Joseph, L. & Ramaswamy. Anti-allergic, anti-pruritic and anti-inflammatory activities of Centella asciatica extracts. Afr. J. Tradit. Complement. Altern. Med., 6(4):554-9, 2009.
45. Gerstner ER, Fine RL; Fine (June 2007). "Increased permeability of the blood-brain barrier to chemotherapy in metastatic brain tumors: establishing a treatment paradigm". J. Clin. Oncol. 25 (16): 2306–12.
46. Giday M, Asfaw Z, Elmqvist T, Woldu Z.(2003): An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia; J Ethnopharmacol. 2003 Mar; 85(1):43-52.
47. Gilani AH, Atta ur R. Trends in ethnopharmacology. Journal of Ethnopharmacology.2005; 100 (1-2):43-49.
48. Gibb, R. K., Taylor, D. D., Wan, T., O´Connor, D. M., Doering, D. L., Gercel-Taylor, C., 1997: Apoptosis as a measure of Chemosensitivity to Cisplatin and Taxol Therapy in Ovarian Cancer Cell Lines. Gynecologic Oncology 65: 13-22.
49. Gohil, K. J.; Patel, J. A. & Gajjar, A. K. Pharmacological Review on Centella asciatica: A Potential Herbal Cure-all. Indian J. Pharm. Sci., 72(5):546-56, 2010.
50. Gnanapragasam, A.; Ebenezar, K. K.; Sathish, V.; Govindaraju, P. & Devaki, T. Protective effect of Centella asciatica on antioxidant

tissue defense system against adriamycin induced cardiomyopathy in rats. Life Sci., 76(5):585-97, 2004.

51. Goldman B (February 2003). "Multidrug resistance: can new drugs help chemotherapy score against cancer?".J. Natl. Cancer Inst. 95 (4): 255–7.
52. Grace OM, Davis SD (2002).Kigelia Africana (Lam.) Benth.Record from protabase. Oyen LPA, Lemmens RHMJ Wageningen, Netherlands. Inmagic DB/Text Webpublisher PRO: 1 records (http://database.prota.org/search.htm).
53. Griggs, J., Metcalfe, J. C., Hesketh, R., 2001: Targeting tumour vasculature: the development of combretastatin A4. The Lancet Oncology 2: 82-87.
54. Guo JS, Cheng CL, Koo MW (2004). Inhibitory effects of C. asciatica water extract and asiaticoside on inducible nitric oxide synthase during gastric ulcer healing in rats. Planta Medica, 70: 1150-1154.
55. Gupta, R. & Flora, S. J. Effect of Centella asciatica on arsenic induced oxidative stress and metal distribution in rats. J. Appl. Toxicol., 26(3):213-22, 2006.
56. Gurib-Fakim, A., 2006: Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27: 1-93.
57. Hail, N. Jr. (2005) Mitochondria: A novel target for the chemoprevention of cancer. Apoptosis: An International Journal on Programmed Cell Death, 10, 687-705.
58. Hanahan D, Weinberg RA; Weinberg (January 2000). "The hallmarks of cancer".Cell 100 (1): 57–70.
59. Harbourne, J.B. (1983). Phytochemical Methods.A Quide to modern Technique of Plants Analysis. Chapman and Hall, London.
60. Harborne, J.B., Phytochemical methods (3rd edition) Chapman and Hall, London (1978).
61. Hirano, T., Abe, K., Gotoh, M., Oka, K., 1995: Citrus flavone tangeretine inhibits leukaemic HL-60 cell growth. British Journal of Cancer 72: 1380-1388.
62. Hodgkin, P.D., Bartell, G., Mamchak, A., Doherty, K.V., Lyons, A.B., Hasbold, J., 1997. The importance of efficacy and partial agonism in evaluating models of B lymphocyte activation. Int. Rev. Immunol. 15, 101.
63. Hodgson S (January 2008). "Mechanisms of inherited cancer susceptibility".J Zhejiang UnivSci B9 (1): 1–4.
64. Huang, S. S.; Chiu, C. S.; Chen, H. J.; Hou, W. C.; Sheu, M. J.; Lin, Y. C.; Shie, P. H. & Huang, G. J. Antinociceptive activities and the mechanisms of anti-inflammation of asiatic Acid in mice. Evid.Based Complement. Alternat. Med., 2011:895857, 2011.
65. Hutchinson J &Dalziel JM (1963).Flora of West Tropical Africa. II. Millbank, London crown agents for oversea Government and Administration 4, 221-221.
66. Houghton PJ, Traditional plant medicines as a source of new drugs. In: Trease and Evans Pharmacognosy. Edited by W.C. Evans. Edinburgh, Saunders 2005, 125-134
67. Houghton P.J., Photion A., Uddin S., Shah P., Browning M., Jackson S.J., Retsas S. Activity of Extracts of Kigelia Pinnata against Melanoma and Renal Carcinoma cell lines. Planta medica 60 (5): 430 – 433, 1994.
68. Inamdar, P. K.; Yeole, R. D.; Ghogare, A. B. & de Souza, N. J. Determination of Biologically Active Constituents in Centella asciatica.J. Chromatogr. A, 742(1-2):127-30, 1996.
69. Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J Ethnopharmacol. 2004; 90: 33 -38.
70. Jo EH, Kim SH, Ra JC, Kim SR, Cho SD, Jung JW, Yang SR, Park JS, Hwaang JW, Aruoma OI, Kim TW, Lee YS, Kang KS (2005). Chemopreventive properties of the ethanol extracts of Chinese licorice (GlycyrrhizaUralensis) root: Induction of apoptisis and G1 cell cycle arrest in Mcf-7 human breast cancer cells. Cancer Letters.pp. 1- 9.
71. Joshi, L., Van Eck, J. M., Mayo, K., Di Silvestro, R., Blake, M. E., Ganapathi, T., Haridas, V., Gutterman, J. U., Arntzen,, C. J., 2002: Metabolomics of Plant Saponins: BioprospectingTriterpene Glycoside Diversity with Respect to Mammalian Cell Targets. OMIC, A Journal of Integrative Biology 6 (3): 235- 246.

72. Kehe K, Balszuweit F, Steinritz D, Thiermann H; Balszuweit; Steinritz; Thiermann (September 2009). "Molecular toxicology of sulfur mustard-induced cutaneous inflammation and blistering".Toxicology 263 (1): 12–9.
73. Kafaru, E., 1994: Immense Help From Nature`s Workshop. Elika Health Services Ltd., Academic Press Plc. Lagos, Nigeria. pp. 1-27.
74. Kamatenesi MM, Acipa A and Oryem-Origa H. J Ethnobiol Ethnomed 2011;7(7):
75. Kasaikina OT, Kortenska VD, Marinova EM, Rusina IF, Yarisbheva NV (1997). Russ.Chem. Bull. 46:1070-1073.
76. Kaufmann SH, Earnshaw WC (2000). Induction of apoptosis by cancer chemotherapy, Exp cell Res, 256. P. 42-9.
77. Kimura Y., Sumiyoshi M., Samukawa K., Satake N., Sakanaka M. (2008) Facilitating action of asiaticoside at low doses on burn wound repair and its mechanism. Eur J Pharmacol. 3, 415-423.
78. Kanadaswami C, Lee L, Lee PH, Hwang J, Ke F, Huang Y, Lee M. The antitumor activities of flavonoids In Vivo, 2005; 19(5): 895-909.
79. Koleva II, Niederlander HAG, Van Beek TA (2000). An online HPLC method for detection of radical scavenging compounds in complex mixtures. Anal. Chem. 72:2323-2328.
80. Kokwaro, O., 1976: Medicinal Plants of East Africa. East African Literature, Nairobi.
81. Krishnaiah D, Devi T, Bono A, Sarbatly R (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plant Res. 3(2): 067 – 072.
82. Kuo PL, Lin TC, Lin CC. The antiproliferative activity of aloe emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. Life Sci 2002;71:1879-92.
83. Kurosaka, K., Takahashi, M., Watanabe, N. and Kobayashi, Y. (2003) Silent cleanup of very early apoptotic cells by macrophages. Journal of Immunology, 171, 4672-4679.
84. Kushi LH, Doyle C, McCullough M, et al. (2012). "American Cancer Society Guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity". CA Cancer J Clin 62 (1): 30–67.
85. Lai C, Mas R, Nair N, Majid M, Mansor S, Navaratnam V. Typhonium flagelliforme inhibits cancer cell growth in vitro and induces apoptosis: an evaluation by the bioactivity guided approach. J Ethnopharmacol.2008;118:14–20.
86. Lall N, Meyer JJM (2000). Antibacterial activity of water and acetone extracts of the roots of Eucleanatalensis. J. Ethnopharmacol. 72:313-316.
87. Lambert, J.D. Hong, J., Yang, G.Y., Liao, J. and Yang, C.S. (2005) Inhibition of carcinogenesis by polyphenols: Evidence from laboratory investigations. American Journal of Clinical Nutrition, 81, 284S-291S.
88. Lamidi M, Digiorgio C, Delmas F, Favel A, Mve-Mba CE, Rondi ML, Ollivier E, Nze-Ekekang L, Balansard G (2005). In Vitro Cytotoxic, Antileishmanial and Antifungal activities of ethnopharmacologically selected Gabonese plants. J. Ethnopharmacol., 102: 185-190.
89. Lamorde Mohammed, J R S Tabuti, Celestino Obua et al. (2010) Medicinal plants used by traditional medicine practitioners for the treatment of HIV/AIDS and related ailments in Uganda, 43-53. In Journal of Ethnopharmacology 130 (1)
90. Lind M.J., M.J. (2008). "Principles of cytotoxic chemotherapy".Medicine 36 (1): 19–23.
91. Lodish H, Berk A, Zipursky SL et al. (2000). Molecular Cell Biology.4th edition.The Role of Topoisomerases in DNA Replication. New York: W. H. Freeman.
92. Luqmani YA (2005)."Mechanisms of drug resistance in cancer chemotherapy".Med Princ Pract. 14 Suppl 1: 35–48.
93. Lee, K.-H., 1999: Anticancer drug design based on plant-derived natural products. Journal of Biomedical Science 6:236-250.
94. Li Y; Guo C; J Yang; J Wei; J Xu; S Cheng. Food Chem., 2006, 96, 254–260.
95. Makin G, Hickman JA; Hickman; Merritt; Fisher (July 2000). "Apoptosis and cancer chemotherapy".Cell Tissue Res.301 (1): 143–52.
96. Mander, M., 1998: Marketing of Indigenous

Medicinal Plants in South Africa. A case study in KwaZulu-Natal. FAO, Rome.

97. Markowitz, edited by William N. Rom ; associate editor, Steven B. (2007). Environmental and occupational medicine (4th ed.). Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins. p. 745.ISBN 978-0-7817-6299-1.
98. Mattos, D. et al. Model to evaluate the toxic effect of drugs: vincritine effect in the mass of organs and in the distribution of radiopharmaceuticals in mice. Mutation Research, v.496, p.137-143, 2001
99. Maquart, F. X.; Chastang, F.; Simeon, A.; Birembautt, P.; Gillery, P. & Wigrowski, Y. Triterpenes from Centella asciatica stimulate extracellular matrix accumulation in rat experimental wounds. Eur. J. Dermatol., 9(4):289-96, 1999.
100. Maquart FX, Bellon G, Gillery P, Wegrowski Y, Borel JP. Stimulation of collagen synthesis in fibroblast cultures by a triterpene extracted from Centella asiatica. Connective tissue research. 1990 Jan 1;24(2):107-20.
101. Martin KR. Targeting apoptosis with dietary bioactive agents. Experimental Biology and Medicine. 2006 Feb 1;231(2):117-29.
102. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer research. 1986 Dec 1;46(12 Part 1):6387-92.
103. Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come?. Pharmaceutical research. 2010 Jun 1;27(6):950-61.
104. Mengome LE, Feuya GR, Eba F and Nsiemyo.E (2009).Antiproliferative Effect of Alcohol Extract of some Gabonese medicinal plants on human colonic cancer cells. African Journal of Traditional, Complementary and Alternative Medicines, vol. 6 No 2, 112-117.
105. Mladosievicova B, (2007): Pathophysiology of malignant disease: Chap. 10; BJU Int, pg 647-650
106. Montvale, (1998). Medical Economic, "Physicians' Desk Reference," 52nd ed. pg 212-215.
107. Morris K (2002). South Africa tests traditional medicines. Lancet Infect. Dis. 2(6): 319.
108. Mutua PM, Gicheru MM, Makanya AN, Kiama SG, MUTUA P, GICHERU M, MAKANYA A, KIAMA S. Anti-Proliferative Activities of Centella Asiatica Extracts on Human Respiratory Epithelial Cells in vitro. Int. j. morphol. 2013 Dec 1;31(4):1322-7.
109. Neuwinger HD. African traditional medicine: a dictionary of plant use and applications. With supplement: search system for diseases. Medpharm; 2000.
110. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Last 25 Years. Journal of natural products. 2007 Mar 23;70(3):461-77.
111. Newman DJ, Cragg GM, Holbeck S, Sausville EA. Natural products and derivatives as leads to cell cycle pathway targets in cancer chemotherapy. Current cancer drug targets. 2002 Dec 1;2(4):279-308.
112. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Natural product reports. 2000;17(3):215-34.
113. Nichenametla, S.N., Taruscio, T.G., Barney, D.L. and Exon, J.H. (2006) A review of the effects and mechanisms of polyphenolics in cancer. Critical Reviews in Food Science and Nutrition, 46, 161-183.
114. Nitiss JL (May 2009). "Targeting DNA topoisomerase II in cancer chemotherapy". Nature Reviews Cancer9 (5): 338–50.
115. Njoroge, G. N., Bussman, R. W., 2006: Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). Journal of Ethnobiology and Ethnomedicine2 (8): 1-7.
116. Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, Zolkifli NA, Hendra R, Ho YW. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from Jatropha curcas Linn. Journal of Medicinal Plants Research. 2011 Jan 4;5(1):49-57.
117. Owolabi OJ, Omogbai EK. Analgesic and anti-inflammatory activities of the

ethanolic stem bark extract of Kigelia africana (Bignoniaceae). African Journal of Biotechnology. 2007 Mar 5;6(5):582.

118. Park HJ, Kim MJ, Ha E, Chung JH. Apoptotic effect of hesperidin through caspase 3 activation in human colon cancer cells, SNU-C4. Phytomedicine. 2008; 15: 147–151.

119. Parker WB. Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chemical reviews. 2009 May 29;109(7):2880-93.

120. Parkin, D.M., Bray, F., Ferlay, J. & Pisani, P. 2005, "Global Cancer Statistics, 2002", CA: A Cancer Journal for Clinicians, vol. 55, pg. 74-108.

121. Perera FP (November 1997). "Environment and cancer: who are susceptible?".Science278 (5340): 1068–73.

122. Pieters L, Vlietinck AJ (2005). Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds? J. Ethnopharmacol. 100: 57-60.

123. Pettit GR, Toki BE, Herald DL, Boyd MR, Hamel E, Pettit RK, Chapuis JC. Antineoplastic Agents. 410. Asymmetric Hydroxylation of trans-Combretastatin A-4 1. Journal of medicinal chemistry. 1999 Apr 22;42(8):1459-65.

124. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garcia-Kendal D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. Experientia. 1989 Feb 1;45(2):209-11.

125. Pettit, G. R, Singh, S. B., Schmidt, J. M., 1988: Isolation, structure, synthesis, and antimitotic properties of combretastatins B-3 and B-4 from Combretumcaffrum. Journal of Natural Products 51 (3): 517-527.

126. Pettit GR, Cragg GM, Singh SB. Antineoplastic agents, 122. Constituents of Combretum caffrum. Journal of natural products. 1986 Dec;50(3):386-91.

127. Priyavardhini S, Vasantha K , Tresina Soris P and Mohan VR. Int J Pharm Tech Res 2012; 4(1):44-48.

128. Promega corporation, 2006; Protocol and application cell viability.

129. Punturee K, Wild CP, Kasinrerk W, Vinitketkumnuen U. Immunomodulatory activities of Centella asiatica and Rhinacanthus nasutus extracts. Asian Pacific Journal of Cancer Prevention. 2005 Jul 21;6(3):396.

130. Ramos S. Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. Molecular nutrition & food research. 2008 May 1;52(5):507-26.

131. Reed JC. Dysregulation of apoptosis in cancer. Journal of clinical oncology. 1999 Sep;17(9):2941-.

132. Reed JC, Pellecchia M. Apoptosis-based therapies for hematologic malignancies. Blood. 2005; 106: 408-418.

133. Riss TL, Moravec RA. Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays. Assay and drug development technologies. 2004 Feb 1;2(1):51-62.

134. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. The Journal of nutrition. 2004 Dec 1;134(12):3479S-85S.

135. Saikumar P, Dong Z, Mikhailov V, Denton M, Weinberg JM, Venkatachalam MA. Apoptosis: definition, mechanisms, and relevance to disease. The American journal of medicine. 1999 Nov 1;107(5):489-506.

136. Sampson JH, Raman A, Karlsen G, Navsaria H, Leigh IM. In vitro keratinocyte antiproliferant effect of Centella asiatica extract and triterpenoid saponins. Phytomedicine. 2001 Jan 1;8(3):230-5.

137. Schenkel E.P., et al..Produtos de Origem vegetal e o Desenvolvimento de Medicamentos. In: Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R. Farmacognosia: da Plantaao Medicamento. Editora da UFSC, Cap.15, 291-320, 1999.

138. Shkreta L., U. Froehlich, E.R. Paquet, J.Toutant, S.A. Elela, and B. Chabot. 2008. Anticancer drugs affect the alternative splicing of Bcl-x and other human apoptotic genes. Mol Cancer Ther 2008; 7(6):1398–409.

139. Shukla A, Rasik AM, Dhawan BN. Asiaticoside-induced elevation of antioxidant levels in healing wounds. Phytotherapy

Research. 1999 Feb 1;13(1):50-4.

140. Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK, Dhawan BN. In vitro and in vivo wound healing activity of asiaticoside isolated from Centella asiatica. Journal of ethnopharmacology. 1999 Apr 1;65(1):1-1.
141. Siddik ZH. Mechanisms of Action of Cancer Chemotherapeutic Agents: DNA-Interactive Alkylating Agents and Antitumour Platinum-Based Drugs. The cancer handbook. 2002.
142. Siddique YH, Ara G, Beg T, Faisal M, Ahmad M, Afzal M. Protective role of Centella asiatica L. extract against methyl methanesulphonate and cyclophosphamide induced genotoxic damage in cultured human lymphocytes. Recent Progress in Medicinal Plants. 2007;19(1):369-81.
143. Sofowora LA (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan. 55-71.
144. Stevigny C, Bailly C, Quetin-Leclercq J. Cytotoxic and antitumor potentialities of aporphinoid alkaloids. Current Medicinal Chemistry-Anti-Cancer Agents. 2005 Mar 1;5(2):173-82.
145. Sun SY, Hail N, Lotan R. Apoptosis as a novel target for cancer chemoprevention. Journal of the National Cancer Institute. 2004 May 5;96(9):662-72.
146. Tabuti JR, Lye KA, Dhillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. Journal of Ethnopharmacology. 2003 Sep 30;88(1):19-44.
147. Tatiana AN. Medicinal Chemistry and Pharmaceutical Technology Ethno botany and Traditional Medicine of Pygmies baka. Scientifica Acta. 2008;2(2):129-33.
148. Taraphdar AK, Roy M, Bhattacharya RK. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. Curr Sci. 2001 Jun 10;80(11):1387-96.
149. Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer control. 2003 Mar;10(2):159-.
150. TREASE G, EVANS W. Pharmacognosy. 14th Edt, Publ. Brown Publ. Uko OJ, Usman A and Ataja AM 2001 Some biological activities of Garcinia kola in growing rats. Veterinarski ARHIV. 1983;71(5):287-97.
151. Trease, GE., Evans, W.C., Pharmacognsy, 11th edition Brailliar Tiridel Can. Macmillian publishers, (1989).
152. Vogel, M; Schwarze-Zander, C; Wasmuth, JC; Spengler, U; Sauerbruch, T; Rockstroh, JK (July 2010). "The treatment of patients with HIV".Deutsches Ärzteblatt international 107 (28–29): 507–15.
153. Walker K, Croteau R. Taxol biosynthetic genes. Phytochemistry. 2001 Sep 30;58(1):1-7.
154. Waxman DJ, Schwartz PS. Harnessing apoptosis for improved anticancer gene therapy. Cancer research. 2003 Dec 15;63(24):8563-72.
155. Weiss CR, Moideen SV, Croft SL, Houghton PJ. Activity of extracts and isolated naphthoquinones from Kigelia pinnata against Plasmodium falciparum. Journal of natural products. 2000 Sep 22;63(9):1306-9.
156. World Cancer Report 2014.World Health Organization. 2014. pp. Chapter 1.1. ISBN 9283204298.
157. World Health Organization. Traditional medicine (1989). Fact sheet No. 134. www.who.int/ Mediacentre/factsheets/ fs134/cn/.
158. Wyllie, A.H., Bellamy, C.O., Bubb, V.J., Clarke, A.R., Corbet, S., Curtis, L., Harrison, D.J., Hooper, M.L., Toft, N., Webb, S. and Bird, C.C. (1999) Apoptosis and carcinogenesis. British Journal of Cancer, 80, 34-37.
159. Hu YW, Liu CY, Du CM, Zhang J, Wu WQ, Gu ZL. Induction of apoptosis in human hepatocarcinoma SMMC-7721 cells in vitro by flavonoids from Astragalus complanatus. Journal of ethnopharmacology. 2009 Jun 22;123(2):293-301.
160. Yoshida, M.; Fuchigami, M.; Nagao, T.; Okabe, H.; Matsunaga, K.; Takata, J.; Karube, Y.; Tsuchihashi, R.; Kinjo, J.; Mihashi, K. & Fujioka, T. Antiproliferative constituents from Umbelliferae plants VII. Active triterpenes and rosmarinic acid

from Centella asciatica.Biol. Pharm. Bull., 28(1):173-5, 2005.

161. Yue QX, Liu X, Guo DA; Liu; Guo (August 2010). "Microtubule-binding natural products for cancer therapy".Planta Med. 76 (11): 1037–43.
162. Yu Z, Li W, Liu F. Inhibition of proliferation and induction of apoptosis by genistein in colon cancer HT-29 cells. Cancer letters. 2004 Nov 25;215(2):159-66.
163. Yu J, Liu H, Lei J, Tan W, Hu X, Zou G. Antitumor activity of chloroform fraction of Scutellaria barbata and its active constituents. Phytotherapy Research. 2007 Sep 1;21(9):817-22.
164. Zaidan MR, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed. 2005 Dec;22(2):165-70.
165. Zainol MK, Abd-Hamid A, Yusof S, Muse R. Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of Centella asiatica (L.) Urban. Food Chemistry. 2003 Jun 30;81(4):575-81.
166. Zakaria ZA, Rofiee MS, Mohamed AM, Teh LK, Salleh MZ. In vitro antiproliferative and antioxidant activities and total phenolic contents of the extracts of Melastoma malabathricum leaves. Journal of acupuncture and meridian studies. 2011 Dec 31;4(4):248-56.