Safety and anti-proliferative activity of *Prunus africana, Warburgia stuhlmannii* and *Maytenus senegalensis* extracts in breast and colon cancer cell lines

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to the almighty God, He provided me with strength, grace, patience and favor, to have me complete my studies.

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The success of this research was only made possible by the sacrifices of many people along this journey and I am truly grateful.

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ABBREVIATIONSANDACRONYMS

ABCIC: African Biodiversity Conservation and Innovations Center.

ACUC: Animal Care and Use Committee.

AIDS: Acquired Immune Deficiency Syndrome.

ANOVA: Analysis of variance.

BPH: Benign prostate hyperplasia.

CTMDR: Centre for Traditional Medicine and Drug Research.

CQ: Chloroquine.

DALYs: Disability-adjusted-life-years.

DHT: Dihydrotestosterone

DMSO: Dimethyl sulphoxide.

DNA: Deoxyribonucleic acid.

CC_{50}: Cytotoxic concentration at 50%.

EDTA: Ethylenediaminetetraacetic acid.

ERC: Ethical Review Committee.

HBV: Hepatitis B virus.

HELcells: Human embryonic lung cells.

HIV: Human immunodeficiency virus.

HPV: Human papilomavirus.

IAEA: International Atomic Energy Agency.

IARC: International Agency for Research in Cancer.

IC_{50}: Inhibitory concentration at 50%.

ISO: International Organization for Standardization

KEMRI: Kenya Medical Research Institute.

LD_{50}: Lethal dose at 50%.

MIC: Minimum inhibitory concentration.

MRSA: Methicilin resistant *Staphylococcus aureus.*

MTT: (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide.

NCAPD: National Coordinating Agency for Population and Development.

NSAID: Nonsteroidal anti-inflammatory drug.

SD: Standard Deviation.

SEM: Standard Error of Mean.

SI: Selectivity Index.

SPSS: Statistical Package for Social Sciences.

SSC: Scientific Steering Committee.

TMDDP: Traditional Medicine and Drug Development Program.

UV: Ultra violet.

WHO: World Health Organization.

ABSTRACT

Cancer is the leading cause of death worldwide. In Kenya, different cancer cases have been witnessed all over the country. The emphasis of diseases like malaria, Human Immunodeficiency Virus (HIV) and tuberculosis has resulted in the neglect of non-communicable diseases like cancer. This is characterized by lack of awareness, inadequate facilities, qualified personnel and financial shortages. Locally, majority of the population relies on traditional medicine as an alternative treatment since the conventional health system provides for only 30% of the population. Although medicinal plants in Kenya have been used for treatment of cancer by the traditional healers, there are no reports of studies carried out to verify their healing claims as well as their safety. The objective of this study was to determine the safety and anti-proliferative activity of *Prunus africana*, *Warburgia stulhamannii* and *Maytenus senegalensis* extracts in breast (4T1 ATCC®CRL-2539™) and colon (ATCC® CRL-2638™) cancer cell lines. The *in vitro* assays involved determination of the cytotoxic concentration levels (CC_{50}) of the plant extracts on Vero cells as well as calculating the inhibitory concentration (IC_{50}) of the plant extracts on breast and colon cancer cell lines.The extracts with the highest selectivity index (SI) to have low IC_{50} in the breast and colon cancer cell lines and high CC_{50} in Vero cells were used in the *in vivo assays* which involved acute oral toxicity studies, conducted on 8 weeks old Swiss albino mice to calculate the median lethal dose (LD_{50}). The safest effective extracts were of leaf methanol extracts of leaves from *Prunus africana* whose triplicate results showed an average IC_{50} of 164.64±4.14 (n=3) µg/ml in the breast cancer cell lines and 21.33±0.5 (n=3) µg/ml in the colon cancer cell lines, as well as the stem bark water extracts from *Warburgia Stuhlmannii*whose triplicate results showed an average IC_{50} of 332.79±7.53 (n=3) µg/ml in the breast cancer cell lines and 107.20±2.50(n=3) µg/ml in the colon cancer cell lines.

Both extracts had an average CC_{50} of >1000 (n=3) µg/ml in Vero cells. Based on positive cytotoxicity results on the two extracts, acute oral toxicity studies were conducted on 8 weeks old female Swiss albino mice. This revealed no signs of acute toxicity after administration with LD_{50} of >5000mg/kg body weight, therefore the extracts were considered to be practically non-toxic. The findings of this study may form basis for the development of a candidate drug that is effective, less toxic and more affordable.

CHAPTER ONE

INTRODUCTION

1.1: Background of the study

Cancer is one of the leading causes of death in the world. According to GLOBOCAN, about 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012. The most commonly diagnosed cases worldwide were those of lung, accounting for 1.8 million (13% of the total), breast (1.7 Million, 11.9%) and colorectal (1.4 million, 9.7%). There will be a substantive increase to 19.3 million new cases by 2025 (Bray *et al.,* 2013).

Worldwide, in 2008, it was estimated that approximately 169.3 million years of healthy life was lost due to cancer, with prostate, breast and lung cancer being the major contributors to the total disability-adjusted-life-years lost (DALYs) in most regions of the world, causing a total cancer burden of 18-50% (Soerjomataram *et al.,* 2012).

In Africa, cancer accounts for over one million new cases yearly (South African medical journal, 2007) and this is attributed to changes in lifestyle including smoking, unhealthy eating, lack of physical exercises (WHO, 2008) and increase in population (Ferlay *et al.,* 2010). However, despite its increasing burden, Cancer is not a major priority in developing countries (Silas*et al.,* 2008) and this is largely attributed to limited resources and other pressing public health concerns like communicable diseases such as HIV/AIDS, malaria and tuberculosis. There is also lack of awareness among policy makers, the general public and international private or public health agencies concerning its current and future impact (Parkin*et al.,* 2008; Silas*et al.,* 2008).

In Kenya, cancer is the third leading cause of death with a rate of 18,000 deaths per year. It is also estimated annually that the incidence of cancer is about 28,000 cases and the annual mortality rate is above 22,000. Amongst those affected, 60% are

below 70 years. The risk of getting cancer before attaining the age of 75 years is around 14%, while that of dying from cancer is 12%.

According to the Nairobi Cancer Registry, amongst all cancers, breast cancer accounted for 23.3%, cervical cancer for 20% and prostate cancer for 9.4%. Roughly 2,354 women were diagnosed with cervical cancer and 65% of them died of the disease in 2006 (Kenya Ministry of Public Health, 2009).

Treatment of cancer involves a series of interventions like psychosocial support, surgery, radiotherapy, chemotherapy and hormone therapy, all geared to the goal of prolonging and improving the quality of life of the patient (WHO, 2008). Most anti-cancer drugs make cancer patients receiving chemotherapy to suffer from side effects such as hair loss and anemia (Malcolm, 2001).

As cancer incidences rise dramatically in developing countries, the already limited resources and equipment are overstretched with shortages of equipment and qualified staff making it difficult to effectively treat and manage it (Mohamed, 2003). The need for radiotherapy in developing countries is much greater since most patients present themselves when cancer is at its later stages (WHO, 2008). Access to radiotherapy is however severely limited. For instance, facilities for radiotherapy are only accessible to 23 of Africa's 53 countries, reaching less than 5% of the total African population (Silas *et al.,* 2008).

Medicinal plants have been used since 300 BC (Ayensu, 1978) and for thousands of years, plants and other natural products have been used to treat a variety of diseases and as a result, a number of modern drugs have been developed from them (Samuelsson, 1997). According to the World Health Organization, roughly 80% of the world's inhabitants rely on traditional medicine for their primary health care (Hartwell, 1982). Plants have long been used in cancer treatment (Hartwell, 1982). Secondary metabolites have proved to be reliable sources of new medicinal compounds (Hartwell, 1982). So far 60% of anti-cancer drugs used currently were obtained either from plants, marine organisms or microorganisms (Cragg *et al*., 2005) and scientists are still trying to research on the unexplored plant species (Hartwell, 1982).

The discovery of vinca alkaloids like vinblastine, vincristine and the cytotoxic podophyllotoxins in 1950s in plants began the extensive research of anti-cancer drugs from plant sources (Cassady & Duoros 1980). A combination of vinblastine and vincristine with other cancer chemotherapeutic drugs have been used in the treatment of cancers like leukemias, lymphomas, advanced testicular cancer, breast and lung cancer as well as Kaposi's sarcoma (Cragg & Newman, 2005).

An isomer of podophyllotoxin, epiphyllotoxin (Stahelin, 1973) was isolated from the roots of *Podophyllum* species, *Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich to have active anti-tumor activities (Stahelin, 1973).

Cancer being a major challenge globally (Grey and Sener, 2006), has prompted the discovery of significant medicinal plants of natural anti-cancer compounds with promising biological activities (Roja & Rao, 2000).So far, about 30 compounds derived from plants have been proven to be clinically active against various types of cancer cells (Joyce et al., 2011), this is a very small portion as it is anticipated that plants can provide potential bioactive compounds for the development of new methods to combat cancer diseases (Shoeb, 2006).

The use of herbal medicine is increasingly finding more relevance today, especially with the recognition that there are challenges in the treatment of some medicinal conditions such as diabetes and cancer (Kigen *et al.,* 2013). The practice of traditional medicine is as old as the human race and several drugs have been derived directly or indirectly from plants. Medicinal plants have therefore been important sources of research and development of new drugs (Ebadi, 2006). Currently, many plants are being investigated for potential therapeutic effects including the graviola plant, which has shown evidence of anticancer activity (Torres *et al.,* 2012).

In Africa, clear data on the integration of traditional medicine in the healthcare systems are lacking much as the region is a rich source of medicinal plants. Extracts of plants from the continent has given rise to important medications that has assisted in management of some difficult health conditions. Notable examples include *Catharanthus reseus,* which yields anti-tumor agents such as Vinblastine and Vincristine (KEMRI Natural Products Newsletter, 2014). Several other researches on

medicinal plants are conducted at KEMRI'S Centre for Tradictional Medicine and Drug Research (CTMDR) under the Traditional Medicine and Drug Development Program (TMDDP) using documented scientific evidence on medicinal plants and use measure to apply to their safety and efficacy (KEMRI Natural Products Newsletter, 2014).

The three medicinal plants**:** *Prunusafricana, Warburgiastuhlmannii*and *Maytenussenegalensis*are commonly used traditionally in Kenya as ethnobotanical information claims that they have anti-cancer properties although these therapeutic claims as well as their safety have not been scientifically proven. It is these claims therefore that this study has to verify.

So far there are over 200 different types of cancers but lung, breast, prostate and colon cancers account for more than half of all cases (Grey & Sener, 2006).

1.2: Problem statement

Globally, cancer is a major public health burden, accounting for one in eight deaths overall, more than HIV/AIDS, tuberculosis and malaria combined (Grey & Sener, 2006). According to GLOBOCAN, about 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012 compared with the 12.7 million and 7.6 million in 2008. These prevalence estimates from 2012 show that there were 32.6 million people over the age of 15 years alive that had a cancer diagnosed in the previous 5 years. The most commonly diagnosed cancers worldwide were those of lung, accounting for 1.8 million (13% of the total), breast (1.7 million, 11.9%) and colorectal (1.4 million, 9.7%). It is estimated that there will also be a substantive increase to 19.3million new cancer cases by 2025 (Bray *et al.*, 2013). In Kenya, the risk of acquiring cancer before attaining 75 years is 14% with breast (34 per 100,000) and cervical (25 per 100,000) cancers leading in women, while in men, prostate (17 per 100,000) and esophagus (9 per 100,000) are common (Kenya, Ministry of Public Health, 2009). The developing world, including Kenya is experiencing a dramatic rise in cancer cases.

More than half of all cancer prevalence (56.8%) and deaths (64.9%) in 2012 were from less developed regions in the world, and this will increase further by 2025 (Bray *et al*., 2013). This poses a threat due to overstretching of the already limited resources, and the fact that the expensive treatment is unaffordable.

Unlike conventional cancer treatments like surgery, radiotherapy, chemotherapy, which have adverse effects on patients, compounds of plant origin have proven to be safer and portray promising results towards fighting cancer. The major challenge is that many of these plants have not been studied (Hartwell, 1982).

1.3: Justification

In Africa, 90% of the population relies on traditional medicine for primary healthcare (Miller, 1990). According to the National Coordinating Agency for population and Development (NCAPD), the conventional health system in Kenya provides for only 30% of the population. This means that more than two-thirds of Kenyans rely on traditional medicine for their healthcare needs (NCAPD, 2007). Plants have been used for many years in the treatment of cancer and compounds from several plants have shown to have anti-cancer activity (Cragg*et al.,* 2005). So far, only up to 60% of anti-cancer drugs currently used were obtained either from plants, marine organisms or micro-organisms (Cragg*et al.,* 2005). The benefit of using the drug extracts from medicinal plants is that they are usually safer than their synthetic alternatives and are also more affordable (Iwu*et al.,* 1999). Most cancer drugs available in the market are toxic as they kill both the normal and cancer cells, there is therefore need to look for a safer drug with high specificity to target cancer cells only.

The purpose of this study was to evaluate the safety and anti-proliferative activity against breast and colon cancer cell lines of three medicinal plants: *Prunusafricana, Warburgia stuhlmannii* and*Maytenussenegalensis*. These plants are used by herbalists in Kenya to treat ailments, including cancer. It is hypothesized that they contain anti-cancer activity, although no known laboratory studies have been done.

1.4: Research question(s)

1. What is theanti-proliferative activity in the crude extracts from leaves and stem bark of *Prunus africana, Warburgiastuhlmannii*and *Maytenussenegalensis*?
2. What is the *in vitro* toxicity level of the crude extracts from the three plants in Vero cells?
3. What is the level of acute oral toxicity of the three plant extracts in mice?

1.5: Null hypotheses

Water and methanol extracts from the leaves and stem bark of *Prunus africana, Warburgiastuhlmannii*and*Maytenussenegalensis*do not exhibit any anti-proliferative activity against breast and colon cancer cell lines.

1.6: Objectives

1.6.1: General objective

To determine the safety and anti-proliferative activity of *Prunusafricana,Warburgia stuhlmannii* and*Maytenussenegalensis*extracts in breast and colon cancer cell lines.

1.6.2: Specific objectives

i. To determine the anti-proliferative activity of the crude extracts from leaves and stem bark of *Prunus africana, Warburgiastuhlmannii*and *Maytenus senegalensis* against breast and colon cancer cell lines.
ii. To determine the level of toxicity (CC_{50}) of the three plant extracts on Vero cells.
iii. To determine the acute oral toxicity (LD_{50}) of the three plant extracts in Swiss albino mice.

CHAPTER TWO

LITERATURE REVIEW

2.1: Cancer

The term cancer specifically refers to a new growth which has the ability to invade surrounding tissues, metastasize (spread to other organs) and which may eventually lead to the patient's death if untreated (Johns Hopkins Medicine, 2015). It usually arises as a result of a defect in one or more of the genes responsible for cell division. Damage to those genes can make the cells uncontrollably divide, to form a tumor (a lump of abnormal tissue) (Moura *et al.,* 2001).

2.2: Cancer burden.

Globally, cancer is now the leading cause of disease (Ferlay *et al.*, 2008). It is predicted that by 2025, there will be 19.3 million new cases per year. The most commonly diagnosed cancers worldwide were those of lung, accounting for 1.8 million, (13% of total), breast (1.7 million, 11.9%) and colorectal (1.4 million, 9.7%), (Bray *et al.,* 2013). According to the International Agency for Research in Cancer (IARC), at least 15 different types of cancers are related to smoking, and these are acute myeloid leukemia, liver, stomach, cervix, uterine, pancreatic, kidney, bladder, esophagus, paranasal sinuses, nasal cavity, nasopharynx, lip, pharynx, oral cavity, larynx and lung cancer (IARC,2007). Lung cancer has also been seen to be one of the most common cancers since 1985, and now 50% of cases in women and about 80% in men have been attributed to cigarette smoking (Parkin *et al.,* 2005). Breast cancer is also the most common cause of cancer deaths among women. In 2012, 1.7 million women were diagnosed with breast cancer and there were 6.3 million women alive who had been diagnosed with the disease in the previous 5 years.

Since the estimates from 2008, breast cancer incidence has increased by more than 20%, while mortality has increased by 14%. 522,000 deaths from breast cancer have been reported in 2012 alone, and the most frequently diagnosed cancer among women on 140 out of 184 countries worldwide. It now represents one out of four of all cancers in women.

The developing world is experiencing a dramatic rise in the cancer cases. More than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions in the world (Bray *et al.,* 2013). This is partly because of a shift in lifestyle and partly because clinical advances to combat the disease are not reaching women living in these regions (Bray *et al.,* 2013). This poses a threat because there is overstretching of the already limited resources as well as equipment. There is also a shortage of qualified staff making it a major challenge to effectively manage and treat the disease (Mohamed, 2003). Cancer has not been given priority as a public health concern like other communicable diseases like malaria, HIV and tuberculosis and therefore fewer resources are allocated to combat the disease (American Cancer Society, 2011).

Cancer is also an emerging public health scourge in Africa with 542,000 deaths and 715,000 incidences in 2008 alone; these numbers are however predicted to double by 2030 with approximately 970,000 cancer deaths and 1.28 million incidences (Ferlay *et al.,* 2008). The major contributing factors to these are the increase in population, aging, poor lifestyles and behavior change due to enhanced economic development (Ferlay *et al.,* 2008).

In Kenya, cancer is ranked third in causing deaths after cardiovascular and infectious diseases. It contributes to 7% of all the annual mortalities (22,000), with an estimate of 39,000 new cases and more than 27,000 deaths annually. The risk of acquiring the disease before attaining 75years being 14%, while that of dying at 12% (Kenya Ministry of Public Health and Sanitation & Ministry of Medical Services, 2009). In women, the leading cancers are breast (34 per 100,000) and cervical (25 per 100,000) while in men, prostate (17 per 100,000) and esophageal (9 per 100,000) are most common.

According to the Nairobi Cancer registry, 23.3% of all registered cases were of breast cancer, prostate accounted for 9.4% while cervical cancer was 20%. In 2006, 2,354 women were diagnosed as having cervical cancer resulting in 65% of them dying from the disease. Health systems in Kenya have placed too much emphasis towards preventing and controlling communicable diseases yet non-communicable

diseases like cancer, cardiovascular illnesses and chronic respiratory diseases have posed a greater challenge. As per the Regional Cancer Registry at KEMRI, almost 80% of reported cancer cases are at advanced stages, therefore very little gets to be achieved in terms of treatment, and this is attributed to lack of awareness, poor health and diagnostic facilities as well as shortages in both human and financial resources (Kenya Ministry of Public Health and Sanitation & Ministry of Medical Services, 2009).

2.3: Etiology of cancer

2.3.1: Cancer causing genes

For cell division to occur, four major types of genes have to be involved, and for tumors to occur, there must be faulty copies of more than one of these genes:

i. Oncogenes: these, under normal circumstances play an important role in cell division (ontogenesis). When activated, they speed up the rate of cell growth. When one of these oncogenes is damaged, they permanently remain in an 'on' state and continuously cause rapid division of cells.
ii. Defect in tumor suppressor genes: Normally, these prevent ontogenesis. An important tumor suppressor gene is p53.
iii. A defect in suicide genes makes cancerous cells to keep multiplying. Suicide genes are responsible in programming damaged cells to undergo apoptosis to prevent further damage to neighboring cells.
iv. Damage to a gene that makes the DNA repair protein is reduces it ability, and over a period of time, errors could occur.

2.4: Other causes of cancer.

These include:

i. Genetic factors which happen when defective tumor suppressor genes and cancer causing genes are inherited.
ii. Medication, especially immunosuppressants and ankylating agents.
iii. Lifestyle changes like alcohol, tobacco use, lack of exercise and eating unhealthy foods.
iv. Environmental as well as occupational exposure to chemicals like asbestos, benzene, vinyl chloride and ionizing UV radiation.

v. Viruses e.g. the Human Papilloma Virus (HPV), Hepatitis-B-virus (HBV) and Epstein- Burr Virus.

2.4: Diagnosis and treatment

Cancer diagnosis involves a series of assessments and diagnostic investigations like cytology, histopathology, biochemistry, imaging, endoscopy as well as other laboratory studies. Upon confirmation of the diagnosis, a series of therapeutic measures are embraced, with multidisciplinary treatments all aimed at improving the patient's quality of life. Some of them include radiotherapy, surgery, chemotherapy, hormonal therapy or a combination of all (Kenya Ministry of Public Health and Sanitation & Ministry of Medical Services, 2009).

2.5: Alternative or contemporary medicine

African traditional medicine is believed to be one of the oldest and diverse systems. Africa is considered as the cradle of mankind, with rich botanical and cultural diversity having different approaches in using traditional medicine for healing purposes (Gurib-Fakim, 2006). 90% of the population in Africa relies on traditional healers to meet their healthcare needs and this is because the synthetic anti-cancer drugs are beyond reach to the common man due to costs(Miller, 1990).

In Kenya, the conventional system provides for only 30% of the population, this means that more than two-thirds rely on traditional medicine for their health care needs (National Coordinating Agency for Population and Development, 2007).

It has also been a tradition in Africa that the ethnopharmacological and botanical knowledge of its uses are orally passed down from one generation to the next (Kokwaro, 1976). Ethnopharmacological information helps in providing the basis of finding new potential medicinal plants (Farnsworth, 1991; Wood-Sheldon, 1997).

2.6: Plant derived anti-cancer compounds

Plants contain a mixture of different classes of compounds which have portrayed various anti-cancer activities (Thurson, 2006). The isolation of vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle (*catharanthus roseus*) introduced a new era of the use of plant materials as anti-cancer agents.

They were the first agents to advance into the clinical use for the treatment of cancer. Vinblastine and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast, lung cancer and Kaposi's sarcoma (Cragg & Newman, 2005).

The discovery of Paclitaxel from the bark of the pacific yew, *Taxus brevifolia* Nutt. (*Taxaceae)* provided additional evidence of the success in natural product drug discovery (Cragg & Newman, 2005).Various parts of *Taxus brevifolia* and other *Taxus* species like the *Taxus Canadensis* Marshall and *Taxus baccata* L. have been used by several Native American tribes for the treatment of some non-cancerous cases but *Taxus baccata* was reported to be used in the Indian ayurvedic medicine for the treatment of cancer (Cragg & Newman, 2005). Paclitaxel is significantly active against ovarian cancer, advanced breast cancer, small and non-small cell lung cancer (Rowinsky *et al.*, 1992).

Camptothecin, isolated from the Chinese ornamental tree *camptothetica acominate* Decne (*Nyssaceae*), was advanced to clinical trials in the 1970s but was dropped because of severe bladder toxicity (Potmeisel M & Pinedo H, 1995). Topotecan and Irinotecan are semi-synthetic derivatives of camptothecin and are used for the treatment of ovarian and small cell lung cancers and colorectal cancer respectively (Creemers *et al.*, 1996; Bertino, 1997).

Epipodophyllotoxin is an isomer of podophyllotoxin which was isolated as the active anti-tumor agent from the roots of *podophyllum* species, *podophyllum peltatum* Linnaeus and *podophyllum emodi* Wallich (*Berberidaceae*), (Stahelin, 1973). Etoposide and toniposide are the two semi-synthetic derivatives of Epipodophyllotoxin and are used in the treatment of lymphomas, bronchial and testicular cancers (Cragg & Newmann, 2005; Harvey, 1997).

Homoharringtonine was isolated from the Chinese tree *cephalotaxus harringtonia* var *drupacea* (Itokawa *et al.*, 2005; Powell *et al.*, 1970).

A racemic mixture of harringtonine and Homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia (Cragg & Newmann, 2005; Kantarjian *et al.*, 1996).

Elliptinium, a derivative of ellipticine, isolated from a Fijian medicinal plant *bleekeria vitensis* A. C. sm., is marketed in France for the treatment of breast cancer (Cragg & Newman, 2005).

2.7:*Prunus africana*

2.7.1: Description

Prunus africana, commonly known as pyegum or Africa cherry, belongs to the *Rosaceae* family (Kokwaro, 1993). It is widely distributed in various Kenyan provinces especially in the Mount Kenya forest (Kokwaro, 1993). The plant is also found in various parts of the Eastern Cape Province, KwaZulu/Natal and Mpumalanga, often in forested margins and riverine vegetation. Other countries possessing *P. africana* include: Madagascar, guinea, Cameroon, Zaire, Uganda, Mozambique and eastern Zimbabwe. Pharmaceutical companies in France (Prosynthèse), Germany (Madaus), Italy (Indena Spa; Inverni Della Buffa) and in Spain have heavily utilized this species for commercial purposes (Cunningham & Mbekum, 1993).

2.7.2: Chemical compounds

It was documented in 1963 that there was cyanogenic glycoside amygdalin in the fruit, leaf and bark of *P. africana* and from then, growing interests in the use of the bark extracts in the treatment for Benign Prostatic Hyperplasia (BPH) steered several studies on the bark extracts. These studies revealed the presence of trans-ferulic acid esters of 2, 3 and 4, long chain (12-22) fatty acids like palmitic acid, long chain aliphatic alcohols like n-docosanol, n-tetracosanol, pentacyclic triterpenoids, mainly of oleanolic and ursolic acid type and phytosterols like β-sitosterol 1,5 (18%), 3-O-glycoside, β-sitostenone, compesterol and daucosterol (Awang, 1997 and Cristoni *et al.,* 2000).

Literature available shows that triterpenic acids such as derivatives of ursolic and leanoic acid have been isolated with the help of phytochemical studies (Fourneau *et al.,* 1996).

2.7.3: Medicinal uses

In Kenyan traditional medicine, *P. africana* is used to treat chest pain, fever and malaria (Kokwaro, 1993). Stem barks are boiled in water and two glasses taken in the mornings and evenings. It has also been used as remedy for diarrhea, allergies, stomach ache, prostate gland and kidney diseases (Pojal, 1990; Iwu, 1993). Manufactured extracts from the stem bark of *P. africana* have been used for the treatment of enlarged prostate cancer and severe cases of Benign Prostate Hyperplasia (BPH) in modern medicine (Sunderland & Obama, 1999, Schipmarn, 2001).

Extracts from the bark of *P. africana* tree have been reported to treat malaria, gastralgia, chest pain, heart burn and madness (Jiofack *et al,* 2010). The bark extracts in Europe are used to treat BPH. According to clinical data, it has been used to relief stage 1 and 2 lower urinary tract symptoms of BPH, in cases where there was negative diagnosis of prostate cancer; the symptoms include urinary retention, polyuria and nocturia (WHO, 2002).

2.7.4: Biological activity

Studies have shown that *P. africana* has biological properties against *Mycobacterium,* with dichloromethane and ethyl acetate extracts of the plant's bark showing an MIC value of 6.25 and 1.56 respectively (Ali *et al.,* 2001; Kumarasamy *et al.,* 2002; Hamill *et al.,* 2003; Bii *et al.,* 2008).

A study aimed at evaluating the in vitro anti-fungal and anti-microbial properties of methanol and hexane extracts of *P. africana* showed that the methanol extracts were highly active against *Streptococcus pneumoniae, Staphylococcus aureus* ATCC25923, Methicilin Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeroginosa* ATCC 27852 (Bii *et al.,* 2010).

Positive results have also been noted in BPH patients undergoing treatment with the standardized bark extracts of *P. africana*, and this included marked reduction in frequent urination and residual volume of urine and an increase in the volume of voided urine and flow rate as compared to the placebo. Other effects demonstrated by *P. africana* include reduced histamine-induced vessel permeability and reduction in inflammation and edema. These effects have been achieved because of the ferulic acid esters (hypocholesterolemic), pentacyclic triterpenes (immunostimulant, anti-edema) and phytosterols (anti-estrogen, anti-inflammatory), which are compounds found in the plant (WHO, 2002).

The phenomenon of *P. africana* sterols/lipids from bark extracts being used as treatment for BPH is not clearly understood, however, some or all of the following mechanisms have been suggested to be responsible (WHO, 2002):

i. The inhibition of 5α-reductase that prevents the conversion of testosterone to dihydrotestosterone (DHT).
ii. Inhibition of aromatase, which prevents the conversion of DHT to androstandiol therefore preventing the synthesis of estrogen/estradiol.
iii. Synthesis of leukotrienes being blocked by the inhibition of 5-lipoxygenase.
iv. Reduction of edemas by inhibiting β-glucoronidase and glycosyl transferase.

2.8: *Warburgia stuhlmannii*

2.8.1: Description

Warburgia stuhlmannii belongs to the *canellaceae* family. It is a rare tree found in forests from coastal regions and wooded grasslands, and it grows at an altitude of about 0-400m. The species is only found in Kenya and Tanzania (Orwa *et al.*, 2009).

2.8.2: Uses of *Warburgia stuhlmannii*

For its products, it can be used in food, its barks, leaves and fruit have a hot taste; the bark is used traditionally as a spice.

It has also been used as timber in making wooden materials and furniture because of its beautiful appearance, luster, smell and texture. The oil from its wood has also been extracted to make perfumes.

For medicinal purposes, the bark has been used as a remedy for tooth aches and rheumatism. Its pulverized bark, when mixed with honey, is used as cough medicine and exudates from its bark, when mixed with an egg, boiled and consumed have been used for constipation (Orwa *et al.,* 2009).

The stem bark obtained from *Warburgia stuhlmannii* has also been used in the treatment of both anti-tumor and anti-inflammatory diseases in traditional medicine (Beentje, 1999).

In a study conducted to biologically screen some Kenyan medicinal plants, it was found that *Warburgia stuhlmannii* had a lethality value (LC_{50}) of 8ug/ml (Nguta *et al.,* 2011).

This was consistent with the existing phytochemical information on the plant to possess anti-tumor and cytotoxic compounds (Manguro *et al.,* 2003). Methanolic and water extracts from *Warburgia stuhlmannii* have exhibited anti-plasmodial activity making the plant an agent for anti-malarial drugs (African Biodiversity Conservation and Innovations Center, 2001).

2.8.3: Chemical compounds of *Warburgia stuhlmannii*

An investigation of the Methanolic extracts of leaves from *Warburgia stuhlmannii* led to the isolation of two new drimane-type sesquiterpene glycosides i.e. mukadial 6-O-β-D-glucopyranoside and two new flavonol glycosides, 3'-5'-O-dimethylmyrecetin 3-O-β-D-2'',3''-diacetylglucopyranoside and 3'-o-methylquercetin 3-O-β-D-2'',3'',4''-triacetylglucopyranoside. The known compounds i.e. mukadial, diacetylugandesolide, quercetin, kaemferol, kaemferol 3-O-α-rhamnopyranoside, quercetin 3-O-β-D-glucopyranoside, kaempferol 7-O-β-D-glucopyranoside, myricetin 3-O-α-L-rhamnopyranoside, quercetin 3-O-α-L-rhamnopyranoside, quercetin 3-o-sophoroside and isorhamnetin 3-O-β-D-

glucopyranoside were also obtained from the extract of *Warburgia stuhlmannii* (Lawrence *et al.,* 2003).

2.9: *Maytenus senegalensis*

2.9.1: Description

Maytenus senegalensis also commonly known as the 'spike thorn', is synonymous to *Gymnosporia senegalensis* and it belongs to the *celastraceae* family (Farnsworth and Soejarto, 1991). Geographically *Maytenussenegalensis* is found in Arabia, Afghanistan and India. It is also widespread in the savannah regions of tropical Africa (Jansen and Mendes, 1991).

2.9.2: Chemical compounds

Important bioactive secondary metabolites have been isolated from the cellastraceae family and these include alkaloid amines such as cathine and benzylisoquinolide alkaloids. Celastaceae members are commonly tanniferous, containing anthocyanins, but they can also be saponiferous although rarely can they be cyanogenic and without iridoid compounds (Hutchings *et al.,* 1996).

Amongst other compounds isolated from this species, triterpenes and triterpenoid quinonemethides are of great importance because they portray a wide variety of biological activity (Mueller & Mechler, 2005). Constituents of β-amyrin, lupine derivatives and quinoid pigments are also typical extracts.

The compounds that have been isolated from the *Maytenus*genus include the ansa macrolide, maytansine and related macrolides like normaytansine, maytanprine and maytanbutine (Hutchings *et al.,* 1996). Others include spermadine alkaloids like celacinnine and celallocinine and nicotinoyl sesquiterpene alkaloids like maytoline and maytolidine as well as catechin, procynidines and phenoldienne triperpenoids (Hedberg *et al.,* 1982).

Other compounds isolated from the *Maytenus* species have (-)-4-methylepigallocatichin(oura-tea-proanthocyanidin) and maytenoic acid (Brüning & Wagner, 1978, Abraham *et al.*,1971, Delle Monache *et al.*, 1976). Ethanolic extracts of its stem showed cytotoxic effects against carcinoma in cell culture and leukemia in mice (Tin-wa *et al.*, 1971).

Studies have also shown that compounds from *M. senegalensis* leaves contained alkaloids, alkanes, alkanols, terpenes, steroids and phenol compounds (Mueller and Mechler, 2005). Its stem-bark had tannins detected (Muregi *et al.*, 2007). The terpenoid content of the active fractions from *Maytenussenegalensis* was found to be associated with anti-leishmanial and antiplasmodial properties (El Tahir *et al.*, 1998, 1999). When screened for antiviral activities, the ethanolic and water extracts of *Maytenus senegalensis* exhibited inhibitory effects against HIV-1 protease with IC_{50}values of 104 and 88µg/ml respectively (Otake *et al.*, 1995).

2.9.3: Medicinal uses of *Maytenus senegalensis*

In some African regions, the roots and bark of *M. senegalensis* are used in traditional medicine for treatment of several illnesses including chest pain, rheumatism, snake bites, diarrhea, eye infection, dyspepsia and wounds (Matu & van Staden, 2003, Okine *et al.*, 2005). In Sudan the aqueous extract of the stem-bark of *M. senegalensis* (*celastraceae*) is commonly used in the treatment of tumors, dysentery and snakebites. Its vernacular name is “Dabalab” (Kupchan and Smith, 1977, Brüning & Wagner, 1978, Shirota *et al.*, 1996).

Upon brewing, the roots are used for tooth ache, skinning of wounds and gonorrhea. Its leaves are also used for eye infections, tooth ache, stomatitis, gingivitis, gastric ulcers and bilharzias. Different species of *M. senegalensis* have also been reported in traditional medicine to have been used as anti-inflammatory and analgesic agents after oral or in topical administration (Neuwinger, 2000) showing pain inhibition of up to 72%, in the anti-inflammatory activity of the leaves and roots, inhibition of edema, 3 hours after injection of Carrageenan was 64% and 66% respectively (Sanogo *et al.*, 2006).

In western Kenya, among the Bukusu community, the fruit, leaf and stem bark of *Maytenussenegalensis* is used against livestock ticks after application on the animal's body surfaces (Wanzala *et al.,* 2012).

2.9.4: Pharmacological effects.

Previous biological studies have shown that the roots and stem extracts demonstrated anti-plasmodial activity *in vitro* against a cloroquine-sensitive strain of *Plasmodium falciparum* (D10) (Clarkson *et al.,* 2004). Extracts from the stem bark of *M. senegalensis* have also been demonstrated in vitro anti-leishmanial activity against the promastigotes of leishmania major reference vaccine strain (5AKSH) (El Tahir, 1998).

The leaf and bark extracts of *M. senegalensis* have also shown to inhibit cyclo-oxygenase-1, an enzyme that synthesizes anti-inflammatory mediators like thromboxanes and prostaglandins (Matu & van Staden, 2003).

The root-bark extract of *M. senegalensis* also contains maytenoic acid, whose properties have proven anti-bacterial activities against *Bacillus subtilis, Escherechia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* (Lindsey *et al.,* 2006). Fractions from both the stem and stem barks from *M. senegalensis* have been shown to inhibit growth of various causative agents of the genitourinary tract infections like *Klebsiella pneumoniae, Proteus vulgaris, Escherechia coli, Staphylococcus aureus* and *Pseudomonas aeroginosa* (Mbatchou & Adoum, 2010).

Acetone extracts of the aerial parts of this species have been discovered to be active against the strain H37Rv, 0.5mg/ml of *Mycobacterium tuberculosis* (Lall & Meyer, 1999).

Different species of the genus *Maytenus* have been used in African traditional medicine to prepare infusions for anti-inflammatory and analgesic remedies for oral and topical administration (Jorge *et al.,* 2004).

Pristimerin and Aytenoic acid from *Maytenus* genus have been known tosignificantly suppress inflammation (Abraham *et al.,* 1971; Sosa *et al.,* 2007). Although there has been few biological studies to evaluate the anti-inflammatory activity of the African *Maytenus* species, the anti-inflammatory activity in the American species like *M. ilicifolia* (Joyce *et al.,* 2004), *M. aquifolium* (Kimura *et al.,* 2000), *M. boavia* (Backhorse *et al.,* 1994) and *M. rigida* (dos Santos *et al.,* 2007) have been verified. It is thought that this activity is due to the presence of phenol and triterpene metabolites. In a recent study, the anti-inflammatory activities of *M. heterophyla* and *M. senegalensis* ethanol extracts (70%) were investigated in Winstar albino rats using carcigeenan-induced paw edema method and the extracts portrayed significant anti-inflammatory activity, reducing edema by 51% and 35% respectively (da Silva *et al.,* 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Study site

This study was conducted at the Centre for Traditional Medicine and Drug Research (CTMDR) and the animal facility at the Kenya Medical Research Institute (KEMRI).

3.2: Study design

This was an experimental laboratory-based study nested in an ongoing cancer project at the Centre for Traditional Medicine and Drug Research (CTMDR).

3.3: Laboratory procedures

3.3.1: Plant materials

The leaves and stem barks of *Maytenus senegalensis* and*Warburgia stulhamannii* were collected from Kwale County while those of *Prunus africana* from Nyeri County. Five kilograms of the plant parts were air dried in mesh bags and voucher specimens and deposited at the East African Herbarium, National Museums of Kenya. The plant parts were then delivered to Kenya Medical Research Institute, Centre for Traditional Medicine and Drug Research (CTMDR). A taxonomist was involved during plant identification and collection.

Table 3.1: Plant species, voucher numbers and plant parts collected.

Botanical name	Family	Voucher number	Part used
Prunus africana	Rosaceae	SW00017	leaf and stem bark
Warburgia stuhlmannii	Canellaceae	SW00026	leaf and stem bark
Maytenus senegalensis	Celastraceae	SW00027	leaf and stem bark

3.3.2: Preparation of the plant extracts

The plant materials were dried at room temperature (25^{o}C) pulverized using a laboratory mill (Christy and Norris Ltd., Chelmsford, England) and packed air tight in polythene bags. Each plant sample was separately extracted using both water and methanol. For water extraction, 100g of the dried ground plant materials were soaked in 1000ml of distilled water and put in a water bath at 70^{o}C for 1 hour, filtered and lyophilized in a Freeze Dryer (Edwards freeze dryer Modulyo). For the methanol extraction, 100g of the dried plant materials was percolated with 1000ml of methanol at room temperature for 3 days. The methanol extracts were filtered through Whatman filter paper no. 1 and concentrated to dryness under reduced pressure using a rotary evaporator (J.B. Harborne, 1984). The extracts were then weighed, labeled and stored in air tight bijou bottles at 4 ℃ prior to use. 100mg of the extracts were dissolved in 1ml DMSO to make a stock solution of 100,000µg/ml in 100% DMSO sterilized by filtration (at pore size of 0.2µm) before testing. The working solution was made by diluting1 part of the stock solution to 99 parts of Earl's Minimum Essential Medium containing 2% Fetal Bovine Serum (FBS) (maintenance medium), which was 10µl of the extract in 990µl of media to give a start concentration of 1000µg/ml in 1% DMSO which was used in the MTT ((3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay. The percentage yield was calculated as follows:

$$\text{percentage yield} = \frac{\text{weight of plant after extraction}}{\text{weight of plant before extraction}} \times 100\%$$

3.3.3: Cell culturing

The mouse mammary breast cancer cell line (4T1 ATCC®CRL-2539™), mouse colon cancer cell line (CT26.WT-ATCC® CRL-2638™) and Vero cells (monkey kidney cells) were obtained from the American Type Culture Collection (ATCC) to represent the human cancer cells. They were revived and cultured in T-75 flasks with Earl's Minimum Essential Media (EMEM), all supplemented with penicillin& streptomycin(1%) and 10% Fetal Bovine Serum maintained at 37˚C in a humidified atmosphere of 5% CO_2 to achieve a monolayer.

3.3.4: 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay for cytotoxicity

The *In vitro* cytotoxicity test was carried out using a rapid calorimetric assay (Mosmann, 1983) which is based on the capacity of succinate dehydrogenase enzyme in the mitochondria of living cells to reduce the yellow water soluble substrate MTT into formazan, which is measured spectrophotometrically (Masters, 2000; Wilson, 2000). Upon attainment of confluence, Cells were detached by trypsinization, and the number of viable cells determined by Trypan blue exclusion test (cell density counting). A hemocytometer was used to aid in counting viable cells, which were seeded at 2×10^5/ml cell suspension for the Vero cells and 1×10^5/ml cell suspension for the 4T1 cells and colon cancer cells on 96-well plates and incubated at 37ºC in 5% CO_2 for 24 hours. The test sample extracts were then added to the plates and incubated for 48 hours at 37^{o}C with 5% CO_2.At the end of the incubation time, 10µl of MTT dye (5mg of MTT, dissolved in 1ml serum free medium (Phosphate Buffered Saline (PBS)) was added to all the cells and incubated for another 4 hours. All media was then removed from the plates and 100µl of 100% DMSO added.

The plates were then read on a scanning multi well spectrophotometer (Multiskan Ex labssystems) at 562nm and 690nm as reference. Data was analyzed as follows, for both the Vero cells, colon and 4T1 cells:

$$\% \text{ Cell viability} = \left[\frac{OD_{sample562} - OD_{690}}{OD_{control562} - OD_{690}}\right] \times 100$$

Where OD = optical density (Mosmann, 1983)

Data was then transferred onto a graphic programme (Excel work sheet) and plotted as dose-response curves, from which IC_{50} (concentration required to cause visible alterations in 50% of intact cells) was estimated on the colon and 4T1 cells, while CC_{50}(concentration required to kill 50% of the intact cells) was estimated on Vero cells.

Podophyllotoxin resin from the *Podophyllumhexandrum*plantwas used as the standard reference drug to represent plant derived anti-cancer drugs with an initial concentration of 1000µg/ml in 1% DMSO.

The procedures were done in triplicate and the cytotoxic results (CC_{50}) determined whether mice would be used in this study for acute oral toxicity assays as only the extracts with low IC_{50} and high CC_{50} were selected.

3.3.3: Study animals

The Swiss albino mice from the KEMRI animal facility weighing 20±2g were used in this study. The animals were moved to an experimental room for acclimatization for one week before the experiment.

Five mice of the same sex (female) aged 8 weeks were housed in 15×21×29 cm steel cages. They were bedded with wood shavings and equipped with a continuous flow of nipple watering devices. The mice were fed with pellets (Mice Pellets UNGA® feeds, Kenya) and water *ad libitum.* The wood shaving dressings in the cages were changed on daily basis.

The animals were handled as humanely as possible and in the same manner as before the onset of the experiment (they were provided with feeds and water). At the end of the experiment, the animals were immediately euthanized in a CO_2chamber and incinerated (William, 1976).

3.3.4: Drug administration

The basis of this study was to calculate LD_{50}. A total of 55 Swiss Albino mice were used in the study as in 3.3.3 above aged 6 weeks weighing between 20±2g were used in the study. Each test consisted of 25 mice, 5 mice for each dosage labeled as group 1-5 (Group 1-500mg/kg, Group 2-889.53mg/kg, Group 3-1581.6mg/kg, Group 4-2812.5mg/kg and Group 5-5000mg/kg). For the water extracts, the dosages were prepared by dissolving each one of them in distilled water. For the methanol extracts, a stock solution was first prepared by mixing 700µl of Tween 80 with 300µl of analytical ethanol, after which a working solution was prepared by mixing 100µl of the stock solution with 900µl of distilled water.

5 mice were used as negative control and these were given 0.2ml of distilled water. Each mouse received only a single oral dose of 0.2ml of the drug in the entire experiment. The mice were deprived of feeds 12 hours prior to introduction of the drug and 3 hours after.

The animals were observed over a period of 24-48 hours for signs of acute oral toxicity, for example reduced activeness, convulsions, writhing, decreased motor activity, decreased body/limb tone, decreased respiration, mortality rate and survival period, in this study, none of these were noted.

The Lorke formula $r = (n-1)\sqrt{L/l}$ was used in calculating the oral dosages whereby:

L = largest dose n = the number of dosages

l = smallest dose r = ratio.

Since 5 dosages were used in the assay, with 5000mg as the highest dose and 500mg as the lowest, using the Lorke formula,

r = (5-1)$\sqrt{5000 \div 500}$ = 4$\sqrt{10} = 1.778$

1st dose = 5000mg/kg/day.

2nd dose = 5000mg/kg/day ÷1.778 = 2812.15mg/kg/day.

3rd dose = 2812.15 mg/kg/day ÷ 1.778 = 1581.6mg/kg/day.

4th dose = 1582mg/kg/day ÷1.778 = 889.53mg/kg/day.

5th dose = 890mg/kg/day ÷ 1.778 = 500mg/kg/day.

The setup is shown as in table 3.2.

Table 3.2: Drug administration design

Groups.	**Dose levels (mg/kg).**	***P.africana*.**	***W. stuhlmannii.***	***M. senegalensis.***	**Controls (distilled water).**
1	500	5 mice	5 mice	5 mice	-
2	889.53	5 mice	5 mice	5 mice	-
3	1581.6	5 mice	5 mice	5 mice	-
4	2812.15	5 mice	5 mice	5 mice	-
5	5000	5 mice	5 mice	5 mice	-
-	-	-	-	-	5 mice

3.3.5: LD_{50} determinations

Determination of LD_{50} was carried out using the Lorke formula (Lorke, 1983).Five dose levels of each compound were administered orally to the Swiss Albino mice as in experiment 3.3.4.

The animals were observed over a period of 24-48 hours for signs of acute toxicity and mortality. The number of deaths within this period was to be noted and recorded. They were also observed twice daily for 14 days, with their weights recorded before drug administration and after the 14 days. Classification of toxicity was described based on the scale of Loomis and Hayes, 1996 as shown in table 3.3

Table 3.3: Classification of toxicity by Loomis and Hayes (1996)

Dosage (mg/kg)	Classification of toxicity
1 or less	Extremely toxic
1-50	Highly toxic
50-500	Moderately toxic
500-5000	Slightly toxic
5000-15000	Practically non-toxic
More than 15000	Relatively harmless

3.4: Data management and analysis

Data was entered in notebooks and files kept in safe custody in the laboratory. It was then keyed in MS Access, which acted as the database. During analysis data was exported to the Excel worksheet from which IC_{50} and CC_{50}were calculated. Results were expressed as mean ± Standard Error of Mean (SEM), while the differences in weight for the mice studies and means was analyzed statistically using the Student's t-test.

Differences between means was considered statistically significant at $p<0.05$. Descriptive statistics was used to summarize the data and determine the trends.

A measure of central tendencies was also computed. Summary tables were used as tools for data presentation.

3.5: Ethical approvals

Ethical clearance was sought from Kenya Medical Research Institute (KEMRI) Scientific Steering Committee (SSC), Animal Care and Use Committee (ACUC), and Ethical Review Committee (ERC) before study implementation. The clearance letters are attached in Appendix I, II and III.

3.6 Intellectual Property Rights

There were no intellectual property rights issues involved in this study because the medicinal plants used were obtained from their natural habitats using available literature. The ethnobotanical and ethnomedicinal information on medicinal plants is well documented and is in the public domain. The purpose of this research project was to determine the antiproliferative activity of *Prunus africana, Warburgiastuhlmannii*and *Maytenus senegalensis* in breast and colon cancer cell lines while the nesting project was exploiting the anti-cancer properties of three medicinal plants traditionally used in Kenya i.e. *aloe vera*, *Prunus africana* and *Moringa oleifera.*Therefore all the resultant interventions, discoveries and their associated property rights in this research project was independent of the nesting project SSC no. 2434.

CHAPTER FOUR

RESULTS

4.1: Extraction

A total of 12 extracts from the leaf and stem bark of three plant species representing 3 families were extracted using methanol and water. Table 4.1 shows the percentage yields of the 12 plant extracts.

Table 4.1: Plant species and percentage yields of water and methanol extracts

Plant	**Part**	**Extraction method**	**Weight after extraction (g)**	**% yield**
Prunus Africana	Leaf	Water	2	2
Prunus Africana	Leaf	Methanol	49	49
Prunus Africana	Stem bark	Water	16	16
Prunus Africana	Stem bark	Methanol	35.04	35.04
Maytenus senegalensis	Leaf	Water	18.65	18.65
Maytenus senegalensis	Leaf	Methanol	39.51	39.51
Maytenus senegalensis	Stem bark	Water	14.38	14.38
Maytenus senegalensis	Stem bark	Methanol	29.78	29.78
Warbugia stuhlmannii	Leaf	Water	24.49	24.49
Warbugia stuhlmannii	Leaf	Methanol	77.63	77.63
Warbugia stuhlmannii	Stem bark	Water	24.12	24.12
Warbugia stuhlmannii	Stem bark	Methanol	44.82	44.82

4.2: IC_{50} results with the 4T1 cells

The concentration that inhibited growth in 50% of the cells (IC_{50}) was calculated for the 4T1 cells. Methanol extracts of *P. africana* stem bark, *M. senegalensis* stem bark and *W. stuhlmannii* leaf had the lowest IC_{50} values of 26.37±3.54, 32.96±2.91 and 75.30±6.31µg/ml respectively, while the reference drug, *Podophyllum hexandrum* resin had IC_{50} of 3.14±0.19µg/ml. Table 4.2 shows the results of each plant extract together with the reference drug, *P. hexandrum.*

Table 4.2: IC_{50} results of the plant extracts with 4T1 cells.

Plant extracts	**IC_{50}(µg/ml)**	**SI**
Prunus africana leaf water	570.89±11.21	1.75
Prunus africana leaf methanol	164.64±4.14	6.07
Prunus africana stem bark water	133.51±2.13	0.42
Prunus africana stem bark methanol	26.37±3.54	7.46
Maytenus senegalensis leaf water	>1000	1
Maytenus senegalensis leaf methanol	256.41±4.77	1.81
Maytenus senegalensis stem bark water	>1000	1
Maytenus senegalensis stem bark methanol	32.96±2.91	2.26
Warburgia stuhlmannii leaf water	>1000	1
Warburgia stuhlmannii leaf methanol	75.30±6.31	2.44
Warburgia stuhlmannii stem bark water	332.79±7.53	3.00
Warburgia stuhlmannii stem bark methanol	123.69±1.58	1.25
Podophyllum hexandrum	3.14±0.19	318.47

4.5: IC_{50} results with colon cancer cells

IC_{50} was also calculated for the colon cancer cell lines. The IC_{50} varied with the plant extract and the solvent used for extraction. The lowest IC_{50} was registered from methanol extract of *M. senegalensis* stem bark, *M. senegalensis* leaf, *W. stuhlmannii* stem bark and *P. africana* leaf with IC_{50} values of 2.32±0.17, 4.18±0.14, 13.94±0.27 and 21.33±0.75µg/ml respectively, while the reference drug, *P. hexandrum* resin had IC_{50} value of >1000µg/ml. Table 4.3 shows the IC_{50} results together with the reference drug, *P. hexandrum.*

Table 4.3: IC_{50} results of the plant extracts with colon cancer cells

Plant	**IC_{50} in (µg/ml)**	**SI**
Prunus africana leaf water	716.75±3.32	1.40
Prunus africana leaf methanol	21.33±0.75	46.88
Prunus africana stem bark water	83.53±1.58	0.67
Prunus africana stem bark methanol	176.90±0.89	1.11
Maytenus senegalensis leaf water	87.52±0.31	11.43
Maytenus senegalensis leaf methanol	4.18±0.14	111.01
Maytenus senegalensis stem bark water	461.06±6.84	2.17
Maytenus senegalensis stem bark methanol	2.32±0.17	32.15
Warburgia stuhlmannii leaf water	371.56±11.35	2.69
Warburgia stuhlmannii leaf methanol	149.51±0.94	1.23
Warburgia stuhlmannii stem bark water	107.20±2.50	9.33
Warburgia stuhlmannii stem bark methanol	13.94±0.27	11.07
Podophyllum hexandrum	>1000	1

4.5: CC_{50} results with VERO cells

The concentration of plant extracts that killed (reduced cell viability) in 50% of the cells (cytotoxic concentration, CC_{50}) was calculated. The water and methanol extracts from the leaves of *P.* africana, water extracts of the leaf and stem bark of *M.* senegalensis, water extracts from the leaves and stem bark of *W. stuhlmannii* and the reference drug *P. hexandrum* all exhibited CC_{50} values of >1000 µg/ml. Table 4.4 shows the results

Table 4.4: CC_{50} results of the plant extracts with VERO cells

Plant	**CC_{50}(µg/ml)**
Prunus africana leaf water	>1000
Prunus africana leaf methanol	>1000
Prunus africana stem bark water	55.64±4.41
Prunus africana stem bark methanol	196.84±4.62
Maytenus senegalensis leaf water	>1000
Maytenus senegalensis leaf methanol	464.04±0.02
Maytenus senegalensis stem bark water	>1000
Maytenus senegalensis stem bark methanol	74.59±3.21
Warburgia stuhlmannii leaf water	>1000
Warburgia stuhlmannii leaf methanol	184.08±6.08
Warburgia stuhlmannii stem bark water	>1000
Warburgia stuhlmannii stem bark methanol	154.37±0.77
Podophyllum hexandrum	>1000

4.6: Selectivity index

The Selectivity index ($SI=CC_{50}/IC_{50}$) was calculated from the CC_{50} ratio of the normal Vero cells and IC_{50} of the cancerous (4T1, CT26.WT) cells. SI value indicates selectivity of the sample to the cell lines tested. Samples with SI value greater than 3 were considered to have high selectivity. From the SI column in table 4.2 and 4.3, methanol extract of *P. africana* stem bark and leaf had SI>3 (7.46, 6.07), against 4T1 cancer cell lines while methanol extracts of *M. senegalensis* leaf, *P. africana* leaves, *M. senegalensis* stem bark, water extract of *M. senegalensis* leaf, *W. stuhlmannii* stem bark and methanol extract of *W. stuhlmannii* stem bark had SI greater than 3 (111.01, 46.88, 32.15, 11.43, 9.33, 11.07 respectively) against colon cancer cell lines. All the other extracts had SI values less than 3 and were therefore considered non selective to the other specific cancer cell lines.

4.7: Acute oral toxicity

4.7.1: *Warburgia stuhlmannii*

There was no mortality recorded within 48 hours and during the 14 day period of observation in all mice groups that received the water extracts from the stem bark of *W. stuhlmannii*, LD_{50} was therefore >5000mg/kg body weight. There was an increase in body weight in all mice groups as shown in table 4.5. Mice that received 5000mg/kg (group 5) had a significant difference in weight compared to the control group ($p<0.05$). Those that received 500mg/kg (group 1), 889.53mg/kg (group 2), 1581.6mg/kg (group 3) and 2812.5mg/kg (group 4) had no significant difference compared to the control group ($p>0.05$). Table 4.5 shows the results.

Table 4.5: Weights of mice before and after oral administration of *Warburgia stuhlmannii*

Dosage levels	Mice weight in (g) before drug administration	Mice weight in (g) after drug administration
Group 1	21	22
(500mg/kg/day)	20	28
(0.5mg)	22	28
	22	30
	21	23
Group 2	21	28
(890mg/kg/day)	22	29
(0.89mg)	22	26
	22	30
	20	28
Group 3	18	27
(1582mg/kg/day)	22	28
(31.63mg)	21	26
	22	31
	21	26
Group 4	22	24
(2812mg/kg/day)	19	21
(56.24mg)	19	27
	21	26
	20	23
Group 5	18	20
(5000mg/kg/day)	20	24
(100mg)	22	29
	21	26
	22	24
Negative control	22	27
	21	27
	20	28
	22	30
	19	26

Table 4.6: Comparisons between means of weight differences with the control

Comparing Groups Vs Control	Mean (Control Mean 6.8)	SE	95% CI	P-value
Group 1(500mg/kg)	5	1.594	(-1.86, 5.46)	0.291
Group 2 (889.53mg/kg)	6.8	0.938	(-2.16, 2.16)	1
Group 3 (1581.6mg/kg)	6.8	1.086	(-2.51, 2.51)	1
Group 4 (2812.5mg/kg)	4	1.281	(-0.15, 5.75)	0.06
Group 5(5000mg/kg)	4	1.114	(0.23, 5.37)	0.036

*Mean difference is significant at the 0.05 level.

4.7.2:*Prunus africana*

Mice that received a single dose of methanol extracts from the leaves of *P. africana* ranged from 500 to 5000mg/kg body weight. There was no mortality recorded within 48 hours and during the 14 day period of observation, LD_{50} was therefore >5000mg/kg body weight. There was a general increase in body weight in all mice groups as shown in table 4.8; this is because all the dosages did not affect weight gain. Those that received 1581.6mg/kg/day (group 3), 2812.5mg/kg (group 4) and 5000mg/kg (group 5) had a significant difference in weight compared to the control group ($p<0.05$).The weights of mice that received 500mg/kg (group 1) and 889.53mg/kg (group 2) had no significant difference with that of the control group ($p>0.05$). Table 4.7 shows the results.

Table 4.7: Comparisons between means of weight differences with the control

Comparing Groups Vs Control	Mean (Control Mean 6.8)	SE	95% CI	P-value
Group 1(500mg/kg)	4.2	1.371	(-0.56, 5.76)	0.095
Group 2 (889.53mg/kg)	5	1.114	(-0.77, 4.37)	0.145
Group 3 (1581.6mg/kg)	3.4	1.342	(0.31, 6.49)	0.035
Group 4 (2812.5mg/kg)	3	1.068	(1.34, 6.26)	0.007
Group 5(5000mg/kg)	2.8	0.883	(1.96, 6.04)	0.002

*Mean difference is significant at the 0.05 level.

Table 4.8: Weights of mice before and after administration of *Prunus africana*

Dosage levels	Mice weight in (g) before drug administration	Mice weight in (g) after drug administration
Group 1	21	26
(500mg/kg/day)	21	23
(0.5mg)	19	27
	18	23
	21	22
Group 2	21	27
(890mg/kg/day)	19	22
(0.89mg)	21	24
	20	25
	18	26
Group 3	18	21
(1582mg/kg/day)	22	24
(31.63mg)	21	22
	20	23
	18	26
Group 4	20	24
(2812mg/kg/day)	18	24
(56.24mg)	21	22
	21	23
	22	24
Group 5	18	20
(5000mg/kg/day)	20	24
(100mg)	22	29
	21	26
	22	24
Negative control	22	27
	21	27
	20	28
	22	30
	19	26

CHAPTER FIVE

DISCUSSION

5.5: Cytotoxicity studies

Vero cells have been recommended for cytotoxicity studies and for the analysis of cell-substrate interactions in biomaterial research (ISO, 1992; Kirkpatrick, 1992). This study shows investigations of anti-cancer potential of three plant species which has not been studied in Kenya, by screening for cytotoxic activity against healthy cells and two mouse model cancer cell lines, 6 out of 12 extracts showed low or no toxicity against the control cell lines (Vero cells), this includes water and methanol extracts from the leaves of *P. africana*, water extracts of the leaf and stem bark of *M. senegalensis,* water extracts from the stem bark of *W. stuhlmannii*, whereas the other 6 showed toxicity ranging from 5.64 to 464.04µg/ml. Among the extracts that showed no toxicity on the control cells but showed the highest selective cytotoxicity against 4T1 and CT26 was the methanol extract from the leaf of *P. africana.*

Methanol extract of the stem bark of this plant (*P. africana)* had the highest selective cytotoxicity against 4T1 cells. There is possibility of the leaf and stem bark of *P. africana* extracts having similar phytochemicals and hence causing similar activities. The stem bark of this plant has been used traditionally for the treatment of benign prostate hyperplasia (BPH) (Kokwaro, 1993). The selective cytotoxicity shown by this plant could be attributed to the summation effects of many compounds present in the extract. The pharmacology of some compounds from *P. africana* has been reported (Andro & Riffaud, 1995; Marandola *et al.,* and Bombardelli, 1997). These include cyanogenic glycoside amygdalin in the bark, leaf and fruit of this species which was documented by 1962 (Awang, 1997).

The methanol extract from the stem bark of *M. senegalensis* showed moderately high toxicity against breast cancer (4T1) cell lines and high toxicity against Vero cells. However, the methanol extract of both the stem bark and leaf of *M. senegalensis* were the most cytotoxic amongst the 12 plant extracts tested against colon cancer cell

lines and had low toxicity against Vero cells, these showed the most potent selective cytotoxicity.

Screening studies on antitumor properties of the root and stem extracts revealed the *in vitro* cytotoxic activity against carcinoma cells and *in vivo* anti-leukemia effects.

Interestingly, plants of the genus *Maytenus* are used in South America to prepare infusions or decoctions as anti-inflammatory and analgesic remedies for topical and oral administration. Evidence for the *in vivo* anti-inflammatory activity of *M. senegalensis* root extracts has been scientifically established. Such extracts contain maytenoic acid, which was found to be an anti-inflammatory triterpene twice as active as the NSAID indomethacin (Sosa *et al.*, 2007; da Silva *et al.*, 2011). This plant species has been used traditionally as an anti-inflammatory. Compounds isolated from the *Maytenus* genus include mayteine and maytansine, these alkaloids are much documented for their anti-tumor activity. Other isolated compounds include spermidine alkaloids and nicotinyl sesquiterpene alkaloids as well as catechin procyanidins phenoldienone triterpenoids (Da Silva *et al.*, 2010).

The methanolic extracts from the stem bark of *W. stuhlmannii* also showed high cytotoxic activity against colon cancer cell lines and high Selectivity Index. The stem bark of *W. stuhlmannii* has previously been used in the treatment of both anti-tumor and anti-inflammatory diseases in traditional medicine (Beentje, 1994). The biological activity of this extract may be attributed by the presence of different compounds like mukadial 6-O-β-D-glycopyranoside and flavonol glycosides (Lawrence *et al.*, 2003).

This study provides an important basis for further investigation into the isolation, characterization and mechanism of cytotoxic compounds from some of the screened plant extracts, thus these plants could be used as a source of new lead structures in drug design to combat cancer.

5.6: Acute oral toxicity

The importance of medicinal plants in traditional health care practices, providing clues to new areas of research and biodiversity conservation is now well recognized (Sanjay*et al.,* 2006). On administration of single doses ofvaried concentrations of *W. stuhlmannii* to mice, no mortality was observed even at thehighest concentration tested.These findings concur with previous studies which have shown that one of the active compounds in *W. stuhlmannii* was polygodial, a sesquiterpene dialhehyde (Kubo *et al.,* 1976). This compound is not mutagenic, as was determined by three variants of the Ames *Salmonella* test (Anke, 1991) and further confirmed by the mammal-based V79/HGPRT method (Morales *et al.,* 1992).

This is unique in that many other sesquiterpene dialdehydes possessing strong biological activity are mutagenic (Anke 1991; Forsby *et al*., 1991). This indicates that *W. stuhlmannii* is safe for use and the findings are also in agreement with studies that found that the stem bark of *W. stulmannii* was used traditionally as a spice (Beentje, 1994).

There was also no mortality noted in mice that received the methanol extracts from the leaves of *P. africana*. Previous studies have demonstrated that *P. africana* extracts are non-toxic. Chloroform extracts of *P. africana* did not cause clinical signs or pathology in rats at daily oral dosages of up to 1000mg/kg for 8 weeks (Gathumbi *et al.,* 2002). A different study that involved acute and chronic toxicity of *P. africana* in mice and rats showed no adverse reactions observed after itragastric administration of a single dose of a lipophilic extract of the trunk bark (1–6g/kg body weight in mice and 1–8g/kgbody weight in rats). There were also no adverse reactions observed in mice and ratsafter chronic intragastric administration of the extract (60 and 600mg/kg bodyweight, respectively, daily for 11 months) (Bombardelli & Morazzoni, 1997).

Toxicology studies in human trials demonstrated a low incidence of toxicity; this explains why the bark extracts from *P. africana* have been used in treatingprostate disorders in men both traditionally and in modern medicine (APA citation, 2015).

The results of this study indicate that no mortality was noted even with the highest concentration among all the mice that received both the methanol extracts from the leaves of *P. africana* and the water extracts from the stem bark of *W. stuhlmannii* at all dose levels. Therefore based on the scale of Loomis and Hayes classification of toxicity (1996), both plant extracts were relatively harmless with LD_{50} of >5000mg/kg body weight.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1: Conclusion

1. Anti-proliferative activity of the plant extracts was experienced with the methanol extracts from the stem bark of *Prunus africana* exhibiting low IC_{50} of 26.37±3.54 µg/ml in the breast cancer cell lines. Methanol extracts from the stem
   bark of *Maytenus senegalensis* and *Warburgia stuhlmannii* had low IC_{50} values of 2.32±0.17 µg/ml and 13.94±0.27 µg/ml respectively.
2. Plant extracts that exhibited CC_{50} values of >1000µg/ml on Vero cells were methanol and water extracts from the leaves of *P.africana*, water extracts from the leaves of *M. senegalensis* together with the water extracts from the leaves and stem bark of *W. stuhlmannii.*
3. The LD_{50} of the plant extracts used for the acute oral toxicity studies were the methanol extracts from the leaves of *P. africana* and water extracts from the stem bark of *W. stuhlmannii.*

6.2: Recommendations

1. This study managed to explore the safety and anti-proliferative activity of the three plant extracts in breast and colon cancer cell lines, there is need to expose the extracts to a wider variety of other different cancer cell lines as there could be ineffective plant samples in this study that are more effective only to specific cancer cells.
2. Phytochemical analysis of the effective plant extracts also needs to be done to identify the compounds in them that make them effective.
3. This study also did not find out the safety and anti-proliferative activity in the roots of the three plant extracts as it is possible that some could be having effects.

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APPENDICES

Appendix I: Clearance letter from KEMRI Scientific Steering Committee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/SSC/102709 22nd April, 2014

Patricia Nabende

Thro'
Director, CTMDR
NAIROBI

Forwarded
22/4/2014

REF: SSC No. 2641(Amendment) – Safety and evaluation of anti-proliferative activity of *Prunus africana*, *Warbugia stuhlmannii* and *Maytenus senegalensis* in breast and colon cancer cell lines

Thank you for your letter dated 14th April, 2014 responding to the comments raised by the KEMRI SSC.

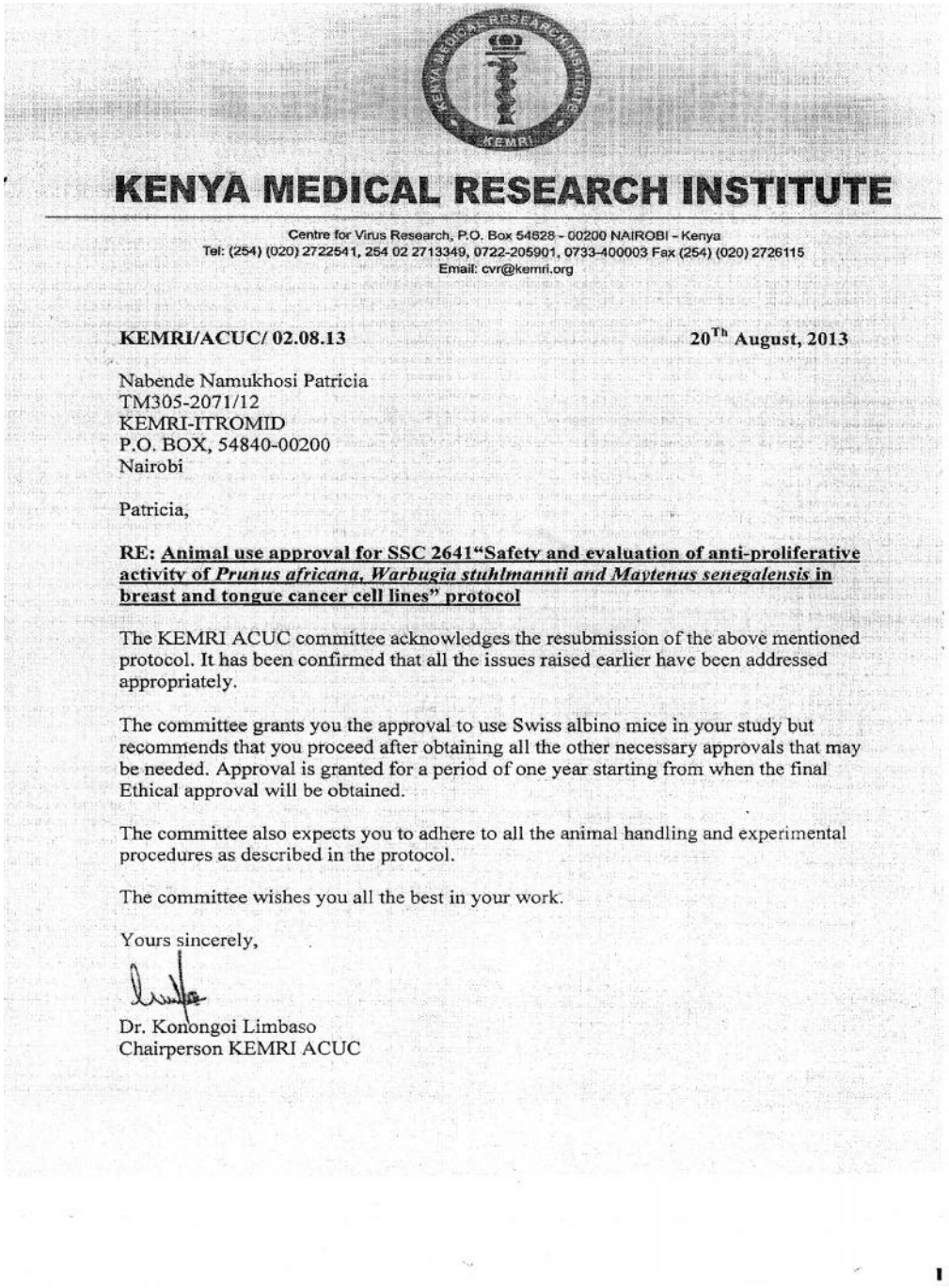
I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
SECRETARY, SSC

In Search of Better Health

AppendixII: Clearance letter from Animal Care and Use Committee



KENYA MEDICAL RESEARCH INSTITUTE

Centre for Virus Research, P.O. Box 54628 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 02 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2726115
Email: cvr@kemri.org

KEMRI/ACUC/ 02.08.13 **20Th August, 2013**

Nabende Namukhosi Patricia
TM305-2071/12
KEMRI-ITROMID
P.O. BOX, 54840-00200
Nairobi

Patricia,

RE: Animal use approval for SSC 2641"Safety and evaluation of anti-proliferative activity of *Prunus africana, Warbugia stuhlmannii and Maytenus senegalensis* in breast and tongue cancer cell lines" protocol

The KEMRI ACUC committee acknowledges the resubmission of the above mentioned protocol. It has been confirmed that all the issues raised earlier have been addressed appropriately.

The committee grants you the approval to use Swiss albino mice in your study but recommends that you proceed after obtaining all the other necessary approvals that may be needed. Approval is granted for a period of one year starting from when the final Ethical approval will be obtained.

The committee also expects you to adhere to all the animal handling and experimental procedures as described in the protocol.

The committee wishes you all the best in your work.

Yours sincerely,

Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

Appendix III: Clearance letter from KEMRI Ethics Review Committee

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **May 20, 2014**

TO: **PATRICIA NABENDE**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. JENNIFFER ORWA,**
ACTING DIRECTOR, CTMDR,
NAIROBI

Forwarded 27/05/2014
for Director CTMDR

Dear Madam,

RE: **SSC PROTOCOL No. 2641 (*REQUEST FOR AMENDMENT 1*): SAFETY AND EVALUATION OF ANTI-PROLIFERATIVE ACTIVITY OF PRUNUS AFRICANA, WARBUGIA STUHLMANNII AND MAYTENUS SENGALENSIS IN BREAST ANC COLON CANCER CELL LINES**

This is to inform you that at the 227th meeting of the KEMRI Ethics Review Committee held on 20th May, 2014, the request for amendment to the above referenced research proposal was discussed.

The Committee noted:

I. Section 13.0 has been added for limitation and potential bias
II. Section 15.0 time frame: the section has been to reflect the changes from tongue cancer cells to colon cancer cells
III. Page 12, second sentence from the top of the page has been revised; it now reads 'colon' cancer and not 'tongue' cancer.

The committee concluded that the suggested amendments are justified and will not result in increased risk to the participant. The amendments are therefore granted **approval** for implementation. You may continue with your study.

You are required to submit any further requests for changes to this version of the protocol to the SSC and ERC for review and approval prior to implementing any additional changes.

Yours faithfully,

EAB

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

Appendix IV: Publication

European Journal of Medicinal Plants
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Anti-proliferative Activity of *Prunus africana,Warburgia stuhlmannii* and *Maytenus senegalensis*Extracts in Breast and Colon Cancer Cell Lines

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PNN and SWW conceived the study and participated in the design of the study. Author PNN carried out the studies and analyzed the data. Authors PNN, SMK, JKM and SWW wrote the paper. All authors read and approved the final manuscript.

Article Information

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(1) Thomas Efferth, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Germany.
(2) Marcello Iriti, Faculty of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Review
(1) Fathilah Abdul Razak, Department of Oral Biology & Biomedical Sciences, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia.

(2) Mondher Boulaaba, Centre de Biotechnologie de Borj-Cédria, Laboratoire des Plantes Extremophiles, Tunisie.
(3) Mariano Bizzarri, Department of Experimental medicine, University La Sapienza, Roma, Italy.
(4) Anonymous, Morocco.
(5) Anonymous, Quaid-i-Azam University, Pakistan.

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Original Research Article

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ABSTRACT

Aims: To determine the anti-proliferative activity of*Prunus africana, Warburgia stuhlmanni*iand*Maytenus senegalensis* in breast and colon cancer cell lines and to assess their toxicity levelsbased on responses against Vero cells and the Swiss albino mice.
Study Design: Experimental laboratory-based study.
Place and Duration of Study: Centre for Traditional Medicine and Drug Research, Kenya MedicalResearch Institute, between May 2013 and May 2014.
Methodology: The*in vitro*assays involved determination of the cytotoxic concentration levels(CC_{50}) of the plant extracts on Vero cells as well as calculating the inhibitory concentration (IC_{50}) of the plant extracts on breast and colon cancer cell lines. The drugs with the highest selectivity index (SI) to have low IC_{50} in the breast and colon cancer cell lines and high CC_{50} in Vero cells were used in the *in vivo assays* which involved acute oral toxicity studies, conducted on 8 weeks old Swiss albino mice to calculate the median lethal dose (LD_{50}).
Results: The safest and effective drugs were methanol extracts of leaves from*Prunus africana*whose results showed an average IC_{50} of 164.64±4.14 µg/ml in the breast cancer cell lines and 21.33±0.5 µg/ml in the colon cancer cell lines, as well as the stem bark water extracts from *Warburgia stuhlmannii,* whose results showed an average $IC_{50}$of 332.79±7.53 µg/ml in the breast cancer cell lines and 107.20±2.50 µg/ml in the colon cancer cell lines. Both extracts had an average CC_{50} of >1000 µg/ml in Vero cells. Based on positive cytotoxicity results on the two extracts, acute oral toxicity studies were conducted on 8 weeks old female Swiss albino mice. This revealed no signs of acute toxicity after drug administration with LD_{50} of >5000 mg/kg body weight, therefore the extracts were considered to be safe.
Conclusion: The methanol extract from the leaves of*Prunus africana*and the water extracts fromthe stem bark of *Maytenus senegalensis* were safe for use in the murine model. These extracts also showed a level of anti-proliferative activity in both breast and colon cancer cells without being toxic to Vero cells. This information forms a basis for the development of the extracts as safer alternative therapies for the management of cancer.

Keywords: Prunus africana; Warburgia stuhlmannii; Maytenus senegalensis; IC_{50}; CC_{50}; LD_{50}.

1. INTRODUCTION

Cancer is one of the leading causes of death in the world. According to GLOBOCAN, about 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012. The most commonly diagnosed cases worldwide were those of lung, accounting for 1.8 million (13% of the total), Breast (1.7 Million, 11.9%) and colorectal (1.4 million, 9.7%). There will be a substantive increase to 19.3 million new cases by 2025 [1]. In Africa, cancer accounts for over one million new cases yearly [2], however, despite its increasing burden, Cancer is not a major priority in developing countries [3]. In Kenya, cancer is the third leading cause of death, with an annual mortality rate of above 22,000 and incidences of about 28,000 cases [4].

As cancer incidences rise dramatically in developing countries, the already limited resources and equipments are overstretched, making it difficult to effectively treat and manage it [5]. Access to radiotherapy is however severely limited. For instance, 55% of all cancers in Africa require radiotherapy, but facilities are only accessible to 23 of Africa's 53 countries, reaching less than 5% of the total African population [3].

For thousands of years, plants and other natural products have been used to treat a variety of diseases and as a result, a number of modern drugs have been developed from them [6]. So far, about 30 compounds derived from plants have been proven to be clinically active against various types of cancer cells [7], this is a very small portion as it is anticipated that plants can provide potential bioactive compounds for the development of new methods to combat cancer diseases [8]. The discovery of vinca alkaloids, vinblastine, vincristine and cytotoxic podophyllotoxins in 1950s in plants began the extensive research of anti-cancer drugs from plant sources [9]. A combination of vinblastine and vincristine with other cancer chemotherapeutic drugs have been used in the treatment of cancers like leukemias, lymphomas, advanced testicular cancer, breast and lung cancer as well as Kaposi's sarcoma [10]. An isomer of podophyllotoxin, epiphyllotoxin was isolated as having active anti-tumor activities from the roots of *Podophyllum* species,*Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich [11].
In Africa, 90% of the population relies on traditional medicine for primary healthcare [12]. The conventional health system in Kenya provides for only 30% of the population, this means that more than two-thirds of Kenyans rely on traditional medicine for their healthcare needs [13]. The benefit of using the drug extracts from medicinal plants is that they are usually safer than their synthetic alternatives and are also more affordable [14]. Most cancer drugs available in the market are toxic as they kill both the normal and cancer cells, there is therefore need to look for a safer drug with high specificity to target cancer cells only.medicine fortreatment of several illnesses including chest pain, rheumatism, snake bites, diarrhea, eye infection, dyspepsia and wounds [25,26]. In Sudan, the aqueous extract of the stem bark is commonly used in the treatment of tumors, dysentery and snake bites [27,28]. A study on the anti-inflammatory activities of *M. heterophyla* and *M. senegalensis* using carcigeenan-induced paw edema method on Winstar albino rats had portrayed significant anti-inflammatory activity that reduced edema by 51% and 35% respectively [29]. However, the many therapeutic claims over these three plants, their safe use have not been scientifically proven. It is these claims that this study has to verify.

2. MATERIALS AND METHODS

Prunus africana, Warburgia stuhlmannii and *Maytenus senegalensis* are commonly usedtraditionally in Kenya as ethnobotanical information claims that they have anti-cancer properties. *Prunus africana,* commonly known as pyegum or African cherry, is widely distributed in various Kenyan provinces especially in the Mount Kenya forest [15]. It was documented in 1963 that a cyanogenic glycoside, amygdalin was isolated in the fruit, leaf and bark of the plant. In Kenyan traditional medicine, *P. africana* is used to treat chest pain, fever and malaria [15]. Stem barks have been used as remedy for diarrhea, allergies, stomach ache, prostate gland and kidney diseases [16,17].

2.1 Plant Materials

Five kilograms of the leaves and stem barks of
M. senegalensis and *W. stulhamannii* werecollected from Kwale County while those of *P.africana* from Nyeri County. They were air driedin mesh bags and voucher specimens deposited at the East African Herbarium, National Museums of Kenya. The plant parts were then delivered to Kenya Medical Research Institute, Centre for Traditional Medicine and Drug Research (CTMDR). A taxonomist was involved during identification of the plants and collection (*P. africana*-SW00017, *W. stulhamannii*-SW00026 and *M. senegalensis*-SW00027).

2.2 Preparation of the Plant Extracts

The plant materials were dried at room temperature (25ºC) pulverized using a laboratory mill (Christy and Norris Ltd., Chelmsford, England) and packed air tight in polythene bags. Each plant sample was separately extracted using both water and methanol. For water extraction, 100g of the dried ground plant materials were soaked in 1000ml of distilled water and put in a water bath at 70ºC for 1 hour, filtered and lyophilized in a Freeze Dryer (Edwards freeze dryer Modulyo). For the methanol extraction, 100g of the dried plant materials was percolated with 1000ml of methanol at room temperature for 3 days. The methanol extracts were filtered through Whatman filter paper no. 1 and concentrated to dryness under reduced pressure using a rotary evaporator [30]. The extracts were then weighed, labeled and stored in air tight bijou bottles at 4°C prior to use. 100mg of the extracts were dissolved in 1 ml DMSO to make a stock solution of 100,000 µg/ml in 100% DMSO, sterilized by filtration (at pore size of 0.2 µm) before testing. The working solution was made by diluting 1 part of the stock solution to 99 parts of Earl's Minimum Essential Medium containing 2% Fetal Bovine Serum (FBS) (maintenance medium), which was 10 µl of the extract in 990 µl of media to give a start concentration of 1000 µg/ml in 1% MSO which was used in the MTT assay.

3. Cell Culturing

The mouse mammary breast cancer cell line (4T1 ATCC®CRL-2539™), mouse colon cancer cell line (CT26.WT-ATCC® CRL-2638™) and Vero cells (monkey kidney cells) were obtained from the American Type Culture Collection (ATCC), revived and cultured in T-75 flasks with Earl's Minimum Essential Media (EMEM), all supplemented with penicillin, streptomycin and 10% Fetal Bovine Serum maintained at 37˚C in a humidified atmosphere of 5% CO_2 to achieve confluence.

2.4 MTT Assay for Cytotoxicity

The *in vitro* cytotoxicity was carried out following a rapid calorimetric assay [31], which is based on the capacity of succinate dehydrogenase enzyme in the mitochondria of living cells to reduce the yellow water soluble substrate MTT into insoluble formazan, which is measured spectrophotometrically [32,33]. Upon attainment of confluence, Cells were detached by trypsinization, and the number of viable cells determined by Trypan blue exclusion test (cell density counting). A hemocytometer was used to aid in counting viable cells, which were seeded at 2×10^5/ml cell suspension for the Vero cells and 1×10^5/ml cell suspension for the 4T1 cells and colon cancer cells on 96- well plates and incubated at 37ºC in 5% CO_2 for 24 hours. The test sample extracts were then added to the plates and incubated for 48 hours at 37^oC with 5% CO_2. At the end of the incubation time, 10µl of MTT dye (5mg of MTT, dissolved in 1ml serum free medium (Phosphate Buffered Saline (PBS)) was added to all the cells and incubated for another 4 hours. All media was then removed from the plates and 100µl of 100% DMSO added. The plates were then read on a scanning multi well spectrophotometer (Multiskan Ex labs systems) at 562 nm and 690 nm as reference. Podophyllotoxin resin from the *Podophyllumhexandrum* plant was used as the standardreference drug. The cytotoxic results (CC_{50}) determined whether mice would be used in this study for acute oral toxicity assays as only the extracts with low IC_{50} and high CC_{50} were selected.

2.5 Drug Administration

The drugs were administered in 5 groups as follows: Group 1-500 mg/kg, Group 2-889.53 mg/kg, Group 3-1581.6 mg/kg, Group 4-2812.5 mg/kg and Group 5-5000 mg/kg). For the water extracts, the dosages were prepared by dissolving each one of them in distilled water. For the methanol extracts, a stock solution was first prepared by mixing 700 µl of Tween 80 with 300 µl of analytical ethanol, after which a working solution was prepared by mixing 100µl of the stock solution with 900 µl of distilled water. 5 mice were used as negative control and these were given 0.2 ml of distilled water. Each mouse received only a single oral dose of the drug in the entire experiment. The mice were deprived of feeds 12 hours prior to introduction of the drug and 3 hours after. The animals were observed over a period of 24-48 hours for signs of acute oral toxicity, for example reduced activeness, convulsions, writhing, decreased motor activity, decreased body/limb tone, decreased respiration, mortality rate and survival period, in this study, none of these were noted.

2.6 LD_{50} Determination

Determination of LD_{50} was to be carried out using the Lorke formula [34]. Five dose levels of each compound were administered orally to the Swiss Albino mice and observed over a period of 24-48 hours for signs of acute toxicity and mortality. The number of deaths within this period was to be noted and recorded. They were also observed twice daily for 14 days, with their weights recorded before drug administration and after the 14 days. Classification of toxicity was described based on the scale of Loomis and Hayes, 1996 [35].

2.7 Data Management and Analysis

The *in vitro* cytotoxicity results were expressed as mean ± Standard Error of Mean (SEM), while the *in vivo* studies had the differences in weight for the mice studies and means analyzed statistically using the Student's t-test. Differences between means were considered statistically significant at $p<0.05$.

3. RESULTS

3.1 Extraction

A total of 55 female Swiss Albino mice aged 6 weeks weighing between 20±2g were used in the study. Each test consisted of 25 mice, 5 mice for each dosage labeled as group 1-5 (Group 1-500

A total of 12 extracts from the leaf and stem bark of 3 plant species representing 3 families were extracted using methanol and water. Table 3.1 shows the percentage yields of the 12 plant extracts.

3.2 IC_{50} Results with the 4T1 Cells

The concentration that inhibited growth in 50% of the cells (IC_{50}) was calculated for the 4T1 cells. Methanol extracts of *P. africana* stem bark, *M.senegalensis* stem bark and *W. stuhlmannii* leafhad the lowest IC_{50} values of 26.37±3.54, 32.96±2.91 and 75.30±6.31 µg/ml respectively, while the reference drug, *Podophyllumhexandrum* resin had $IC_{50}$of 3.14±0.19 µg/ml.Table 3.2 shows the results of each plant extract together with the reference drug, *P. hexandrum*.

3.3 IC_{50} Results with Colon Cancer Cells

IC_{50} was also calculated for the colon cancer cell lines. The IC_{50} varied with the plant extract and the solvent used for extraction. The lowest IC_{50} was registered from methanol extract of *M.senegalensis* stem bark, *M. senegalensis* leaf, *W. stuhlmannii* stem bark and *P. africana* leafwith IC_{50} values of 2.32±0.17, 4.18±0.14, 13.94±0.27 and 21.33±0.75 µg/ml respectively, while the reference drug, *P. hexandrum* resin had IC_{50} value of >1000 µg/ml. Table 3.3 shows the IC_{50} results together with the reference drug,*P. hexandrum.*

3.4 CC_{50} Results with VERO Cells

The concentration of plant extracts that killed (reduced cell viability) in 50% of the cells (cytotoxic concentration, CC_{50}) was calculated. The water and methanol extracts from the leaves of *P.* africana, water extracts of the leaf and stem bark of *M.* senegalensis, water extracts from the leaves and stem bark of *W. stuhlmannii* and the reference drug *P. hexandrum* all exhibited CC_{50} values of >1000 µg/ml. Table 3.4 shows the results

Table 3.1. Plant species and percentage yields of water and methanol extracts

Plant	Part	Extraction method	Weight after extraction (g)	% yield
Prunus africana	Leaf	Water	2	2
Prunus africana	Leaf	Methanol	49	49
Prunus africana	Stem bark	Water	16	16
Prunus africana	Stem bark	Methanol	35.04	35.04
Maytenus senegalensis	Leaf	Water	18.65	18.65
Maytenus senegalensis	Leaf	Methanol	39.51	39.51
Maytenus senegalensis	Stem bark	Water	14.38	14.38
Maytenus senegalensis	Stem bark	Methanol	29.78	29.78
Warbugia stuhlmannii	Leaf	Water	24.49	24.49
Warbugia stuhlmannii	Leaf	Methanol	77.63	77.63
Warbugia stuhlmannii	Stem bark	Water	24.12	24.12
Warbugia stuhlmannii	Stem bar	Methanol	44.82	44.82

Table 3.2. IC_{50} results of the plant extracts with 4T1 cells

Plant extracts	IC_{50}(µg/ml)	CC_{50}(µg/ml)	SI
Prunus africana leaf water	570.89±11.21	>1000	1.75
Prunus africana leaf methanol	164.64±4.14	>1000	6.07
Prunus africana stem bark water	133.51±2.13	55.64±4.41	0.42
Prunus africana stem bark methanol	26.37±3.54	196.84±4.62	7.46
Maytenus senegalensis leaf water	>1000	>1000	1
Maytenus senegalensis leaf methanol	256.41±4.77	464.04±0.02	1.81
Maytenus senegalensis stem bark water	>1000	>1000	1
Maytenus senegalensis stem bark methanol	32.96±2.91	74.59±3.21	2.26
Warburgia stuhlmannii leaf water	>1000	>1000	1
Warburgia stuhlmannii leaf methanol	75.30±6.31	184.08±6.08	2.44
Warburgia stuhlmannii stem bark water	332.79±7.53	>1000	3.00
Warburgia stuhlmannii stem bark methanol	123.69±1.58	154.37±0.77	1.25
Podophyllum hexandrum	3.14±0.19	>1000	318.47

Table 3.3. IC_{50} results of the plant extracts with colon cancer cells

Plant	IC_{50} in (µg/ml)	CC_{50} (µg/ml)	SI
Prunus africana leaf water	716.75±3.32	>1000	1.40
Prunus africana leaf methanol	21.33±0.75	>1000	46.88
Prunus africana stem bark water	83.53±1.58	55.64±4.41	0.67
Prunus africana stem bark methanol	176.90±0.89	196.84±4.62	1.11
Maytenus senegalensis leaf water	87.52±0.31	>1000	11.43
Maytenus senegalensis leaf methanol	4.18±0.14	464.04±0.02	111.01
Maytenus senegalensis stem bark water	461.06±6.84	>1000	2.17
Maytenus senegalensis stem bark methanol	2.32±0.17	74.59±3.21	32.15
Warburgia stuhlmannii leaf water	371.56±11.35	>1000	2.69
Warburgia stuhlmannii leaf methanol	149.51±0.94	184.08±6.08	1.23
Warburgia stuhlmannii stem bark water	107.20±2.50	>1000	9.33
Warburgia stuhlmannii stem bark methanol	13.94±0.27	154.37±0.77	11.07
Podophyllum hexandrum	>1000	>1000	1

Table 3.4. CC_{50} results of the plant extracts with VERO cells

Plant	CC_{50}(µg/ml)
Prunus africana leaf water	>1000
Prunus africana leaf methanol	>1000
Prunus africana stem bark water	55.64±4.41
Prunus africana stem bark methanol	196.84±4.62
Maytenus senegalensis leaf water	>1000
Maytenus senegalensis leaf methanol	464.04±0.02
Maytenus senegalensis stem bark water	>1000
Maytenus senegalensis stem bark methanol	74.59±3.21
Warburgia stuhlmannii leaf water	>1000
Warburgia stuhlmannii leaf methanol	184.08±6.08
Warburgia stuhlmannii stem bark water	>1000
Warburgia stuhlmannii stem bark methanol	154.37±0.77
Podophyllum hexandrum	>1000

3.5 Selectivity Index

The Selectivity index ($SI=CC_{50}/IC_{50}$) was calculated from the CC_{50} ratio of the normal Vero cells and IC_{50} of the cancerous (4T1, CT26.WT) cells. SI value indicates selectivity of the sample to the cell lines tested. Samples with SI value greater than 3 were considered to have high selectivity. From the SI column in table 3.2 and 3.3, methanol extract of *P. africana* stem bark and leaf had SI>3 (7.46, 6.07), against 4T1 cancer cell lines while methanol extracts of *M.senegalensis* leaf, *P. africana* leaves, *M. senegalensis* stem bark, water extract of *M. senegalensis* leaf, *W. stuhlmannii* stem bark andmethanol extract of *W. stuhlmannii* stem bark had SI greater than 3 (111.01, 46.88, 32.15, 11.43, 9.33, 11.07 respectively) against colon cancer cell lines. All the other extracts had SI values less than 3 and were therefore considered non selective to the other specific cancer cell lines.

3.6 Acute oral Toxicity with *Warburgiastuhlmannii*

There was no mortality observed within 48 hours and during the 14 day period of observation in all the mice groups that received the water extracts from the stem bark of *W. stuhlmannii*, LD_{50} was therefore >5000mg/kg body weight. There was a general increase in the body weight in all mice groups as shown in Table 3.5, this is because the drug dosages did not affect weight gain. Mice that received 5000mg/kg (group 5) had a significant difference in weight compared to the control group ($p<0.05$). Those that received 500 mg/kg (group 1), 889.53 mg/kg (group 2), 1581.6 mg/kg (group 3) and 2812.5 mg/kg (group 4) had no significant difference compared to the control group ($p>0.05$). Table 3.6 shows the results.

Table 3.5. Weights of mice before and after oral administration of *Warbugia stuhlmannii*

Dosage levels	Mice weight in (g) before drug administration	Mice weight in (g) after drug administration
Group 1	21	22
(500 mg/kg/day)	20	28
(0.5 mg)	22	28
	22	30
	21	23
Group 2	21	28
(890 mg/kg/day)	22	29
(0.89 mg)	22	26
	22	30
	20	28
Group 3	18	27
(1582 mg/kg/day)	22	28
(31.63 mg)	21	26
	22	31
	21	26
Group 4	22	24
(2812 mg/kg/day)	19	21
(56.24 mg)	19	27
	21	26
	20	23
Group 5	18	20
(5000 mg/kg/day)	20	24
(100 mg)	22	29
	21	26
	22	24
Negative control	22	27
	21	27
	20	28
	22	30
	19	26

Table 3.6. Comparisons between means of weight differences with the control

Comparing groups vs control	Mean (Control Mean 6.8)	Standard error	95% confidence interval	P-value
Group 1 (500 mg/kg)	5	1.594	(-1.86, 5.46)	0.291
Group 2 (889.53 mg/kg)	6.8	0.938	(-2.16, 2.16)	1
Group 3 (1581.6 mg/kg)	6.8	1.086	(-2.51, 2.51)	1
Group 4 (2812.5 mg/kg)	4	1.281	(-0.15, 5.75)	0.06
Group 5 (5000 mg/kg)	4	1.114	(0.23, 5.37)	0.036

** Mean difference is significant at the 0.05 level*

3.7 Acute Oral Toxicity with *P.africana*

The mice that received a single dose of the methanol extracts from the leaves of *P. africana* ranged from 500 to 5000 mg/kg body weight. There was no mortality observed within 48 hours and during the 14 day period of observation, LD_{50} was therefore >5000 mg/kg body weight.

There was a general increase in body weight in all the mice groups as shown in table 3.7, this isbecause all the dosages did not affect weight gain. Those that received 1581.6 mg/kg/day (group 3), 2812.5 mg/kg (group 4) and 5000 mg/kg (group 5) had a significant difference in weight compared to the control group ($p<0.05$). The weights of mice that received 500 mg/kg (group 1) and 889.53 mg/kg (group 2) had no significant difference with that of the control group ($p>0.05$). Table 3.8 shows the results.

Table 3.7. Weights of mice before and after oral administration of *Prunus africana*

Dosage levels	Mice weight in (g) before drug administration	Mice weight in (g) after drug administration
Group 1	21	26
(500 mg/kg/day)	21	23
(0.5 mg)	19	27
	18	23
	21	22
Group 2	21	27
(890 mg/kg/day)	19	22
(0.89 mg)	21	24
	20	25
	18	26
Group 3	18	21
(1582 mg/kg/day)	22	24
(31.63 mg)	21	22
	20	23
	18	26
Group 4	20	24
(2812 mg/kg/day)	18	24
(56.24 mg)	21	22
	21	23
	22	24
Group 5	18	20
(5000 mg/kg/day)	20	24
(100 mg)	22	29
	21	26
	22	24
Negative control	22	27
	21	27
	20	28
	22	30
	19	26

Table 3.8. Comparisons between means of weight differences with the control

Comparing groups vs control	Mean (Control Mean 6.8)	Standard error	95% confidence interval	P-value
Group 1 (500 mg/kg)	4.2	1.371	(-0.56, 5.76)	0.095
Group 2 (889.53 mg/kg)	5	1.114	(-0.77, 4.37)	0.145
Group 3 (1581.6 mg/kg)	3.4	1.342	(0.31, 6.49)	0.035
Group 4 (2812.5 mg/kg)	3	1.068	(1.34, 6.26)	0.007
Group 5 (5000 mg/kg)	2.8	0.883	(1.96, 6.04)	0.002

** Mean difference is significant at the 0.05 level.*

4. DISCUSSION

4.1 Cytotoxicity Studies

Vero cells have been recommended for cytotoxicity studies and for the analysis of cell-substrate interactions in biomaterial research [36,37]. Our study shows investigations of anti-cancer potential of three plant species which has not been studied in Kenya, by screening for cytotoxic activity against healthy cells and two mouse model cancer cell lines, 6 out of the 12 extracts showed low or no toxicity against normal cell lines (Vero cells), this includes water and methanol extracts from the leaves of *P. africana,* water extracts of the leaf and stem bark of *M.senegalensis,* water extracts from the stem barkof *W. stuhlmannii,* whereas the other 6 showed toxicity ranging from 55.64 to 464.04µg/ml. Among the extracts that showed no toxicity on the normal cells (Vero) but showed the highest selective cytotoxicity against 4T1 and CT26 was**,**the methanol extract from the leaf of *P. africana*. Methanol extract of the stem bark of this plant (*P.africana*) had the highest selective cytotoxicityagainst 4T1 cells. There is possibility of the leaf and stem bark of *P. africana* extracts having similar phytochemicals and hence causing similar activities. The stem bark of this plant has been used traditionally for the treatment of Benign Prostate Hyperplasia (BPH) [15]. The selective cytotoxicity shown by this plant could be attributed to the summation effects of many compounds present in the extract. The pharmacology of some compounds from *Prunusafricana* has been reported [38,39,40].

The methanol extract from the stem bark of *M.senegalensis* showed moderately high toxicityagainst breast cancer (4T1) cell lines and high toxicity against Vero cells. However, the methanol extract of both the stem bark and leaf of *M. senegalensis* were the most cytotoxic amongst the 12 plant extracts tested against colon cancer cell lines and had low toxicity against Vero cells, these showed the most potent selective cytotoxicity. The stem bark of *M.senegalensis* has been used in treatment oftumors in Sudan [27,28]. This plant species has been used traditionally as an anti-inflammatory. Compounds isolated from the *Maytenus* genus include mayteine and maytansine, these alkaloids are much documented for their anti-tumor activity [29].

The methanolic extracts from the stem bark of *W.stuhlmannii* also showed high cytotoxic activityagainst colon cancer cell lines and high Selectivity Index. The stem bark of *W.stuhlmannii* has previously been used in thetreatment of both anti-tumor and anti-inflammatory diseases in traditional medicine [19]. The biological activity of this extract may be attributed by the presence of different compounds like mukadial 6-O-β-D-glycopyranoside and flavonol glycosides [41].

This study provides an important basis for further investigation into the isolation, characterization and mechanism of cytotoxic compounds from some of the screened plant extracts, thus these plants could be used as a source of new lead structures in drug design to combat cancer.

4.2 Acute Oral Toxicity

The results of this study indicate that no mortality was noted even with the highest concentration among all the mice that received both the methanol extracts from the leaves of *P. africana* and the water extracts from the stem bark of *W.stuhlmannii* at all dose levels. Therefore basedon the scale of Loomis and Hayes classification of toxicity [36], both plant extracts were relatively harmless with LD_{50} of > 5000 mg/kg body weight.

5. CONCLUSION

The present study supports the anti-proliferative activity of the three medicinal plants: *P. africana,W. stuhlmannii* and *M. senegalensis* in breastand colon cancer cell lines used in this study, as well as their safety in mice models. This study provides important basis for further investigation in the development of the extracts as safer alternative therapies for the management of cancer.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Ethical clearance was sought from Kenya Medical Research Institute (KEMRI) Scientific Steering Committee (SSC), Animal Care and Use Committee (ACUC), and Ethical Review Committee (ERC) before study implementation.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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