INVITRO PHARMACOPHYSIOLOGICAL ANTICANCER POTENTIAL OF MEDICINAL PLANTS COMMONLY USED IN KAKAMEGA COUNTY, KENYA

A thesis submitted in fulfillment of requirements for Doctor of Philosophy degree of the University of Nairobi (Comparative Mammalian Physiology).

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DECLARATION

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DEDICATION

This work is especially dedicated to all students from poor backgrounds especially Sub-Saharan Africa who strive to achieve their academic dreams, who understand the statement that "Anyone who has ever struggled with poverty knows how extremely expensive it is to be poor".

This work is also dedicated to my treasured dear friend and companion Nelly Kanazi, my children Israel Ochwang'i, John David (JD) and Bethel Rebekah Moraa (Bekah) and my family who have been a source of support and love. My beloved father Wilfred Ochwang'i and Mother Annastancia Serah Moraa, Brother Robert and Justus, Sisters Damarice, Linet, Lydia, Mercy, Diana and Purity.

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ABSTRACT

Cancer is a high mortality disease of public concern. Effectiveness of chemotherapy is often limited by toxicity to untargetted tissues among other serious side effects. Alternative therapy such as herbal remedies for cancer, which may have the potential to offer more efficacy and less side effects have, however, not been rigorously studied or tested. An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several chemotherapeutics from these plants as well as from traditional information on rural herbal remedies. The objective of the present study therefore was to validate the anti-cancer properties of selected medicinal herbal remedies used by the population in Kakamega County, Kenya. Using semi-structured questionnaires administered to 32 randomly selected herbalists from Kakamega County, an ethnobotanical survey identified and documented anti-cancer medicinal plants. 65 plants belonging to 59 genera and 32 families were cited. The findings of this study indicated that diverse medicinal plants were used in the management and treatment of cancer primarily through oral administration. Aqueous and dichloromethane methanol extracts were prepared for the assessment. Using Thin Layer Chromatography (TLC) phytochemical constituents of the selected herbal extracts were analysed.The selected herbal extracts revealed the presence of alkaloids, anthraquinones, xanthines, valepotriates, cardiac glycosides, flavonoids, essential oils, coumarins, lignans, saponins and arbutin drugs. Five human cell culture lines; Leukemia cell lines (CCRF-CEM and CEM/ADR5000), cancer cell lines from the colon (HCT116 (p53+/+)

and HCT116 (p53−/−), Breast cancer cell lines (MDA-MB-231-pcDNA3) and MDA-MB-231-BCRP clone 23), Human glioblastoma multiforme U87MG cells and Kidney cell lines HEK-293; were used to screen for cytotocity of the selected herbal extracts. The cytotoxic activity of the candidate plant extracts on the viability of the various cell lines was done using Rezasurin reduction assay with a single concentration of 40 μg/mL for each extract. Screening results indicated that eight of the thirty four organic plant extracts and two of the aqueous plant extracts showed less than 50% growth proliferation of CCRF-CEM cells. The organic extracts include *Harungana madagascariensis* Lam.ex poir, *Prunus africana* (Hook.f.) kalkman, *Entada abyssinica* Steud.ex A.Rich. , *Phyllanthus fischeri* Pax, *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax, *Bridelia micrantha* (Hochst.) Baill , Futumia *africana* Benth. and *Microglossa pyrifolia* (Lam.) Kuntze. The aqueous extracts include *Bridelia micrantha* (Hochst.) Baill and *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax. Dose response curves with to generate IC50s were generated for candidates with best anticancer activity on a set of eight cancer cell lines, including both sensitive and MDR phenotypes.Promising results were exhibited by extracts of *Prunus africana* (Hook.f.) kalkman and *Harungana madagascariensis* Lam.ex poir.The present study simulated the potential field situation by combining the extracts in equal ratios; weight: volume and screening them against sensitive cancer cells CCRF-CEM. The results indicated excellent results and great potential of combinational therapy, some exceeding the standard anti-cancer drug Doxorubicin. Marked synergistic cytotoxicity were seen with combinations of aqueous extracts of *Harungana madagascariensis* Lam.ex poir and *Prunus africana* (Hook.f.) kalkman compared to their single activities. The present study also investigated the possible primary cellular mechanism

of *Harungana madagascariensis* Lam.ex poir and *Bridelia micrantha (Hochst.) Baill.* cytotoxicity using flow cytometry to measure Reactive Oxygen Species (ROS) and Cell Cycle analysis to determine the possible mechanism of occurrence of cytotoxicity. There was a fold-increase of ROS induction by extract of *Bridelia micrantha (Hochst.) Baill.* and *Harungana madagascariensis Lam.ex poir* against CCRF-CEM cancer cells. High Performance Liquid Chromatography analysis and fractionation (HPLC) and Mass Spectrophotometry (MS) was performed on the extract with the best anticancer activity. UV chromatograms showed there are many bioactive compounds within the extract that we fractionated of *Harungana madagascariensis* Lam.ex poir and *Bridelia micrantha (Hochst.) Baill.* Findings from the study showed that some medicinal plants had excellent anti-cancer activity and therefore offer potential as therapeutic agents against cancer. These findings offer support on the use of selected plants as alternative forms of cancer treatment that are efficacious, safe, practical, reliable, affordable, accessible, self-administered and culturally acceptable.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

"Cancer can be defined as malignant neoplasia encompassing a broad group of ailments all involving unregulated cell growth in which cells divide and grow uncontrollably forming tumors that may metastasize to invade other parts of the body. These neoplastic cells regress highly specialized cells forming more primitive stages which, unlike the normal parent cells, divide continuously although inefficiently", (Anand *et al*., 2008). "The main properties of neoplastic tissue include; sustained proliferative signaling, evading growth suppressors, resisting cell death, enabling replication, inducing angiogenesis, activating invasion and

metastasis, reprogramming energy metabolism and escaping immune destruction", (Anand *et al*., 2008).

According to Jemal *et al* 2011; in 2008 approximately 12.7 million cancers were diagnosed globally (excluding non-melanoma skin cancers and other non-invasive cancers) and 7.6 million people died of cancer worldwide.This figure keeps increasing with time. The World Health Organization has reported that cancers cause 13% of all deaths each year with the most common being: lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths) (WHO, 2010). Age has been cited as the most common risk factor, with those over 65 years being most affected (Coleman *et al* .,2009). 50 Kenyans expire daily from various forms of cancers with about 80,000 cases of cancer diagnosed each year (Pact Kenya Cancer Assessment in Africa and Asia (2010).

"Current treatment regimens include chemotherapy, radiotherapy and surgery. Patient survival varies greatly depending on the type and location of the cancer and the stage of disease at the onset of treatment. Chemotherapy in addition to surgery has proved useful in a number of different cancer types including: breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer, and certain lung cancers. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Chemotherapy drugs include: antimetabolite drugs (fludarabine and hydroxyurea)", (Iwasaki H, *et al.,* 1997), inhibitors of topoisomerase (camptothecin, topotecan) (Cliby WA *et al.,* 2002), alkylating agents (cisplastin, temozolomide) (Jean-Claude BJ *et al.,* 1997), intercalating agents (doxorubicin, daunorubicin) (Goodman MF *et al.,* 1977) and substances that interfere with tubuline (Vinca alkaloids and taxotere) (Gratzner HG *et al.,* 1986).

Radiotherapy is normally employed together with surgery and or chemotherapy and may be used singly for some cancers such as early head and neck. Increasingly complementary and alternative therapies are used. These include herbal remedies for cancer have however not been rigorously studied or tested (Vickers A, 2004). The objective of the present study therefore is to validate these alternative medicines by determining the anti-neoplastic properties of selected medicinal herbal remedies used by the population in Kakamega County, Kenya.

1.1 OBJECTIVE OF THE STUDY

The overall objective was to validate anti-cancer properties of selected medicinal herbal therapies traditionally used by the populace in Kakamega County, Kenya.

1.1.1 SPECIFIC OBJECTIVES

1) To identify and document anti-cancer medicinal plants used in Kakamega County, Kenya.

2) To analyze the major phytochemical constituents of selected (most widely used) anticancer plant species

3) To assess *in vitro* anti-neoplastic cytotoxic activity of the herbal extracts using human cell line cultures.

4) To analyse the potential mechanisms of action of the herbal extract with the highest anticancer cytotoxic activity.

1.2 HYPOTHESIS

1.2.1 Null hypothesis

Medicinal plants used for cancer therapy in Kakamega County lack anticancer properties.

CHAPTER TWO

2.0 GENERAL LITERATURE REVIEW

2.1 The Cancer burden

"Cancer is an emerging public health problem in Africa. About 715,000 new cancer cases and 542,000 cancer deaths occurred in 2008 on the continent, with these numbers expected to double in the next 20 years simply because of the aging and growth of the population. Furthermore, cancers such as lung, female breast, and prostate cancers are diagnosed at much higher frequencies than in the past because of changes in lifestyle factors and detection practices associated with urbanization and economic development", (Jemal *et al* ., 2012). Boyle and Levin, 2008 have reported that Africa's cancer burden is increasing due to aging and growth of the population as well as increased prevalence of risk factors associated with economic transition (Boyle and Levin, 2008). It is projected that the population with those aged more than 60 years is set to increase in Africa from 1.03 billion to 1.52 billion by 2030; an age at which cancer mostly occurs (United Nations Population Division, 2008). "Despite this growing cancer burden, cancer continues to receive a relatively low public health priority in Africa, largely because of limited resources and other pressing public health problems, including communicable diseases such as acquired immunodeficiency syndrome (AIDS)/human immunodeficiency virus (HIV) infection, malaria, and tuberculosis.

Surgery and/or radiation are the most important methods of treating early stage (local) cancers, including cancers of the breast, colorectum, cervix, head and neck,esophagus, stomach, and prostate", (Sankaranarayanan and Boffetta, 2010).The access to such treatments

in Africa is not wide spread because of limited skilled workers, equipment, and radiation facilities. (Barton *et al*., 2006).

2.2 Hallmarks of cancer

"The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of human tumors. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Underlying these hallmarks are genome instability which generates the genetic diversity that expedites their acquisition, and inflammation which fosters multiple hallmark functions. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list; reprogramming of energy metabolism and evading immune destruction. In addition to cancer cells, tumors exhibit another dimension of complexity; they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the ''tumor microenvironment''. Recognition of the widespread applicability of these concepts will increasingly affect the development of new means to treat human cancer", (Hanahan and Weinberg, 2011). These capabilities of cancer cells pose a challenge to both modern and traditional therapeutic intervention leading to development of multidrug resistant cancer types. Resistance of cancer cells to various drugs is frequently brought by the ATP-dependent efflux pump P-glycoprotein (MDR1, P-gp, and ABCB1) with the capacity to efflux a wide range of cancer drugs. Its expression confers cross-resistance termed "multidrug resistance" (MDR) to various drugs. "Strategies to overcome this resistance have been actively sought for more than 30 years, yet clinical solutions do not exist", (Hall *et al.*, 2009). MDR is a quite a

challenge in cancer treatment worldwide and has been seen to cause chemotherapy failure in over 90% of patients with metastatic cancer (Liu *et al*., 2012; Longley and Johnston, 2005). ATP-binding cassette (ABC) transporters are a major cause of MDR phenotypes with P-gp and BCRP as the most important ones (Chen *et al*., 2016; Efferth, 2001; Gillet *et al*., 2007). As clinical trials to overcome MDR with synthetic molecules failed so far (Amiri-Kordestani *et al*., 2012), the search for novel compounds is going on. Natural compounds reached special attention to combat MDR. Natural product inibitors of ABC transporter-mediated drug efflux have been described (Gatouillat *et al.*, 2015; Reis *et al*., 2016; Su *et al.*, 2015; Teng *et al*., 2016). Another possibility to kill MDR cells is to by bypassing drug efflux by using cytotoxic natural compounds, which are not substrates of P-glycoprotein or BCRP and which are therefore not transported out of the cells by ABC transporters (Kuete *et al*., 2015a; Kuete *et al.*, 2015b; Kuete *et al.*, 2015c; Kuete *et al.*, 2015d). Phytochemicals are appealing to kill MDR cells, because they may reveal novel chemical structures that enlarge the chemical space (Abdelfatah and Efferth, 2015; Hu *et al*., 2015; Xia *et al*., 2015) and phytotherapy and natural products are frequently considered as well tolerated without severe side effects. Instead of screening plants in a non-directed manner, it may be more promising to focus on plants used in traditional medicine. Compared to plants used in traditional Chinese medicine, which have been intensively investigated during the past two decades (Efferth *et al*., 2016), the cytotoxic activity of African plants is still sparsely reported in the international literature (Kuete and Efferth, 2015).

2.3 Anticancer herbal therapies

Ethnopharmacology may be broadly defined as the study of the indigenous drugs from plants and animals used in past and present cultures (Bruhn *et al*., 1982). Extraction of several

chemotherapies can be traced to herbal remedies currently traditionally used notably digoxin, taxol, vinblastine, nabilone and artemesin. (UNESCO, 1998). A greater percentage of the currently used anticancer and antihypertensive drugs are of plant origin thus an important source of research and development of new chemotherapeutics (Cragg *et al*., 2005; Newman *et al*., 2003; Ebadi, 2006). A variety of herbal remedies reported to have anticancer effects are not extensively used as alternative therapies (Cassileth and Chapman, 1996). Plants products have been utilized since time memorial in treatment of various ailments including cancer (Syam *et al*., 2011). Many plants such as *Typhonium flagelliforme* (Mohan *et al.*, 2008) and *Murraya koenigii* (Syam *et al.*, 2011) have been reported to have active agents against cancer in laboratory studies.

Africa is a rich source of medicinal plants from which various bioactive compounds are extracted notably *Catharanthus roseus* which yields anti-tumour agents such as vinblastine and vincristine (Lucy and Edgar 1999). "Vinblastine and vincristine are the bisindole alkaloids isolated from the Madagascar periwinkle, Catharanthus roseus G. Don. (Apocynaceae) introduced a new era of the use of plant material as anticancer agents. They were the first plant derived agents to advance into clinical use for the treatment of cancer" (Cragg and Newman, 2005). Vinblastine and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of different types of cancers, including leukemias, lymphomas, testicular cancer, breast and lung cancers, and Kaposi's sarcoma (Cragg and Newman, 2005). Ellipticine is an antitumor alkaloid isolated from a Fijian medicinal plant Bleekeria vitensis A.C. Sm, is marketed in France for the treatment of breast cancer (Cragg and Newman, 2005).

Some of the medicinal plants from an ethnobotanical survey in Embu and Mbeere regions of Kenya documented to treat breast and prostate cancer include: *Vitex doniana, Flueggea virosa, Ovariodendron anisatum, Launea cornuta, Grewia villosa, Maytenus obscura and Prunus africana* (Kareru *et al*., 2007). Anectodal evidence indicates that most Kenyan communities have herbal anticancer remedies. There is therefore a need to not only carry out ethnobotanical survey studies that identify the plants used but also evaluate their anti-cancer efficacy.

2.4 Bioactivity of anticancer therapeutics

Chemotherapy is a mainstay of cancer treatment. Due to increased drug resistance and the severe side effects of currently used therapeutics, new candidate compounds are required for improvement of therapy success (Wiench *et al*., 2012). The postulated mode of action of most anticancer plant extracts include impairing mitosis or by causing apoptosis (Chong *et al*., 2009). Genistein, a compound found in parsley and soy foods has been shown to inhibit protein-tyrosine kinase *in vitro* by disrupting signal transduction and inducing cell differentiation to the specialized non-cancerous nature (Markovits *et al*., 1989; López-Lazaro *et al*., 2007). "Resistance to cell death and reprogramming of metabolic pathways are two hallmarks of human cancer cells as well as major causes of chemotherapy inefficacy. Mitochondria are key structures for both these traits: (i) mitochondria are crucial for cellular energy production and cell survival; (ii) mitochondria are major regulators in the intrinsic apoptotic pathway. Mitochondrial membrane permeabilization (MMP) and the subsequent release of mitochondrial death effectors (e.g., cytochrome c) are key events for caspase activation and apoptosis. The induction of mitochondrial apoptosis can be triggered by various intracellular stimuli such as Ca2+ overload or high levels of reactive oxygen species

(ROS)" (Wiench *et al*., 2012). Cancer cells display decreased mitochondrial activity and instead shift to aerobic glycolysis for ATP production, a phenomenon known as the Warburg effect (Gogvadze *et al*., 2008). Cancer cells are often more resistant to activation of the mitochondrial apoptotic pathway due to overexpression of antiapoptotic Bcl-2 family proteins (Chen *et al*.,2010) or stabilization of the mitochondrial membrane against apoptosis-associated permeabilization (Kroemer and Pouyssegur,2008). Another trait associated with cancer cells is elevated ROS levels, probably caused by mitochondrial dysfunction (Brandon *et al*., 2006) Therefore, it is conceivable that cancer cells have a lower tolerance to further oxidative insults induced by ROS-generating drugs (Guizzunti *et al*., 2012). The present study measured Reactive Oxygen Species by Flow Cytometry to elucidate the mechanism of bioactivity of the medicinal plants.

CHAPTER THREE

3.0 ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS USED FOR TREATMENT AND MANAGEMENT OF CANCER IN KAKAMEGA COUNTY, KENYA

3.1 INTRODUCTION

"Cancer is a broad group of various diseases typified by unregulated cell growth. In cancerous state cells division and growth is uncontrollable resulting in tumours that, if malignant, may metastasise to other parts of the body. These neoplastic cells originate from highly specialized cells through a process of regression to a simpler; more primitive stage and which unlike the normal parent cells; divide continuously. The main properties of neoplastic tissue include; sustained proliferative signalling, evasion of growth suppressors, resistance to cell death,

replication, angiogenesis, invasion and metastasis, reprogramming energy metabolism and escaping immune destruction. The causes of rise in cases of cancer are not known. However, increased cancer risk is known to correlate with tobacco use, certain infections, radiation, lack of physical activity, age, poor diet, obesity, and environmental pollutants. These factors may damage genes directly or combine with existing genetic faults within cells to cause the disease", (Anand and Kunnumakara, 2008). Approximately 13% of all deaths globally each year are due to cancer of which the most common is lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths) (WHO, 2010). Although cancer may be evident at any age, invasive cancer commonly occurs over the age of 65 (Coleman *et al.,* 2009). In Kenya, mortality from various forms of cancer is approximated at 50 per day with about 80,000 cases diagnosed each year (Pact Kenya Cancer Assessment in Africa and Asia, 2010). Current treatment regimens include chemotherapy, radiotherapy and surgery (Vickers, 2004). "Patient survival varies greatly depending on the type, location and stage of the disease at the onset of treatment. Chemotherapy in addition to surgery has proved useful in a number of different cancer types including breast, colorectal, pancreatic, osteogenic sarcoma, testicular, ovarian and certain lung cancers. However, the effectiveness of chemotherapy is often limited by toxic effects on other non-target tissues. Consequently, complementary and alternative therapies such as herbal therapies are increasingly used. Such interventions have however not been rigorously studied or tested", (Vickers, 2004).

Although information on indigenous medicinal plants used for many diseases has been recorded by several authors (Glover, 1996; Kokwaro, 1993; Kaendi, 1997; Lindsay and Hepper, 1978), little work has been done on anticancer plants in Kenya. The present study

documented medicinal plants traditionally used by communities in treatment and management of cancer. The study was carried out in Kakamega County, Kenya, a populous region adjacent to a tropical forest, the Kakamega rain forest.

3.2. MATERIALS AND METHODS

3.2.1 Study area

Kakamega County located in the Western part of Kenya is the second most populous county in Kenya with a population of 1,660,651 and an area of 3224.9 km2 (Kenya Open Data, 2011) (Fig. 1). The area is tropical with high rainfall (1250–1750 mm per annum) and temperature range of 10.3–30.8 °C with an average of 20.5 °C. Kakamega Forest National Reserve, with the only tropical rainforest in Kenya, is found in Kakamega County. The County has a total of 55 health facilities; 12 hospitals, 15 health centres, 20 dispensaries and 8 clinics. However, the medical services in the county are inaccessible to the majority of the people due to high costs, inadequate or poorly equipped health facilities, staff shortage and lack of maintenance of the health facilities. In Kenya, the average distance to a health facility is 10 km in rural areas and 500 m in urban areas. The doctor patient ratio of 1: 14,246 is indicative of medical personnel shortage (Kenya Open Data, 2011). The study area is adjacent to Kakamega rain forest that makes it a rich hub of indeginous medicinal plants.

3.2.2 Data collection

An ethnobotanical survey was rolled out in September 2012 with key respondents being Traditional Medicine Practioners (TMP) and questionnaires were administered to randomly sampled 32 TMP from Kakamega County capturing data on demography of respondents, neoplastic conditions encountered, patient symptoms, diagnosis logic, number of patients previously treated by conventional medical approach, and medicinal plants used for treatment.

The questionnaires (see Appendix 1) also collected information on the medicinal plants; the local name of plant(s) and part (s) used storage, plant habitat, availability, method of preparation, dosage and means of administration, use in cancer treatment, effectiveness, and their perceived effectiveness and toxicity. In order to limit biased information, an introductory seminar with key stakeholders and cultural officers preceded the administration of questionnaires. The participants were assured of confidentiality and use of the data for research purposes only. Signed consents were obtained from the herbalists prior to the interviewing session. Each informant was interviewed *in camera*.

3.2.3 Sampling and identification of medicinal plants used in treatment of cancer

The medicinal plants reportedly used by the TMPs in cancer treatment were collected and verified by a plant taxonomist and voucher specimens deposited at the University of Nairobi School of Biological Sciences Herbarium. Questionnaire transcripts were summarised into meaningful units by computing frequencies and percentages. The dried plant was ground into powder in a fume chamber using a Cunningham grinder and preserved in clearly labeled polythene bags (Gakuya, 2001).

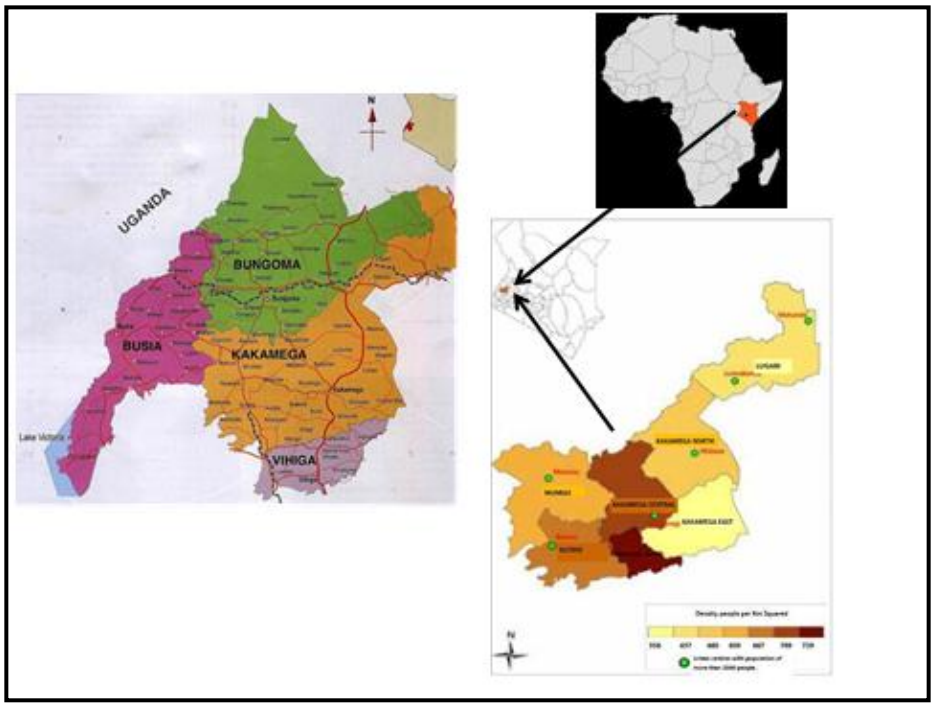

Figure 3.1 Map of Kenya showing Kakamega County

3.3 RESULTS

The following graphical presentation gives the different families of medicinal plants that were cited by carious TMPs.

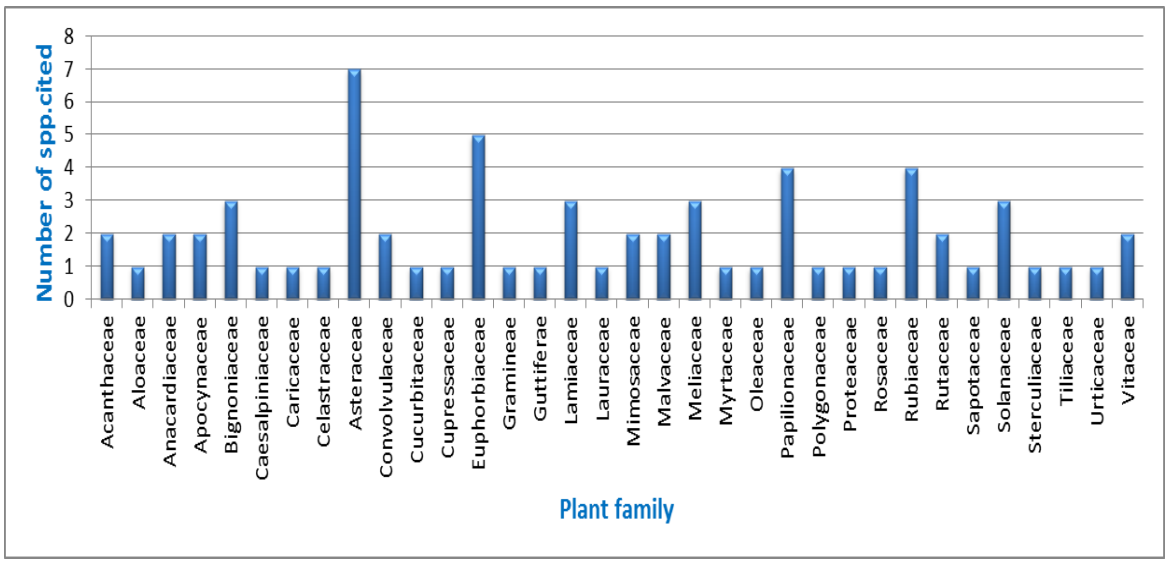

Figure 3.2 Number of plant species per taxonomic family

Table 3.1: Inventory of medicinal plants mentioned to be used in management and treatment of cancer in the study area. The data represents the medicinal plants their botanical names, identitifying voucher specimen number, the cancer treated, family name, luhya name, part(s) used, method of preparation and administration, life form, habitat and number of herbalists mentioning plant spp. (overall percentage).

Family	Botanical name and voucher number	Cancer treated	Luhya name	Part(s) used	Method of preparation and administration	Life form	Habitat	n=Number of herbalists mentioning plant spp. (overall percentage)
Acanthaceae	Dicliptera laxata C.B.Clarke (DO2012/031)	Colorectal cancer	Likhundu/ Eshitoo	Leaves	Boiled in water and taken as a concoction orally, one tablespoon (4.5g) twice daily for one week. Usually boiled together with *Albizia gummifera (J.F.Gmel.)* leaves and *Salvia coccinea (L.) Murr* leaves.	Herb	Compound	2(6.3)

Acanthaceae	Justicia betonica L. (DO2012/045)	Breast/colorectal/skin cancer	Shikuduli	Whole	Powder is mixed with hot water and taken orally as a concoction half a glass (150ml) twice per day. Usually mixed with powder from *Microglossa pyrifolia* leaves and stem bark, *Hippocratea africana (Willd.) Loes.* Leaves and roots and *Prunus africana (Hook.f.)Kalkman* leaves, root bark and stem.	Herb	Wild	1(3.1)
Aloaceae	Aloe volkensii (DO2012/037)	Colorectal/esophageal/Prostate	Linakha	Leaves	Ground to powder or boiled fresh in water and taken orally half a glass three times a day for two months. Usually 40 grams in one litre of water. Also applied fresh on the breast cancer wound three times a day until recovery.	Shrub	Wild and cultivated	4(12.5)

Anacardiaceae	Mangifera indica L.(DO2012/017)	Skin/throat/Breast cancer	Liembe	Roots, leaves and stem bark.	Boiled and taken orally as a concoction one glass (300ml) three times daily for seven days. Usually boiled together with *Harungana madagascariensis poir* stem bark, *Vernonia lasiopus O Hoffin* stem bark and *Spathodea campanulata P.Beauv.ssp.nilotica(See m)* stem bark and roots.	Tree	Wild and compound	2(6.3)
Anacardiaceae	Rhus vulgaris Meikle (DO2012/014)	Stomach/ skin/breast cancer	Sungula	Roots, leaves and fruits	Pound and boiled, mixed with *Carica papaya* roots and taken orally one glass per day until recovery.	Tree	Wild	1(3.1)
Apocynaceae	Tabernaemontana stapfiana Britten (DO2012/021)	Breast cancer	Mdondo	Stem bark	Dried and pound into powder and mixed with alcohol and used topically to wash the wound once daily for one month.	Tree	Wild near rivers	1(3.1)

Apocynaceae	Catharanthus roseus (L.) G.Don (DO2012/068)	Throat/stomach/esophageal cancer	Olubinu	Whole	Taken orally as a concoction half a glass (150 ml) twice a day for three weeks, also pound and applied topically. Usually taken together with *Sesbania sesban (L.) Merr.*whole plant.	Herb	Wild	4(6.3)
Bignoniaceae	Spathodea campanulata P.Beauv.ssp.nilotica(Seem) (DO2012/024)	Cervical/bone/breast/colorectal/skin cancer	Muthulio/ Nandi flame/Mutsuria	Stem Bark, roots and leaves	Powder taken in hot water or in meat soup or in alcohol as a concoction orally, one teaspoonful (4.5g) three times a day for 4 weeks. Sometimes mixed with powder of *Prunus africana (Hook.f.)kalkman* stem bark and roots, *Microglossa pyrifolia (Lam.) Kuntz*e leaves and *Harungana madagascariensis Lam.ex Poir* stem bark and roots.	Tree	Wild and cultivated	7(21.9)

Bignoniaceae	Kigelia africana (Lam.) Benth. (DO2012/007)	Breast/uterine/skin cancer	Omurabe/ Morabe	Stem bark and leaves of aerial part.	Stem bark is boiled and taken orally one glass (300ml) twice a day for three months. Leaves are made into powder and applied topically on the wound.	Tree	Wild	1(3.1)
Bignoniceae	Markhamia lutea (Benth.) K.schum (DO2012/019)	Colorectal cancer	Lusiola/ Shisimbali	Stem bark	Boiled, taken orally as a concoction, half a glass (150ml) two times a day until recovery. Usually boiled with *Albizia gummifera* stem bark and *Conyza sumatrensis (Retz.) E.H Walker* leaves.	Tree	Wild	1(3.1)

Caricaceae	Carica papaya (DO2012/016)	Cervical/colorectal /breast	Lipopayi	Roots, leaves and fruit.	Milky juice from the tree is collected and used to wash the wound. Ground dry pawpaw leaves are applied topically on the wound repeatedly every day for seven days. Powder boiled and taken orally as a concoction one glass three times a day for one and half months. Usually boiled together with *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark and roots, *Conyza sumatrensis (Retz.) E.H Walker* leaves, *Bridelia micrantha (Hochst.)Baill* stem bark and roots and *Aloe spp*.leaves.	Tree	Compound	3(9.4)

Celastraceae	Hippocratea africana (Willd.) Loes. (DO2012/042)	Colorectal/skin/breast cancer	Shikhalikhanga	Leaves and roots	Leaves are made into powder and roots are boiled, the mixture is taken orally as a concoction half a glass (150ml) once a day until recovery. Usually used together with *Zanthoxylum rubescens Hook.f* leaves and root bark, *Bridelia micrantha (Hochst.)Baill* leaves and root bark, *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* leaves and stem bark and *Trichilia emetica Vahl* stem bark and roots.	Shrub	Wild and cultivated	1(3.1)
Compositae/ Asteraceae	Conyza sumatrensis (Retz.) E.H Walker (DO2012/020)	Throat/Breast/squamous cell carcinoma of the gums	Liposhe	Leaves	Boiled and taken orally as a concoction half a glass (150ml) 2 times a day until recovery. Usually boiled with *Albizia gummifera* stem bark and *Markhamia lutea (Benth.)*	Herb	Wild	3(9.4)

					K.schum stem bark.			
Compositae/ Asteraceae	Microglossa pyrifolia (Lam.) Kuntze (DO2012/036)	Colorectal/skin/breast cancer	Ingwe/Ingoyi/Enguu	Leaves ,stem bark and root bark	Leaves are used as powder and taken orally one spoonful (4.5g) daily as a concoction in hot water for one month while the stem bark is boiled and taken orally as concoction half a glass twice per day until recovery. Usually taken together with *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark and roots, *Conyza sumatrensis (Retz.) E.H Walker* leaves and *Juniperus procera Endl.* stem bark.	Shrub	Wild	6(18.8)

Compositae/ Asteraceae	Vernonia lasiopus O Hoffin (DO2012/023)	Colorectal cancer	Shiroho	Stem bark	30gms is boiled in one litre of water and taken orally as a concoction half a glass twice daily for one week. Usually boiled together with *Harungana madagascariensis poi*r stem bark and *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark and roots.	Shrub	Wild	1(3.1)
Compositae/ Asteraceae	Solanecio mannii(Hook.f) C.Jeffrey (DO2012/046)	Skin/breast/colorectal cancer	Livokho	Leaves	Leaves are dried and ground to powder and taken orally as a concoction half a glass (150ml) once per day. Taken together with *Microglossa pyrifolia* stem bark and leaves, *Zanthoxylum rubescens Hook.f* leaves and root bark, *Croton macrostachyus Delile* leaves and *Spermacoce*	Shrub	Wild and cultivated	1(3.1)

					princea leaves.			
Compositae/ Asteraceae	Galinsoga parviflora Cav. (DO2012/003)	Colorectal cancer	Oulfuta	Leaves	Dried and ground and mixed in boiled water, taken orally as a concoction one glass (300ml) twice daily for two weeks. Usually mixed with *Ocimum gratissimum Suave* leaves, *Triumfetta rhomboidea Jacq.* Leaves and *Senna didymobotyra (Fresen.) Irwin and Barneby* leaves.	Herb	Wild	1(3.1)
Compositae/ Asteraceae	Bidens pilosa L.(DO2012/062)	Skin/throat cancer	Igwisi	Roots, leaves and stem	Boiled in water together with roots, leaves and stem of Oxygonum *sinuatum (Meisn.)Dammer* and taken orally as a concoction one glass (150ml) three times a day for 14 days.	Herb	Wild	1(3.1)

Compositae/ Asteraceae	Solanecio nandensis (S.Moore) C.Jeffrey (DO2012/063)	Breast/colorectal cancer		Stem and leaves	Put in nylon paper bag and steam in water bath and apply topically on the wound (especially breat cancer wounds) by rubbing. Used together with *Cyphostemma serpens (A.Rich.)* stem bark and leaves.	Herb	Wild	1(3.1)
Convolvulaceae	Ipomoea cairica (L.) (DO2012/047)	Breast/cervical/skin cancer	Ndirande/ Lilande	Leaves and roots	Dried into powder and applied topically on the wound.	Shrub	Wild	2(6.3)
Cucurbitaceae	Momordica foetida Schumach. (DO2012/054)	Breast/cervical cancer	Libobola	Whole aerial part	Boiled and taken orally as a concoction one teaspoonful (4.5g) per day in tea leaves until recovery. Usually boiled together with *Ipomoea cairica (L.)* roots and *Solanum aculeastrum Dunal* fruit and roots.	Climber	Wild	2(6.3)

Cupressaceae	Juniperus procera Endl. (DO2012/066)	Breast/Throat/Squamous carcinoma of the gum	Omusembe	Stem bark	Powder is made into capsules and taken orally with warm water as a concoction one capsule per day for ten days. Usually mixed with *Carica papaya* roots and leaves, *Conyza sumatrensis (Retz.) E.H Walker* leaves, *Microglossa pyrifolia (Lam.) Kuntze* leaves and *Capsicum frutescens L.* fruit cover.	Tree	Compound	1(3.1)
Euphorbiaceae	Bridelia micrantha (Hochst.)Baill (DO2012/028)	Cervical/Breast/Skin/Colorectal cancer	Shikangania/ kamulonda - ngombe/ shikanyanga	Stem Bark, roots and leaves	Powder mixed in hot water and taken orally as concoction one glass (300ml) 3 times a day for 2 months. Usually taken together with *Prunus africana (Hook.f.) kalkman* stem bark and roots, *Syzygium guineense DC*. Bark, *Spathodea campanulata P.Beauv.ssp.nilotica*	Tree	Wild and cultivated	3(9.4)

					(Seem) stem bark and roots and *Cyphostemma serpens (A.Rich)* stem bark.			
Euphorbiaceae	Tragia brevipes Pax(DO2012/030)	Breast cancer/Leukemia	Isambakhalu	Leaves	Powder taken in hot water orally three glasses (900ml) per day until recovery.	Herb	Wild	1(3.1)
Euphorbiaceae	Croton macrostachyus Delile(DO2012/040)	Colorectal/Skin/Breast	Musutsu	Leaves and stem bark	Powder is mixed in hot water, cooled for five minutes and taken orally as a concoction one glass (300ml) three times a day for two months. Usually taken together with *Zanthoxylum rubescens Hook.f* leaves and root bark, *Prunus africana (Hook.f.) kalkman* leaves and stem bark, *Harungana madagascariensis Lam.ex Poir* stem bark and *Harungana madagascariensis Lam.ex Poir* stem bark.	Tree	Wild and cultivated	2(6.3)

Euphorbiaceae	Phyllanthus fischeri Pax (DO2012/041)	Breast/skin/colorectal	Lusarisari	Leaves and stem bark	Leaves are powdered and bark is boiled, taken orally as a concoction half a glass (150ml) once a day until recovery. Usually mixed together with *Microglossa pyrifolia* leaves and stem bark, *Croton macrostachyus Delile leaves, Harungana madagascariensis Lam.ex Poir* stem bark and *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark.	Shrub	Wild and cultivated	1(3.1)
Euphorbiaceae	Shirakiopsis elliptica (Hochst.)Esser synonym) Sapium ellipticum (Hochst.krauss)Pax (DO2012/035)	Colorectal/esophageal cancer	Musasa	Bark and leaves	Powder is in hot water orally one teaspoonful (4.5g) daily as a concoction for one month. Usually mixed together with *Melia azedarach L* leaves, *Microglossa pyrifolia (Lam.) Kuntze* leaves *Zanthoxylum rubescens*	Tree	Wild	1(3.1)

					Hook.f bark and *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* bark.			
Poaceae (Gramineae)	Cymbopogon citratus (DO2012/069)	Colorectal cancer	Lemon grass	Leaves and stem	20 grams of stem is boiled fresh in one liter of water and leaves pound to powder and taken orally one glass (300ml) thrice a day for seven days.	Herb	Cultivated	1(3.1)
Guttiferae	Harungana madagascariensis Lam.ex poir (DO2012/022)	Colorectal/skin/Breast cancer	Musila/ Munamusayi	Stem bark and roots	30 grams is boiled in one litre of water and taken orally as a concoction, one glass three times daily for two months. Usually boiled together with *Zanthoxylum gilletii (De Wild.) P.G.Waterman* stem bark, Spathodea *campanulata P.Beauv.ssp.nilotica (Seem)* stem bark and roots, *Prunus africana (Hook.f.)kalkman* stem bark and *Vernonia lasiopus O Hoffin* stem bark.	Tree	Wild and cultivated	5(15.6)

Labiatae/ Lamiaceae	Fuerstia africana T.C.E.Fr.(DO2012/057)	Colorectal cancer	Kwa matsai	Leaves, stem and roots	30 grams is pound and boiled in one litre of water and taken orally quarter glass twice a day until finished, this is repeated until recovery. The powder from the plant can also be applied topically.	Shrub	Wild	1(3.1)
Labiatae	Salvia coccinea (L.) Murr (DO2012/032)	Breast/Esophageal /colorectal cancer	Muonyi	Leaves	Boil in water and taken as a concoction orally, 1 tablespoon (4.5g) twice daily for one week. Boiled together with *Dicliptera laxata C.B .Clarke* leaves and *Albizia gummifera (J.F.Gmel.)* leaves. Leaves of this plant are dried indoors and powder from them applied topically on lesions once every two days.	Herb	Wild and cultivated	2(6.3)

Labiatae	Ocimum gratissimum L.Suave wiild O.tomentosum oliv. (DO2012/006)	Colorectal cancer	Ouwali	Leaves	30 grams of fresh leaves dried in the shade, ground and mixed in boiled in one liter of water, taken as a concoction orally one glass twice daily for two weeks. Usually mixed with *Galinsoga parviflora Cav.* leaves, *Senna didymobotyra (Fresen.) Irwin and Barneby* leaves *Triumfetta rhomboidea Jacq* leaves.	Herb	Wild	1(3.1)
Labiatae/ Lamiaceae	Persea americana Mill (DO2012,061)	Colorectal/skin/breast cancer	Liavacado	Leaves	Dried and made to powder and taken orally, 4.5g of powder in half a glass (150ml) thrice a day until recovery, the powder can also be licked.	Tree	Compound	2(6.3)

Mimosaceae	Albizia gummifera (J.F.Gmel.) (DO2012/018)	Throat/skin cancer	Musenzeri / Mukhonzuli	Stem bark and leaves	Boiled, taken orally as a concoction half a glass(150ml) 2 times a day until recovery. Usually boiled together with *Markhamia lutea (Benth.) K.schum* stem bark and *Conyza sumatrensis (Retz.) E.H Walker* leaves.	Tree	Wild	2(6.3)
Papilionaceae(fabaceae)	Glycine wightii (wight &Arn.) (DO2012/048)	Breast cancer	Maua kulangany a	Leaves	Dried and made to powder and applied topically on the wound. Has a high penetrative ability.	Climber	Wild	2(6.3)
Papilionaceae(fabaceae)	Abrus precatorius L.ssp africanus Verdc.(DO2012/052)	Skin cancer	Ndirakalu	Roots and seeds	Boiled and taken orally as a concoction half a glass (150ml) twice daily until recovery. Usually boiled together with Solanum aculeastrum Dunal fruits and leaves.	Climber	Wild	1(3.1)

Papilionaceae(fabaceae)	Sesbania sesban (L.)Merr (DO2012/011)	Throat/uterine/skin cancer	Omukhule /Olukhulila mbusi/Lohori	Whole plant	Taken orally as a concoction half a glass (150ml) twice a day for three weeks, also pound and applied topically.	Shrub	Wild	2(6.3)
Caesalpiniaceae	Senna didymobotyra (Fresen.) Irwin and Barneby (DO2012/004)	Colorectal cancer	Omuvinuvinu/ Luvinu	Leaves	30 grams of fresh leaves dried in the shade, ground and mixed in one litre of boiled water taken orally as a concoction one glass twice daily for two weeks. Taken together with *Galinsoga parviflora Cav* leaves, *Triumfetta rhomboidea Jacq* leaves and *Ocimum gratissimum Suave* leaves.	Shrub	Wild	1(3.1)
Papilionaceae(fabaceae)	Aeschynomene abyssinica (A.Rich.) Vatke (DO2012/058)	Uterine/skin/squamous cell carcinoma of the gums	Olunyili	Leaves	Powder is boiled and given orally as a concoction quarter a glass (75ml) daily for two weeks. Boiled together with *Triumfetta rhomboidea Jacq* leaves and *Sida rhombifolia L.* leaves. For topical	Herb	Wild	1(3.1)

					application use at three days interval.			
Mimosaceae	Albizia coriaria Welw.ex Oliv.(DO2012/009)	Breast/uterine/skin cancer	Omubeli	Bark and leaves	Powder from leaves is made as a paste with water and topically applied twice daily for skin cancer eruptions. The bark is boiled in three litres of water and taken orally two glasses for seven days.	Tree	Wild	1(3.1)
Malvaceae	Sida rhombifolia L.(DO2012/013)	Uterine/skin/squamous cell carcinoma of the gums	Omukusa	Leaves	30 grams of leaves are crushed and dried and made into powder, the powder is boiled in one litre of water and given as a concontion quarter a glass daily for two weeks. Usually boiled together with *Triumfetta rhomboidea Jacq* leaves and *Aeschynomene abyssinica (A.Rich.) Vatke leaves*. For topical application use at three days interval.	Herb	Wild	1(3.1)

Malvaceae	Sida cordifolia L.(DO2012/002)	Skin cancer	Lusatsa	Leaves	Powder is applied topically on the wound and it sticks until it covers the lesion. Applied daily till healing occurs. Mostly used in treating Skin sarcoma. Used together with powder from *Waltheria indica L*. leaves.	Herb	Wild	1(3.1)
Malvaceae/ Sterculiaceae	Waltheria indica L.(DO2012/001)	Skin/uterine/breast cancer	Olundu lukhasi	Leaves	Powder is applied topically on the wound and it sticks until it covers the lesion. Applied daily till healing occurs. Mostly used in treating Skin sarcoma.Used together with powder from *Sida cordifolia* L leaves.	Herb	Wild	2(3.1)
Meliaceae	Melia azedarach L.(DO2012/038)	Colorectal and esophageal cancer	Mwarubaine	Leaves	4.5g powder and taken orally half a glass (150ml) thrice a day, the powder can also be licked.	Tree	Wild and compound	2(6.3)

Meliaceae	Trichilia emetica Vahl (DO2012/039)	Breast/skin/colorectal cancer	Munyama/ irojo/ Musinzi	Stem bark ,bark of roots and leaves	Boiled and taken orally as a concoction half a glass once a day until recovery. Usually boiled together with *Prunus africana (Hook.f.) kalkman* stem bark and roots, Aloe *volkensii* leaves *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* leaves and stem bark and *Harungana madagascariensis Lam.ex Poir* stem bark.	Tree	Wild and cultivated	2(6.3)
Meliaceae	Ekebergia capensis Sparrm.(DO2012/018)	Skin/Throat/Breast cancer	Eshiruma	Stem bark and leaves	Stem is boiled fresh and leaves pound to powder and taken orally one glass (300ml) thrice a day for seven days.	Tree	Wild and cultivated	1(3.1)
Myrtaceae	Syzygium guineense DC.(DO2012/025)	Skin cancer	Musiema	Bark	Powder is taken orally with hot milk or in water with honey as a concoction, one teaspoonful (4.5g) thrice a day for 3 weeks,	Tree	Wild	1(3.1)

					remains of powder after extraction is used to shower with. Taken together with powder from *Prunus africana (Hook.f.)Kalkman* stem bark and roots, *Solanum mauritianum Sco*p bark, *Macadamia tetraphylla* bark and *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* bark and roots.			
Oleaceae	Olea hotch spp.hochstetteri (DO2012/056)	Skin cancer	Omutukuyu/ Mutukuyu	Stem	Boiled and taken orally as a concoction half a glass (150ml) three times a day until recovery. Boiled together with *Zanthoxylum gilletii (De Wild.) P.G.Waterman* stem bark, *Harungana madagascariensis Lam.ex Poir* stem bark, *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark and	Shrub	Wild and cultivated	1(3.1)

					roots and *Prunus africana (Hook.f.)Kalkman* stem bark.			
Polygonaceae	Oxygonum sinuatum (Meisn.)Dammer (DO2012/062)	Skin and throat cancer	Rakaro	Roots, leaves and stem	Boiled in water and taken orally as a concoction one glass (300ml) three times a day for 14 days. Boiled together with *Bidens pilosa L* roots, leaves and stem.	Herb	Wild	1(3.1)
Proteaceae	Macadamia tetraphylla (DO2012/029)	Skin cancer	b)Muyundi	Bark	Powder drunk with hot milk or in water with honey orally as a concoction, one teaspoon thrice a day for 3 weeks, remains of powder after extraction is used to shower with. Usually mixed together with powder from *Syzygium guineense DC*. bark.	Shrub	Compound	1(3.1)

Rosaceae	Prunus africana (Hook.f.)kalkman (DO2012/027)	Colorectal/Breast/ Skin and Prostate cancer	a)Mwilitsa / Ishakulu/ Mwilitsa	Stem bark and roots	Powder boiled in water and taken orally as a concoction half a glass to one glass (300ml) 3 times a day for 10 days to one and half months. Usually boiled together with *Solanum mauritianum Scop* bark. Normally taken after meals. Causes one to have increased appetite.	Tree	Wild and cultivated	5(15.6)
Rubiaceae	Psydrax schimperiana(A.Rich) (DO2012/050)	Breast cancer	Mukomari /Shekoye	Stem bark	30 grams in one litre of water boiled fresh and taken orally half a glass three times a day until recovery Also applied topically on the wound.	Tree	Wild	1(3.1)

Rubiaceae	Spermacoce princea (DO2012/044)	Breast/colorectal/skin cancer	Vikudhuli	Leaves	Powder mixed with water and taken orally as a conconction half a glass once per day. Usually taken together with *Solanecio mannii (Hook.f) C.Jeffrey* leaves.	Shrub	Wild and cultivated	1(3.1)
Rubiaceae	Gardenia volkensii K.schum.ssp.volkensii(DO2012/008)	Breast/uterine/skin cancer	Eshiuna	Bark	Three teaspoonful (4.5g each) of powder in hot water is taken orally in a glass of water (300ml) three times daily for three months.	Tree	Wild	1(3.1)
Rubiaceae	Pavetta abyssinica (DO2012/015)	Colorectal/skin/breast cancer	Ombura	Roots and leaves	Boiled and taken orally as a concoction half a glass daily until recovery. Usually boiled together with *Rhus vulgaris Meikle* roots, leaves and fruits and *Triumfetta rhomboidea Jacq*, leaves.	Shrub	Wild	1(3.1)

Rutaceae	Zanthoxylum rubescens Hook.f (DO2012/033)	Breast/colorectal/skin/esophageal cancer	Shikhuma/ Shigulutsu / Shughoma	Stem bark, Leaves and root bark	Boiled and taken orally as a conconction half glass once a day in the evening after meals, Can also be taken mixed with cinamon.Cinamon improves the shelf life of the drug. Usually boiled together with *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark, *Afrosersalisia cerasifera (Welw.) Aubrev.* stem bark, *Sapium ellipticum (Krauss) Pax* bark and leaves and *Microglossa pyrifolia (Lam.) Kuntze* leaves.	Tree	Wild and cultivated	2(6.3)

Rutaceae	Zanthoxylum gilletii (De Wild.) P.G.Waterman (DO2012/055)	Skin cancer	Shihumba/ Shikuma	Stem bark	The powder is boiled and taken orally as a concoction half a glass (150ml) three times a day for three months, also applied topically. Boiled together with *Harungana madagascariensis Lam.ex Poir* stem bark, *Olea capensis* stem bark, *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark and roots and *Prunus africana (Hook.f.)Kalkman* stem bark.	Tree	Wild and cultivated	1(3.1)
Sapotaceae	Synsepalum cerasiferum DO2012/034)	Colorectal/esophageal cancer	Mukurum uru/ Tsikhulum uru	Stem bark	Powder is taken orally in hot water one spoonfull (4.5g) daily as a conconction for one month. Taken together with *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark, *Sapium ellipticum (Krauss) Pax*	Tree	Wild and cultivated	1(3.1)

					stem bark and roots and *Microglossa pyrifolia (Lam.) Kuntze* leaves.			
Solanaceae	Solanum mauritianum Scop(DO 2012/026)	Colorectal cancer	b)Lifuye/ Liavuya	Bark	Powder boiled in water and taken orally as a concoction half a glass to one glass 3 times a day for 10 days to one and half months. Usually boiled together with Prunus africana (Hook.f.)Kalkman stem bark and roots .Normally taken after meals. Causes one to have increased appetite.	Shrub	Wild	1(3.1)
Solanaceae	Solanum aculeastrum Dunal (DO2012/051)	Skin/breast/cervical cancer	Indalandalua	Fruit or roots	Boiled and taken orally as a concoction with *Abrus precatorius L.ssp africanus* roots one glass three times a day for one and half months.	Shrub	Wild	2(6.3)

Solanaceae	Capsicum frutescens L.(DO2012/065)	Throat/breast/squamous cell carcinoma	Pilepile	Fruit cover	The fruit cover is dried in shade, powdered and sieved to exclude the seeds. Powder is made into capsules and taken orally with warm water as a conconction one capsule per day for ten days. Usually mixed with *Spathodea campanulata P.Beauv.ssp.nilotica (Seem)* stem bark, *Microglossa pyrifolia (Lam.) Kuntze* leaves, *Juniperus procera Endl.* stem bark and *Carica papaya* roots and leaves.	Herb	Compound	1(3.1)
Tiliaceae	Triumfetta rhomboidea Jacq. (DO2012/005)	Colorectal/uterine /Squamous cell carcinoma of the gums.	Likhambi/ Imbululusia(male and female)/Oluyasi	Leaves	Powder is boiled and given as a conconction orally one glass twice daily for two weeks. For topical application use at three days interval. Usually boiled together with *Pavetta abyssinica* roots and leaves,	Shrub	Wild	3(9.4)

					Galinsoga parviflora Cav. leaves *Senna didymobotyra (Fresen.) Irwin and Barneby* leaves and *Ocimum gratissimum Suave* leaves.			
Urticaceae	Urtica massaica Mildbr. (DO2012/010)	Skin/Uterine/Breast cancer	Elaila	Leaves	Leaves are dried indoors and powder applied topically on lesions once every two days until recovery. Mostly used for skin cancer.	Herb	Field crop/Herb	2(6.3)
Vitaceae	Cyphostemma serpens(A.Rich) (DO2012/058)	Cervical/skin/Breast cancer	Lithunzune/ Maombola	Stem bark, Leaves and root bark	Powder from leaves is taken orally in hot water or porridge never in milk as a conconction one teaspoonful three times a day for 3 weeks. Also put in a nylon paper bag and steamed in water bath and applied topically on the wound by rubbing.	Herb	Wild and cultivated	4(12.5)

Vitaceae	Cyphostemma adenocaule (DO2012/043)	Colorectal/breast/ skin cancer	Mukoyego ye	Leaves and root bark	Leaves are powdered and root bark boiled and taken orally as a conconction half a glass (150ml) once a day until recovery. Usually taken together with *Phyllanthus fischeri Pax* leaves and stem bark, Hippocratea africana (Willd.) Loes. leaves and roots, *Spermacoce princea* leaves and *Solanecio mannii (Hook.f) C.Jeffrey* leaves.	Herb	Wild and cultivated	1(3.1)

The respondents comprised thirty two TMPs (22 males and 10 females). Majority of the respondents were mature adults aged between 35 and 74 years with 57% having practiced ethnomedicine for more than 10 years. In the study area, the most commonly treated cancers included skin, stomach and breast cancer. Others included cervical, uterine, prostate and throat cancer. Most of the patients had been diagnosed in a hospital prior to last resort consultations with the TMPs. The respondents identified a total of 65 plants species belonging to 59 genera and 34 families as important in the treatment of cancer (Table 3.1). The key plant species identified were Muthulio (*Spathodea campanulata* P. Beauv. ssp. nilotica (Seem) at 22%, Ingwe/Ingoyi (*Microglossa pyrifolia* (Lam.) Kuntze, (19%), Musila (*Harungana madagascariensis* Lam.ex poir, 16%), Mwilitsa (*Prunus africana* (Hook.f.) kalkman, 16%), Lithunzune (*Cyphostemma serpens* (A.Rich), 13%), Olubinu (*Catharanthus roseus* (L.) G.Don, 13%) and Linakha (*Aloe volkensii* Engl. 13%) (Table 3.1). The highest representative plant families were Leguminosae (7), Compositae (7), Euphorbiaceae (5) and Rubiaceae (4) (Fig. 2). The plant parts most commonly used were leaves (30.7%), stem bark (18.46%). Roots/leaves/stem bark (15.4%), stem bark/leaves (13.85%), leaves/ roots (6.15%), whole plant (4.62%), roots/leaves/fruits, stem bark/ roots, fruit (3.08% each) and stem bark/leaves/aerial part, roots (1.54% each). Most were dried and ground to powder but fleshy plants were also used especially the leaves and aerial part. Mature plants were preferred especially for harvesting of stem bark and roots. The scarcity of medicinal plants during the dry season meant that there was bulk harvesting in the wet season whence they were dried under shade and stored until needed. However majority of the medicinal plants were available year round in Kakamega forest. This study documented, for the first time twenty five medicinal plants used for treatment and management of cancer. These medicinal plant species

include; *Aeschynomene abyssinica* (A. Rich.) Vatke (Leguminosae), *Synsepalum cerasiferum* (Sapotaceae), *Albizia coriaria* Welw.ex Oliv. (Leguminosae), *Aloe volkensii* Engl. (Aloaceae), *Bridelia micrantha* (Hochst.) Baill (Euphorbiaceae), *Croton macrostachyus* Delile (Euphorbiaceae), *Cyphostemma serpens* (A.Rich) (Vitaceae), *Dicliptera laxata* C.B. Clarke (Acanthaceae), *Ekebergia capensis* Sparrm. (Meliaceae), *Gardenia volkensii* K. schum. ssp. volkensii (Rubiaceae), *Glycine wightii* (wight & Arn.) (Leguminosae), *Ocimum gratissimum* Suave (Labiatae), *Olea hotcsh spp. hochstetteri* (Oleaceae), *Pavetta abyssinica* Fresen.(Rubiaceae), *Phyllanthus fischeri* Pax (Euphorbiaceae), *Psydrax schimperiana* (A.Rich) (Rubiaceae), *Rhus vulgaris* Meikle (Anacardiaceae), *Senna didymobotyra* (Fresen.) Irwin and Barneby (Leguminosae), *Solanecio nandensis* (S. Moore) C. Jeffrey (Compositae), *Solanum mauritianum* Scop (Solanaceae), *Spathodea campanulata* P. Beauv. ssp. nilotica (Seem) (Bignoniaceae), *Spermacoce princea* (K. Schum.) Verdc. (Rubiaceae), *Tabernaemontana stapfiana* Britten (Apocynaceae), *Tragia brevipes* Pax (Euphorbiaceae) and *Zanthoxylum gilletii* (De Wild.) P.G.Waterman (Rutaceae).Most medicinal plants were reportedly used as a combination in equal weight ratios. The most common preparations were boiling and maceration of plant parts; drying and pounding to powder. The greatest number of THMPs reported used the oral route administration; however a few TMPs applied the preparation topically. The oral dose of the herbal remedies varied among the respondents but most of reportededly administered one glass (about 200 ml), twice per day for 1–2 weeks or until the patient recovered (Table 3.1). The practitioners emphasised the need for strict adherence of the prescribed dosage to achieve the desired response. The medicinal plants were generally reportedly prepared just before use and storage did not exceed 7 days unless the preparation was in powder form. There were no patient side effects reported by the

respondents with an exception of *Solanum mauritianum* Scop that apparently increased appetite. Analysis of the vegetation used indicated that shrubs (16/65), herbs (19/65) and trees (25/65) were the main sources of potential anticancer medicinal plants in Kakamega County. Most of the reported plants occurred naturally in the wild (n=36), however cultivation was also a source (n=20). Some however occur naturally on farmland (n=6), while others were found in both farmlands and the wild (n=20). A majority of the medicinal plants were reportedly available within Kakamega forest, an important biodiversity resource in Kakamega County (Table 3.1).

Table 3. 2 Cross-references the traditional cancer treatment knowledge of the plant species with published literature of their ethnomedicinal use.

Botanical name and family	Biological activity/chemical constituents	Ethnomedicinal uses
Abrus precatorius L.ssp africanus Verdc.(Papilionaceae)	Isoflavan quinones and hydroquinones; *In vitro* antiprotozoan activity against T. brucei rhodesiense (Hata *et al.,* 2012); secretes the toxin called abrin (lectins) which with antitumor properties is closely related to ricin (Bruneton, 1999).	Used in treatment of flatulent colic and bloat in veterinary medicine (Kasonia *et al.,* 1991,Byavu *et al* .,2000) Seeds have Immunomodulatory and Antitumor Properties (Sujit *et al.,* 2011).Leaves decoction and roots is a remedy for gonorrhoea,also an aphrodisiac also for treatment of cystitis(Kokwaro ,1993),Leaf infusion is drunk like tea as a remedy for fevers(Morris,1996)
Aeschynomene abyssinica (A.Rich.) Vatke (Papilionaceae)	No previous reports	Preparation of the root is drunk for stomach ache.(Kokwaro,1993),Bark chewed for treatment of heart burn (Okello *et al* .,2010)
Synsepalum cerasiferum (Sapotaceae)	No previous reports	Fresh latex applied to wounds for treatment(Kokwaro,1993)

Albizia coriaria Welw.ex Oliv.(Mimosaceae)	Molluscicidal activity is shown by bark extracts (Mengesha *et al.*, 1997), Contains tannins as the active ingredient. The bark is rich in saponins(Orwa *et al.*, 2009)	The stem bark is used for the treatment of excess bleeding, threatened abortion and post-partum haemorrhage,and also in treating cattle diseases and a number of abdominal problems associated with protozoan parasites(Kokwaro,1993;Orwa *et al* .,2009)
Albizia gummifera (J.F.Gmel.) (Mimosaceae)	Oleanane-type triterpenoid saponins, gummiferaosides A−C with cytotoxicity against the A2780 human ovarian cancer cell line (Shugeng *et al* .,2007).Bark has tannins(Orwa *et al* .,2009),Macrocyclic spermine alkaloids isolated from A. gummifera were active against 2 Gram-positive (Bacillus subtilis and Staphylococcus aureus) and 2 Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa)(Orwa *et al* .,2009)	Decoction of the bark is used to treat malaria (Burkill,1995;Beentje,1994),Root paste for treatment of skin diseases ,root extract used to cure skin diseases (Kokwaro,1993)
Aloe volkensii(Aloaceae)	Exudate contains a mixture of the stereoisomers aloin A (barbaloin) and aloin B (isobarbaloin), which are responsible for the laxative properties (Maundu *et al* .,2001),Roots have anthraquinones and pre-anthraquinones (Wyk *et al.*,1995)	Ash from leaves is applied directly to sores or licked for whooping cough. Juice from fresh leaves used to kill jiggers (Kokwaro,1993),Leaves have traditional uses for healing skin lesions, as a purgative and an anti-worming agent (Carter,2011)

Bidens pilosa L.(Compositae/Asteraceae)	Polyacetylenes and flavonoids(Christensen and Lam 1991;Silva *et al* .,2011).”Extracts have anti-hyperglycemic, antihypertensive, antiulcerogenic,hepatoprotective,antipyretic,immunosuppressive and anti-inflammatory, anti-leukemic, anti-malarial,anti-bacterial, antioxidant and antitumor effects”,(Silva *et al* ,2011;Kviecinski *et al* 2008),Anti-leukemic activity (Chang *et al* .,2001)	Leaves used to treat conjuctivitis,Roots are chewed or decocted as a remedy for malaria(Kokwaro,1993)
Bridelia micrantha (Hochst.)Baill (Euphorbiaceae)	Stem bark contains phytochemicals including alkaloids, flavonoids, steroids, tannins and saponins (Okeleye *et al.*, 2011).In vitro anti-helicobacter pylori activity (Okeleye et al., 2011).	Bark is used to treat skin ailments(Okello *et al*., 2010),Decoction from the bark used to treat venereal diseases,stomach ache and tapeworm, and diarrhoea in children(Kokwaro,1993)
Capsicum frutescens L.(Solanaceae)	Alkaloids i.e. Capsaicinoids, principally capsaicin, which ranges from 600 to 13,000 ppm in the fruits (Center for New Crops and Plant Products. 2002;Peusch *et al.,* 1997),insulinotropic (Islam and Choi,2008),antimutagenic and anticarcinogenic activities(Surh ,2002)	As a salve to relieve muscle, joint, and toothache pain, to treat cough, asthma, and sore throat, as a stimulant, and to treat stomach ache, seasickness, and flatulence (Bosland and Votava,2000),the fruit is eaten as a part of the regular diet to prevent malaria(Pradilla,1982)

Carica papaya (Caricaceae)	Antibacterial activity (Emeruwa, 1982),antifungal effect (Giordani *et al* .,1996),Anthelmintic (Satrija *et a*l 1995),Leaves contain alkaloids (Tang, 1979,Pradilla,1982),anti-tumor activity and immunomodulatory effects (Otsuki *et al* .,2010)	Decoction of the leaves is used as a remedy for fevers(Morris,1996),the leaves are used as a febrifuge(Burkill,1985),Mature leaves of Carica papaya (paw paw) are widely used to treat malaria and splenomegaly while the fruit is used against anaemia((Adjanohoun *et al.*, 1996)
Catharanthus roseus (L.) G.Don (Apocynaceae)	More than 130 alkaloids of indole group have been isolated from different parts; amongst which two important alkaloids (Vinblastine and Vincristine used in cancer treatment(Aslam *et al.*, 2010),Cytotoxic Activity,CNS Depressant Activity,Cardiotonic Activity, Antiviral Activity, Antibacterial Activity,Antihyperglycemic Activity,Antihyperchcholesterollemic Activity,Antidiuretic Activity(Aslam *et a.l*,2010)	Whole plant decoction used for treating abdominal pains(Kokwaro ,1993),the plant is used to treat malaria(Mulhovo, 1999) ,used to diabetes, menstrual regulators, hypertension, cancer and antigalactagogue(Aslam *et al* .,2010)
Conyza sumatrensis (Retz.) E.H Walker (Compositae/Asteraceae)	Antimalarial activity (Boniface and Pal ,2012), analgesic properties and anti-inflammatory activities (Asongalem *et al* .,2004),sesquiterpenoids and diterpenoids and cyclooctadienone derivatives(Boti *et al.*, 2007)	Infusion of leaves given to babies and children as a laxative,cleans pimples,chicken pox, small pox and antidote for snake bite (Kokwaro,1993),Leaves used for treatment of postpartum pain and back pain in women (Njoroge *et al* .,2009)

Croton macrostachyus Delile(Euphorbiaceae)	Larvicidal activity (Karunamoorthi and Ilango,2010)	Sap, leaves and bark are used to treat ringworms,dysmenorrhea,diarrhea,fever in cows(Okello *et al.,* 2010).Juice from the boiled roots is drunk as a remedy for malaria by the Kikuyu people(Beentje,1994),Juice from fresh leaves used to hasten blood clotting, root decoction used to treat malaria and venereal diseases (Kokwaro,1993),used for treatment of malaria (Giday *et al* .,2007)
Cymbopogon citratus (Gramineae)	Antimalarial activity (Tchoumbougnang *et al*., 2005,Bidla *et al.*,2004),contains oils namely geranial,neral,myrcene and beta-pinene(Tchoumbougnang et al., 2005),anticarcinogenic ,contain compounds citral (neral and geranial), geraniol and β-myrcene, 6-methyl-5-hepten-2-one and undeca-2-one(Bidinotto *et al.*,2012),Quinones,tanins,alkaloids,flavonoids,saponosids,terpene-sterols (Mukarutakwa,2007)	The leaves are infused as a febrifuge and sudorific,insect repellent: (Burkill,1994)
Cyphostemma adenocaule(Vitaceae)	The root is said to contain tannin. Leaves and fruits contain oxalic acid (Bosch,2004);anti-inflammatory, antimicrobial (Lin *et al,* 1999)and antitumor activities (Opoku *et al* .,2000)	Used in treatment of excessive bleeding; irregular and painful menstruation (Kamatenesi-Mugisha *et al* .,2007)

Cyphostemma serpens(A.Rich) (Vitaceae)	No previous reports	Decoction of leaves used for treatment of abscesses, boils, gynaecological problems, polio and skin disease. Roots used as a tonic (Kokwaro,1993),Leaves used to treat boils (Arwa *et al.,* 2010)
Dicliptera laxata(Acanthaceae)	Antimicrobial activity (Kothai and Befirdu ,2012)	Roots chewed as a cough remedy and stomach ache.Leaf infusion drunk for fever (Kokwaro, 1993),Leaves used for treatment of rashes and itching (Kothai and Befirdu,2012)
Ekebergia capensis Sparrm.(Meliaceae)	Hypotensive effects (Kamadyaapa *et al* .,2009),Seeds contain a crystalline limonoid; ekebergin (a diacylated methyl angolensate derivative) (Taylor,1981),uterotonic activity (Sewram *et al* .,2000),Contains β-sitosterol; oleanonic acid; 3-epioleanolic acid; 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetra-cosatetraene and 7-hydroxy-6-methoxycoumarin (Sewram *et al.,* 2000),antibacterial activity (Lall and Meyer.,1999),seeds have limonoids, capensolactones 1–3 and methyl 3α-hydroxy-3-deoxyangolensate(Mulholland Lourine,1998),Antimalarial,Ekeberin D4 (Bero *et al,* 2009),anthelmintic activity (Eguale,2006)	A preparation of the stem bark is used for treatment of backache and chest in humans and diarrhoea in the cow (Okello *et a*l., 2010), It is also used as an emetic, and for treating dysentery. Root preparations are used to treat headaches, heartburn and for chronic coughs. Leaves are used as a remedy for intestinal worms (van Wyk *et al.,* 1997)
Fuerstia africana T.C.E.Fr.(Labiatae/Lamiaceae)	Extracts from aerial parts have antimicrobial activity (Matu *et al.,* 2012),antivenin activity(Owour and kisangau,2006),Antiplasmodial and cytotoxic activities	Infusion from leaves is drank as a snake bit antidote (Owour and kisangau,2006)

	(Muganga *et al.*, 2010),antimalarial (Bero *et al*. ,2009)	
Galinsoga parviflora Cav.(Compositae/Asteraceae)	Anti-inflammatory (Alice *et al* .,1995),contains flavonoids, patulitrin, quercimeritrin, quercitagetrin and caffeoyl derivatives, has antioxidant activity (Bazylko *et al.*, 2012)	Stems and leaves are chewed to cure colds and sores(Kokwaro,1993),used for wound healing(Schmidt *et al.*, 2009)
Gardenia volkensii K.schum.ssp.volkensii(Rubiaceae)	No previous reports	Fruit infusion drunk as an emetic (Kokwaro,1993),Leaves are used for treating stomach ache (Ribeiro *et al* .,2010)
Glycine wightii (wight &Arn.) (Papilionaceae)	No previous reports	Leaf decoction drunk for treatment of oedema and excessive bleeding due to miscarriage (Kokwaro,1993)
*Harungana madagascariensis Lam.ex poi*r(Guttiferae)	Friedelan-3-one (208) was isolated from the root bark ,has antiplasmodial activity (Bero *et al.*,2009),extracts from the stem bark has anticancer activity (Iwalewa *et al.*, 2009), anti-anaemic, spasmolytic and antibacterial in skin diseases, wounds, natural source of dermatological agents and cosmetics (Tona *et al*., 1998, 2000; EMEA,1999; Lukwa et al., 2001; Okoli *et al.*, 2002; Erah *et al*.,2003; Kamanzi Atindehou *et al*., 2004).Extracts contain phenols, alkaloids, flavonoïds, tannins, saponins (Biapa *et al* .,2007)	A preparation of the bark or the juice of the leaf and roots is taken for treatment of malaria, the leaves are used as a fever remedy(Burkill,1994) Roots are used to hasten development of breasts in young women, infusion of root and bark used to interrupt menses and pound leaf paste used to treat diarrhoea, sore throat and venereal diseases (Kokwaro,1993)

*Hippocratea africana (Willd.) Loe*s. (Celastraceae)	Cytotoxic activity,Schizontocidal activity ,contains alkaloids and flavanoids (Okokon *et al,* 2006)	Used for malaria treatment (Okonon *et al.*,2006)
Ipomoea cairica (L.) (Convolvulaceae)	Lignans with antitumor activity (ED50 25 m for arctigenin) (Paska *et al* .,1996;Schroder *et al.*,1990),analgesic and antioxidative effects (Ferreira et al.,2006)	Decoction from leaves, branches and stem used for treatment of snake bites (Otero *et al.*,2000)
Juniperus procera Endl. (Cupressaceae)	Antioxidant activity (Burits *et al* .,2001),hepatoprotective diterpenoids namely: 4-epi-abietol , ferruginol , hinokiol , sugiol, Z-communic acid and hinokiol-1-one 3β,12-dihydroxyabieta-8,11,13-triene-1-one) , in addition to the sesquiterpene 8α-acetoxyelemol (Alqasoumi and Abdel-Kader ,2012)	Roots are pound and berries chewed for treament of wounds and breathing difficulties (Okello *et al*., 2010)
Justicia betonica L. (Acanthaceae)	Alkaloids (jusbetonin,10H-quindoline),triterpenes and Lignans with anticancer activity (Caprio *et al.*,2000;Subbaraju *et al.*,2004; Corrêa and Alcântara.;2012)	Infusion of leaves used to treat snake bite,shoots infusion drunk for orchiitis and venereal diseases (Kokwaro,1993)
Kigelia africana (Lam.) Benth.(Bignoniaceae)	Has anticancer components which include irridoids,Naphthoquinone,Meroterpenoid naphthoquinones,coumarin derivatves,Lignans,sterols and flavanoids (Sain *et al* .,2009)	A preparation from the leaves is used against malaria,measles,roasted seeds drunk with beer caused sexual organs to enlarge,fruit juice is used for wounds,roasted leaf ash used for high blood pressure (Kokwaro,1993),a preparation of the bark is used against malaria (Morris,1996)
Macadamia tetraphylla(Proteaceae)	Antitumor activity (Preedy *et al.* ,2011),Antioxidant activity, contains tocopherols, tocotrienols, and squalene (Wall,2010)	No previous reports

Mangifera indica L.(Anacardiaceae)	Antimalarial, (Bidla *et a.l,* (2004),mango juice and juice extracts have antioxidant and anticancer activity (Percival *et al.*, 2006),contains flavonoids and xanthones (Ribeiro *et al.,* 2008), "Antiviral, antioxidant, radioprotective, antitumor, immunomodulatory, anti-allergic, antiinflammatory, antidiabetic, lipolytic, antibone resorption, monoamine oxidase inhibiting, antiviral, antifungal ,antibacterial and antiparasitic properties", (Mukazayire *et al*.,2010)	A leaf-decoction is used as a febrifuge in Côte d'Ivoire and Nigeria (Burkill,1985),
Markhamia lutea (Benth.) K.schum (Bignoniceae)	In vitro anti-parasitic activity and low cytotoxicity ,fractionation showed cycloartane triterpenoids, musambins A–C and their 3-O-xyloside derivatives musambiosides A–C (Lacroix *et al* ,2009),Hydroperoxy-cycloartane triterpenoids, Phenylpropanoid Glycosides (Mukazayire et al.,2010)	Leaves infusion is used for treatment of cataracts (Okello *et al*., 2010),Juice from young shoots and leaves used to treat throat diseases and conjuctivitis,bark chewed for backache and fresh leaves infusion drunk and used for cleaning snake bit wounds (Kokwaro,1993)
Melia azedarach L.(Meliaceae)	Anti-inflammatory alkaloids (Lee *et al*,2000),antihelmintic, antimalarial, cathartic, emetic and emmenagogic properties and are also used to treat skin diseases (Orwa *et al*.,2009),anticancer limonoids,fruits have Cytotoxic Tirucallane Triterpenoids (Ntalli *et al*., 2010)	Concoctions of roots ,bark and leaves is used for treatment of any disease (Okello *et al*.,2010),Leaf decoction used for stomach ache, bark used as a broad spectrum antibiotic (Kokwaro,1993)

Microglossa pyrifolia (Lam.) Kuntze (Compositae/Asteraceae)	Antimalarial activity (Adjanohoun *et al.*, 1996), high antiplasmodial activity, furanoditerpenes and geranylgeraniol derivatives. Sinapyl diangelate, 1-acetyl-6E, 10E, 14E geranylgeraniol-19-oic acid and 19-oxo-6E, 10E, 14-geranylgeraniol and weak cytotoxic effects (Köhler, 2002)	A preparation of the leaves in water is drunk as a remedy for malaria ,limb fractures and snake bite wounds, root decoction used for treatment of fever and pneumonia,for prevention of premature birth in women (Kokwaro,1993)
Momordica foetida Schumach.(Cucurbitaceae)	Phenolic glycosides, cucurbitane triterpenoids from a leaf extract , alkaloids and glycosides from the complete plant and sitosteryl glycoside, 5,25-stigmastadien-3b-yl-glucoside and 1b-hydroxyfriedel-6-en-3-one,Antimalarial (Froelich *et al* .,2007),antitumor activity (Nguta *et al.*, 2011)	Roots are boiled for treatment of malaria (Okello *et al.*, 2010),Leaves rubbed to a wound from tortoise bite, roots are used as purgative (Kokwaro,1993)
Ocimum gratissimum L.(O.Suave wiild. O.tomentosum oliv.) (Labiatae)	Antimalarial activity(Ngemenya *et al*.,2004),alkaloids, cardiac glycosides, flavonoids, glycosides, resins, steroidal terpens, and tannins (Mbata and Saikia,2008)	Preparation of the leaves is used as a febrifuge and sudorific,also taken by draught or enema to treat fevers,a preparation of the leaves is taken in baths and draughts for coughs and fever (Burkill ,1995),Leaf decoction used to for treatment of constipation,also applied to livestock infected with eye worm *Thelazia* species (Kokwaro,1993)

Olea hotcsh spp.hochstetteri (Oleaceae)	Coumarin and secoiridoid glucosides have been isolated from the bark. The lignans (–)-olivil and (+)-cyclo-olivil have also been isolated from the bark (Aerts,2011)	Bark is boiled and used for treatment of Cough, TB, difficulty in breathing, malaria. (Okello *et al.,* 2010),Infusion of stem used to cure venerial diseases and sterility in women,vermifuge;stem ash applied to wounds (Kokwaro,1993)
Oxygonum sinuatum (Meisn.)Dammer (Polygonaceae)	Angiogenesis inhibotor,Emodin, an inhibitor of the protein kinase CK2, and coleon A lactone, a rare abietane diterpenoid (Crawford *et al.*,2011)	Leaves used for treatment of conjuctivitis,root decoction used for gonorrhoea treatment,paste from leaves applied to boils and women's breast with mastitis (Kokwaro,1993)
Pavetta abyssinica (Rubiaceae)	No previous reports	Boiled root decoction drunk for indigestion (Kokwaro,1993)
Persea americana Mill (Lamiaceae)	Strong anticancer potential, Chemo-protective potentiality against cyclophosphamide induced genotoxicity (Paul *et al*., 2011), seeds have flavonoids, anthocyanins, condensed tannins, alkaloids and triterpenes in methanol extracts. Sterols and triterpenes were detected in the hexane extract,larvicidal and antifungal activity ,leaves have persin (Leite et al., 2009),unripe fruit has cytotoxic activity against six human tumor cell lines (Oberlies *et al* .,1998)	Leaf aqueous extract used for treatment, management and/or control of a variety of human ailments, including childhood convulsions and epilepsy (Ojewole and Amabeoku,2006)
Phyllanthus fischeri Pax (Euphorbiaceae)	No previous reports	Leaf decoction drunk for gynaecological problems and management of AIDS

		(Kokwaro,1993)
Prunus africana (Hook.f.)kalkman (Rosaceae)	Anti-inflammatory and antioxidant activities, cytotoxic phenolics ;phytosterols (beta-sitosterol, beta-sitostenone, n-docosanol ((Bach *et al* ., 2012)	A preparation of the bark and root is boiled for treatment of urinary tract infections (Okello *et al.,* 2010), pounded bark is mixed with water and used as a remedy for prostate cancer, as a purgative and malaria treatment (Kokwaro, 1993), extracts from P. africana bark are used in the treatment of benign prostatic hyperplasia and prostate gland hypertrophy.
Psydrax schimperiana(A.Rich) (Rubiaceae)	No previous reports	Infusion from bark drunk as a remedy for indigestion (Kokwaro,1993)
Rhus vulgaris Meikle (Anacardiaceae)	Antimicrobial activity (Boily and Puyvelde,1986)	Stem is boiled and applied to wounds, root powder used for gonorrhoea treatment, leaves used for treatment of haemorrhoids and stem decoction used for cleaning wounds (Kokwaro,1993)
Salvia coccinea (L.) Murr (Labiatae)	"Saponins, sugars, bitter principles, phenols, tannins, amino groups, alkaloids and flavonoids. The lyophilized extract from leaves and flowers contained 9.02 % of total polyphenols" (Perez *et al* .,2011)	No previous reports

Shirakiopsis elliptica Hochst.Esser (Euphorbiaceae)	Leaves have strong anticancer activity (Sowemimo *et al.*,2011),phenolics with antioxidant activity (Adesegun *et al.*,2008)	A root-concoction is drunk for malaria(Burkill,1994);Decoction from roots used to cure coughs, Bark infusion cause flattening in humans (Kokwaro,1993)
Senna didymobotyra (Fresen.) Irwin and Barneby (Caesalpinaceae)	No previous reports	Roots, leaves and bark are boiled for treatment of Malaria, skin, fever, typhoid, STI's (Okello *et al*., 2010);decoction of leaves, stem and roots used a s a purgative; infusion of leaves used for treatment of gonorrhoea, as an aperient,for bathing of measles patients, decoction of root and leaves used to treat malaria; overdose of plant is fatal (Kokwaro,1993)
Sesbania sesban (L.) Merr(Papilionaceae)	Antiinflammatory,Triterpenoidal and steroidal saponin in crude saponin extract. Other than saponin, the extract also gave positive test for flavanoids and phenolic compounds (Dande *et al*.,2010)	Leaves used to treat stomach ailments, to cure afterbirth pains and expulsion of placenta (Kokwaro, 1993), preparation from the leaves has wound healing and anti-inflammatory properties ,Juice of fresh leaves is credited with anthelmintic properties (Kirtikar *et al*., 1996)

Sida cordifolia L.(Malvaceae)	Alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins and tannins (Kalaiarasan et al.,2010),natural extract possesses strong antitumour activity on cytotoxicity assay (Kumari *et al* .,2012)	"Bark chewed to stimulate menstruation,plant used as an abortifacient,leaves used as diarrhoea remedy", (Kokwaro,1993)
Sida rhombifolia L.(Malvaceae)	Antitumor activity (Muanza *et al* ,1995)	No previous reports
Solanecio mannii(Hook.f) C.Jeffrey (Compositae/Asteraceae)	Antiplasmodial and cytotoxic (Uwitonze,2010),Alkaloids (Asres *et al* .,2008)	Roots are used as an antihelmintic, a purgative, for dysentry and indigestion. Leaves used as a snake bit antidote (Kokwaro,1993)
Solanecio nandensis (S.Moore) C.Jeffrey (Compositae/Asteraceae)	Alkaloids (Asres *et al* .,2008)	No previous reports
Solanum aculeastrum Dunal (Solanaceae)	Root bark, leaf and fruit are anticancerous (Madhuri and Pandey, 2009),It root's bark contains the constituents steroidal alkaloids.Its berries contain the compounds solamargine, β-solamarine and also solasodine when its berries are green in colour.It contains the compounds saponins (Wanyonyi *et al* .,2002),berries have antitumor activity (Koduro *et al* .,2006)	Used for treating bronchitis,roots used as a remedy for gonorrhoea (Kokwaro,1993)
Solanum mauritianum Scop (Solanaceae)	Contains the alkaloid, solasodine, with unripe green berry having the highest content (2% - 3.5% dry weight) ,Solauricine, solauricidine, and solasodamine (Everist,1981)	Treatment of menorrhagia (Lewu & Afolayan, 2009).

Spathodea campanulata P.Beauv.ssp.nilotica(Seem) (Bignoniaceae)	Leaves used as antimalarial (Makinde *et al*.,1988),the stem bark contains ursolic acid (Amusan *et al*.,1996)	Apreparation from the bark is used for treatment of chest pain, backache and Sexually Transmitted Infections (Okello *et al*., 2010),An infusion from the barl is drunk for liver disorders,sore throat and stomach ache.Bark boiled and used to treat skin rashes and decoction drunk as an anthelmintic (Kokwaro,1993), An infusion of the bark, leaves and flowers are employed "to treat malaria, HIV, diabetes mellitus, oedema, dysentery, constipation, gastrointestinal disorders, ulcers, skin diseases, wounds, fever, urethral inflammation, liver complaints and as a poison antidote ",(Bosch,2002)
Spermacoce princea (Rubiaceae)	No previous reports	Whole plant used for treatment of wounds, leaf decoction drunk for hepatic diseases (Kokwaro,1993)
*Syzygium guineense D*C.(Myrtaceae)	"Leaves have aryophyllene oxide (7%), d -cadinene (7.5%), viridiflorol (7.5%), epi- a -cadinol (9.8%), a -cadinol (12.7%), cis-calamenen-10-ol (14%), citronellyl pentanoate (15.2%), b -caryophyllene (20.1%) and a -humulene (39.5%)(Noudogbessi et al, 2008),Saponins (Abdel –Zaher *et al*, 2005), alkaloids (Li *et al*, 2004) and flavonoids", (Coskul *et al*., 2005, Tanko et al	Infusion from bark and roots used for treatment of stomach ache and as a purgative,fruits used as a remedy for dysentry (Kokwaro,1993)

	.,2007)	
Tabernaemontana stapfiana Britten(Apocynaceae)	Contains bioactive compounds which include alkaloids, flavonoids, coumarins, tannins and saponins(Ruttoh *et al.*, 2009)	"Roots and stem barks are used in the treatment of abdominal problems, sexually transmitted infections and upper respiratory tract infections", (Omino and Kokwaro, 1993)
Tragia brevipes Pax(Euphorbiaceae)	There hasn't been any previous records	Roots are used to increase uterine contraction during labour, and also to treat rheumatism (Kokwaro, 1993), "A leaf extract is drunk to cure gonorrhoea, to kill internal parasites including tapeworm and to treat stomach-ache, diarrhoea and gastroenteritis. A decoction of roots and leaves is drunk to promote conception. Leaves, roots and twigs are used to treat poliomyelitis. The ash of burnt leaves is inhaled to cure elephantiasis", (Bosch,2008)

Trichilia emetica Vahl (Meliaceae)	Terpenoids(Kurubasch aldehyde) (Bero *et al* .,2009),limonoids selective toxicity (Gunatilaka *et al*.,1998),antioxidant phenolic acids (Germano et al ,2006),hepatoprotective activity (Germano *et al* .,2005)	Apreparation of the leaves is drunk to relieve fevers (Morris,1996);bark infusion used for pneumonia treatment,an emetic,decoction of roots for induction of labour, oil from seeds used for jigger infection treatment,rheumatism,leprosy or fractures (Kokwaro,1993)
Triumfetta rhomboidea Jacq.(Tiliaceae)	Triumferol (4-hydroxy-isoxazole) ,essential oils of aerial part include β-caryophyllene (22.4%), kessane (14%) and caryophyllene oxide (13%),flavone glycoside triumboidin (scutellarein-6-xyloside-7-rhamnoside), rosmarinic acid, friedelin and friedelinol in the leaves (Bosch, 2011),significant antitumour and antioxidant activities (Sivakumar *et al*.,2008)	A preparation of the leaves is used as a febrifuge while a root preparation is used in combination with other plants against malaria (Burkill,2000);Leaves used for treatment of burns,roots used for toothache treatment and circumcision wounds,leaf infusion used for abscesses and yaws (Kokwaro,1993)
Urtica massaica Mildbr. (Urticaceae)	Tannins,flavanoids,leucoanthocyanes,anthocyanes,quinines and terpens-sterols (Ranzaho,2007)	Infusion from roots and leaves used for treatment of hepatic diseases,decoction from roots drunk for treatment of stomach ache (Kokwaro,1993)
Vernonia lasiopus O Hoffin (Compositae/Asteraceae)	"Alcoholic extract of the dried aerial parts has elemanolides showing in vitro cytotoxicity against human cancer cell lines", (Koul *et al* .,2003),hepatotoxic ,hepatoprotective	Apreparation of the leaves and roots is employed to treat stomach ache and as a sex stimulant for men,sore throat,stomach ache,malaria and STI

	(Uwitonze,2010)	(Kokwaro,1993),a preparation of the leaves and stem is used for treatment of malaria and control of worms (Kareru *et al*.,2008)
Waltheria indica L.(Sterculiaceae)	Has been shown to contain flavanoids (−)-epicatechin, quercetin, and tiliroside (Rao *et al*., 2005),Antibacterial and antioxidant activity (Mongalo *et al.*, 2012)	Decoction of roots used as a cough syrup and eye ache treatment; stem heated and applied to the head as a remedy for headache(Kokwaro,1993),"Various extracts are used as standard febrifugal, purgative, emollient, tonic, analgesic, and astringent herbal medicines in Africa", (Burkill 2000).
Zanthoxylum gilletii (De Wild.) P.G.Waterman (Rutaceae)	"Coantins alkaloids, aromatic and aliphatic amides, sterols and phenylpropanoids-lignans and coumarins",(Adenisa,2005)	The stem bark is chewed for treatment of stomach ache and toothache while bark decoction used for wound healing and rheumatism (Kokwaro,1993)
Zanthoxylum rubescens Hook.f (Rutaceae)	Alkaloids(Zanthomamide,Lemairamide,7,9-Dimethoxy-2,3-methylenedioxybenzophenanthridine Bis[6-(5,6-dihydrochelerythrinyl)] ether (Bero et al .,2009),stem bark and roots contain amides and alkaloids, The leaves contain an essential oils (Tabuti,2011),strong cytotoxic activity (Adenisa,2005)	"Aromatic leaves are chewed as an emetic in cases of food poisoning,stem bark decoction is taken to treat fevers associated with malaria and to treat urinary tract infections, Bark powder is applied to sores", (Tabuti,2011)

3.4. DISCUSSION

This study identified 65 plants belonging to 59 genera and 34 families used in the treatment of cancer in Kakamega County. Some of these candidate plants have been earlier documented in treatment of cancer; notably *Catharanthus roseus* (L.) G. Don (Aslam *et al*., 2010). Although these plants have other therapeutic uses (Table 3.2), a number of them have been reported to show cytotoxic and anticancer properties (Table 3.2). Many studies have reported isolation of bioactive phytoconstituents in some species; *Abrus precatorius* L. ssp africanus Verdc. (Bruneton, 1999), *Albizia gummifera* (J.F. Gmel.) (Shugeng *et al*., 2007), *Bidens pilosa* L. (Chang *et al*., 2001; Kviecinski *et al.,* 2008; Lima Silva *et al*., 2011), *Capsicum frutescens* L (Surh, 2002), *Carica papaya* (Otsuki *et al*., 2010), *Catharanthus roseus* (L.) G.Don (Aslam *et al*., 2010), *Cymbopogon citratus* (Bidinotto *et al*., 2011), *Cyphostemma adenocaule* (Opoku *et al*., 2000), *Fuerstia africana* T.C.E.Fr. (Raymond *et al.,* 2010), *Harungana madagascariensis* Lam.ex poir (Iwalewa *et al.,* 2009), *Hippocratea africana* (Willd.) Loes. (Okokon *et al*., 2006), *Justicia betonica* L. (Caprio *et al.,* 2000; Correa and Alcantara, 2012; Subbaraju *et al*., 2004), *Kigelia africana* (Lam.) Benth. (Saini *et al*., 2009), *Macadamia tetraphylla* (Preedy *et al.,* 2011), *Mangifera indica* L. (Mukazayire *et al.,* 2010), *Markhamia lutea* (Benth.) K. schum (Lacroix *et al*., 2009), *Melia azedarach* L. (Ntalli *et al.,* 2010), *Momordica foetida* Schumach. (Nguta *et al.,* 2011), *Persea americana* Mill (Paul *et al*., 2011), *Prunus africana* (Hook. f.) kalkman (Bach *et al.,* 2013), *Shirakiopsis elliptica* (Hochst.) Esser (Sowemimo *et al*., 2011), *Sida cordifolia* L. (Kumari *et al.,* 2012), *Solanecio mannii* (Hook. f) C. Jeffrey (Uwitonze, 2010), *Solanum aculeastrum* Dunal (Koduru *et al.*, 2006), *Trichilia emetica* Vahl (Leslie *et al.*, 1998), *Triumfetta rhomboidea* Jacq. (Sivakumar *et al*., 2008), *Vernonia lasiopus* O Hoffin (Koul *et al*., 2003) and *Zanthoxylum rubescens* Hook. f (Adesina, 2005) (Table 3.2).

This study documented an additional twenty five candidate plant species that were mentined for management and treatment of cancer. The high number of candidate species identified in the study area may be due to the high biodiversity associated with one of the few remnant tropical rainforest in Kenya, Kakamega forest. Determination of anticancer activity in these plants is the next necessary step for, notably, *Spathodea campanulata* P. Beauv. ssp. nilotica (Seem), *Cyphostemma serpens* (A. Rich) and *Aloe volkensii* spp. Volkensii the most mentioned plant species, have previously not been reported to possess cytotoxic or anticancer activity. However, some of the plants have been previously reported by *in vitro* and *in vivo* studies to possess strong anticancer and cytotoxic effects namely *Albizia gummifera* (J.F. Gmel.) (Shugeng *et al*., 2007), *Bidens pilosa* L. (Chang *et al*., 2001; Kviecinski *et al.,* 2008; Lima Silva *et al.*, 2011), *Catharanthus roseus* (L.) G. Don (Aslam *et al*., 2010), *Shirakiopsis elliptica* (Hochst.) Esser (Sowemimo *et al*., 2011), *Sida cordifolia* L. (Kumari *et al.,* 2012), *Triumfetta rhomboidea* Jacq. (Sivakumar *et al*., 2008), *Vernonia lasiopus* O Hoffin (Koul *et al.,* 2003) and *Zanthoxylum rubescens* Hook. f (Adesina, 2005). It will be ultimately important to validate the anticancer properties of these medicinal plants.

Fleshy or dried leaves were used by a majority of TMPs interviewed suggesting that these components of the plant contained the highest concentration of the active phytochemical compounds. Stem bark were the second most frequently used. Sustainable methods of harvesting were used by the TMPs to protect the plants from destruction and overutilization.

The majority of respondents reported using the oral route for administration of their formulations for treatment of cancer; however few of the TMPs also prescribed topical application on wounds and sores especially for breast cancer and skin sarcomas. The duration of treatment lasted a minimum of two weeks but were usually administered until recovery. It

is important to note that medicinal plant preparations constituted combinations of different plant parts and methods of preparation. Combination of plant extracts offer a more wide range of biological effects which have been shown to have additive and synergistic effects,a desirable direction in the development of efficacious, safe and cost effective phytopharmacotherapeutic products (Darshan and Doreswamy, 2004).

3.5. CONCLUSION

This present study showed that the inhabitants of the study area use a variety of medicinal plants for cancer intervention. Twenty five plants were reported for the first time as being important in the management of cancer. In addition many of the plants identified have been reported to possess antitumor and cytotoxic properties. The herbal remedies were made from a combination of different plants and plant components indicating a synergistic advantage. It is not surprising that the respondents used medicinal plants to manage cancer as the Kakamega forest, an area of high plant biodiversity, was one of the main sources of these plants. These remedies have gained broad acceptability as they were reportedly reliable. It is therefore imperative to document and preserve such important information as a cultural heritage and to form a basis for scientific validation for development of effective cancer therapeutic drugs. A major challenge in the use of traditional phytoremedies is the lack of proper standardisation, safety and quality control, as well as adulteration with conventional medicines and therefore it is recommended that all bioactive phytochemical compounds be scientifically studied and validated.

CHAPTER FOUR

4.0 PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS OF THE KAKAMEGA COUNTY, KENYA, COMMONLY USED AGAINST CANCER.

4.1 INTRODUCTION

"The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds", (Hill, 1952). Phytochemical constituents of 35 selected herbal extracts were analyzed using Thin Layer Chromatography (TLC) as per Wagner, 1995. These 35 medicinal plants were the most commonly reported out of the 65 medicinal plants collected to have anticancer properties in the ethnobotanical survey as recorded by Ochwang'i *et al,* 2014. Specifically, the compounds analysed were alkaloids, anthraquinones, xanthines, valepotriates, cardiac glycosides, flavonoids, essential oils, coumarins, lignans, saponins and arbutins. "They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas", (Vasu *et al,* 2009). A variety of phytomedicines have been derived from barks, leaves, flowers, roots, fruits, seeds (Criagg and David, 2001). Discovery of complex chemical substances of therapeutic value can be achieved from the medicinal plants (Parekh and Chanda, 2007).

4.2 MATERIALS AND METHODS

4.2.1 Extract preparation

Organic and aqueous extracts were prepared using Dichlomethane: Methanol and water respectively

4.2.1.1 Dichloromethane: Methanol extract

Ten grams of dried and ground powder of the medininal plant was extracted by cold maceration in Dichloromethane- methanol mixture at a ratio of 1:1 for 72 hours at room temperature. The extract was reduced in a rotavapor and thereafter in oven at 40 ^{o}C. The yield of each extract was determined and extract preserved at 4 ^{o}C.

4.2.1.2 Aqueous Extract

The plant powder was macerated in distilled water at the ratio of weight to volume of 1:6 The suspension of the sample was rotated on a shaker for 24 hours at room temperature and filtered using cotton wool and whatman filter paper No1. The aqueous extracts were freeze dried and the resultant lyophilized residues stored at room temperature.

4.2.2 Thin Layer Chromatography (TLC)

Phytochemical screening was carried out using thin layer thromatography as described by Wagner, 1995. 2.5 mg of the extract was weighed and dissolved in 500µl of methanol. Silica gel 60F 254-aluminium foli (0.2 mm layer thickness) (Millipore Corporation, Germany) was used in the phytochemical analysis. The TLC-plates were blow dried shortly before applying the extract samples with starting points applied in a height of 1-1.5 cm; a distance between the starting points of 0.5 cm and a distance of 1cm to the plate boundary. It proved reasonable to apply the samples not only as points but also as bar marks. The glass capillaries used to apply the sample solution corresponded to 1.2 cm liquid column of 5µl. The plate was dried well after every application. The developing chamber was lined with filter paper (the superplasticizer moves faster) and developing the TLC-plates occurs when the chamber was saturated. After the plate was developed, the superplasticizer was flushed off completely by either blow-drying or putting them in the drying cabinet before detection.

4.2.3 Spray solutions

Spray solutions on the Thin Layer Chromatography were prepared and used for the various analyses. Glass spray heads were used if the solution contained sulfuric acid. These solutions include:

4.2.3.1 Anisic aldehyde-sulfuric acid-reagent

0.5 ml anisic aldehyde was slowly mixed with 10 ml acetic acid, 85 ml MeOH and 5 ml conc. sulphuric acid in that order. The solution was kept well in the fridge.

4.2.3.2 Dragendorff-reagent

1.7 g basic bismuth nitrate and 20g tartaric acid was suspended in 40ml water.16g potassium iodide was dissolved in 40ml water and added to the bismuth nitrate suspension. The resultant was stirred for 1 hour and filtrated. The stock solution was kept several days in brown glass bottles in the fridge, protected from light. The spray solution was prepared by mixing 5 ml stock solution with 15ml water.

4.2.3.3 Ethanolic KOH-reagent

5% solution of KOH in EtOH 50% (V/V). This comprised of 2.5gms KOH dissolved in 25ml water and 25ml Ethanol.

4.2.3.4 Iodine-platinate-reagent

0.15 g potassium hexachloroplatinatein was dissolved in 50ml water, while heating. 50ml 6% potassium iodine solution was added and solution kept in the fridge.

4.2.3.5 Potassium permanganate-sulfuric acid-reagent

1 g $KMnO_4$ was carefully dissolved in 30 ml conc. Sulphuric acid and **cooled with ice.**

(Caution: Explosive manganheptoxide is generated)

4.2.3.6. Natural substance-PEG-reagent

Solution A: 1% solution of natural substance reagent A (diphenylboronic acid-aminoethyl ester) in MeOH. The solution was kept in the fridge. **Solution B**: 5% solution of polyethylene glycol 4000 in EtOH.

Solutions A and B are sprayed one after the other on the TLC-plate. The PEG solution increased the detection sensivity.

4.2.3.7. Hydrochloric acid-glacial acetic acid-reagent

40 ml conc. hydrochloric acid was mixed with 10 ml glacial acetic acid.

4.2.3.8. Vanillin-sulfuric acid-reagent

Solution A: 5% solution of conc. sulfuric acid in EtOH (5ml acid mixed with 95ml ethanol).

Solution B: 1% solution of vanillin in EtOH (1 gm Vanillin mixed with 99ml ethanol. Solution A was first sprayed on the TLC-plate and immediately after that solution B was sprayed on the TLC-plate. (It's essential, that the reagents are prepared freshly).

4.2.3.9. Methanolic sulphuric acid

10% metanolic sulphuric acid (V/V) was prepared by mixing 10ml H_2SO_4 with 90ml Methanol. Thirty five plant extract samples were applied, 10 samples per plate with bands of 10mm length with their corresponding references for each class.

4.2.4 ALKALOIDS

Reference solutions: The reference compounds used include: Cinchonidin, Cinchonin and Chinidium dissolved in 500µl of methanol applied at 5 µl at a point.

Superplasticizer: Tuluol/EtOAc/DEA: 5/4/1

Running height: 12 cm

Detection and evaluation: The plate was dried to remove DEA (blow-dry or put it in the drying cabinet at 100°C). The chromatograph was evaluated in UV 254 and UV365 and sprayed with 10% methanolic sulphuric acid (V/V) on the plate afterwards. The chromatograph was evaluated again in UV365 and sprayed with iodine-platinate-reagent on the plate and evaluated in day light.

4.2.5 ANTHRAQUINONES

Reference solutions: 1mg aloin and 1mg franguline is dissolved in at 5 µl at a point.

Superplasticizer: EtOAc / MeOH / H2O 50 / 8.5 / 6.5

Running height: 15cm

Detection and evaluation: The plate was dried (max. 10 min) and sprayed with the freshly prepared solution of nitrosodimethyl aniline in pyridine (0.1%) on it immediately. In this for anthrones no violet or grey-blue zones may appear. The chromatograph was evaluated immediately. Ethanolic KOH-reagent was sprayed on it afterwards and put it in the 100-105°C warm drying cabinet for 15min. The plate was evaluated in daylight and in UV365.

4.2.6 XANTHINES

Reference solutions: Caffein, theophylline and theobromine dissolved in 300µl of methanol applied at 5 µl at a point.

Superplasticizer: Toluol / Isopropanol / NH3 conc. 15 / 30 / 5

Running height: 15cm

Detection and evaluation: The dry TLC-plate was evaluated in UV254 and sprayed with potassium permanganate-sulfuric acid-reagent on it. It was subsequently blow dried and put in

the 50°C warm drying cabinet for 15min. Dragendorff reagent was sprayed on the TLC-plate afterwards and evaluated immediately in daylight.

4.2.7 VALEPOTRIATES

Reference solutions: The sugar coat of one tablet Valmane® was removed and half pulverized in a mortar. 1.5ml ether was added to this powder in an Eppendorf vessel. It was applied 10 µl at a point as bands 15mm length.

Superplasticizer: Toluol / EtOAc: 37.5 / 12.5

Running height: 15cm

Detection and evaluation: The dry TLC-plate was evaluated in UV254 and sprayed with hydrochloric acid-acetic acid-reagent. Heat the plate under supervision in the 110°C warm drying cabinet for 10 min. Evaluation occured in daylight and in UV365. The zone's colours deepened while heating the TLC-plate. That's the cause why continuous supervision is important.

4.2.8 CARDIOACTIVE GLYCOSIDES

Reference solutions: 2 mg Digitoxin and 1 mg Gitoxin was mixed in 3ml MeOH and 2ml ether altogether and applied the mixture 20 µl at a point.

Superplasticizer: EtOAc / MeOH / H2O 44 / 5.5 / 4

Running height: 15 cm

Detection and evaluation: Conc. H_2SO_4 was sprayed on the dried chromatograph and evaluated in daylight. After putting it in the 100°C warm drying cabinet for 3-5 min it was evaluated in UV365.

4.2.9 FLAVANOIDS

Reference solutions: 1mg of rutin, chlorogenic acid and hyperoside was dissolved together in 1ml MeOH. 1mg of caffeic acid was dissolved in 1ml MeOH. 25-30µl of the herbal drug extracts and reference solutions were applied as bands of 10mm length.

Superplasticizer: EtOAc / formic acid / acetic acid / H_2O 50 / 5.5 / 5.5 / 13.5 .The first three components were mixed first and water added slowly while shaking the mixture.

Running height: 15 cm

Detection and evaluation: Remove the superplasticizer completely by blow-drying or putting the plate in the 100°C warm drying cabinet. Evaluate the chromatograph after watching it in UV254 and UV365. Control it after 15-20 min. Afterwards spray natural substance-PEG-reagent on the plate and evaluate in daylight.

4.2.10 ESSENTIAL OILS

Reference solutions: Dissolve respectively 5mg or µl anethole, carvone, 1, 8-cineol (eucalyptol), eugenol, menthol and thymol in 1.0 ml Toluol.

Superplasticizer: Toluol / EtOAc 93 / 7

Running height: 6 cm

Detection and evaluation: After watching the dry TLC-plate in UV254 and UV365, spray vanillin-sulfuric acid-reagent on it and heat it in the 110°c warm drying cabinet for 5-10 min under supervision. The evaluation occurs in daylight.

4.2.11 COUMARIN DRUGS

Reference solutions: Caffeic acid dissolved in Methanol

Superplasticizer: Toluene-Ether 1:1 Saturated with 10% acetic acid

Running height: 15 cm

Detection and evaluation: At UV-365 without chemical treatment.

4.2.12. LIGNANS

Reference solutions: Rutin; chlorogenic acid and hyperoside dissolved in methanol.

Superplasticizer: Chloroform-Methanol-Water (70:30:4)

Running height: 15 cm

Detection and evaluation: After drying the plate spray with PEG reagent and watch under UV 365.

4.2.13. SAPONINS

Reference solutions: Aescin dissolved in methanol.

Superplasticizer: Chloroform-acetic acid-methanol-water; 60:32:12:8

Running height: 15cm

Detection and evaluation: After drying the plate in warm cabinet and watching under UV 365 and 254, spray with Anisaldeyde-sulphuric acid reagent.

4.2.14. ARBUTIN DRUGS

Reference solutions: Arbutin and Rutin in 300 µl Methanol.

Superplasticizer: Ethyl acetate-Methanol-Water (100:13.5:10)

Running height: 15 cm

Detection and evaluation: After drying the plate in warm cabinet and evaluating at UV 256 and 354, spray with Natural products-polyethylene glycol reagent (NP/PEG) at UV 365.

4.3 RESULTS

4.3.1 PHYTOCHEMICAL SCREENING RESULTS

The phytochemical constituents of thirty five medicinal plants were summarized in Table 4.1 below representing the designated sample ID, the botanical name of each medicinal plant and the various phytochemical constituents which include alkaloids, athraquinones, xanthines, valepotriates, cardiac glycosides, flavonoids, essential oils, coumarin drugs, lignans, saponins and arbutin drugs.The results showed the presence of a variety of active phytochemical compounds in the plant part extracts.

Table 4. 3. Scoring of Thin Layer Chromatography (TLC) results of commonly used medicinal plants in Cancer treatment in Kakamega County, Kenya.

The sample identification in Table 4.1 is the same one used in TLC plates (Sample 1-35)

SAMPLE ID	**PLANT SPECIES (Botanical name)**	**PHYTOCHEMICALS CONSTITUENTS** (+ indicating presence and – indicating absence)										
		ALKALOIDS	**ANTHRAQUINONES**	**XANTHINES**	**VALEPOTRIATES**	**CARDIOACTIVE GLYCOSIDES**	**FLAVONOIDS**	**ESSENTIAL OILS**	**COUMARIN DRUGS**	**LIGNANS**	**SAPONINS**	**ARBUTIN DRUGS**
1	*Harungana madagascariensis* Lam.ex poir	+	–	+	+	+	–	+	+	–	–	–

	(Stem bark)											
2	*Fuerstia africana* T.C.E. Fr. (Whole plant)	–	+	+	+	+	+	+	+	+	+	+
3	*Sida rhombifolia* L.(Leaves)	+	–	+	+	+	+	+	+	+	+	+
4	*Zanthoxylum rubescens* Hook. f (Stem bark)	+	+	+	+	+	+	+	–	+	+	–
5	*Bridelia micrantha* (Hochst.)Baill (Stem bark)	+	–	–	–	+	–	–	–	–	–	–
6	*Juniperus procera* Endl. (Stem bark)	+	+	+	+	+	+	+	+	+	+	+
7	*Tragia brevipes* Pax (Leaves)	+	–	+	+	+	+	+	+	+	+	+
8	*Phyllanthus sapialis (Leaves)*	+	+	+	+	+	+	–	+	+	+	+
9	*Conyza sumatrensis* (Retz.)E.H Walker (Leaves)	+	–	+	+	+	+	+	+	+	+	+
10	*Momordica foetida Schumach.* (Whole aerial plant)	+	–	+	+	+	+	+	+	+	+	–
11	*Synsepalum cerasiferum Synonym: Afrosersalisia cerasifera* (Welw.) Aubrev (Stem bark)	–	–	–	–	+	+	–	–	+	+	+
12	*Aloe volkensii*	+	+	+	–	+	+	+	+	–	+	–

	Engl. (Leaves)											
13	*Aeschynomene abyssinica* (A.Rich.) Vatke (Leaves)	+	–	+	+	+	+	+	+	–	+	+
14	*Futumia africana* Benth.(Leaves)	+	–	+	+	+	–	+	+	–	–	–
15	*Cyphostemma serpens* (A. Rich) (Roots)	+	+	+	+	+	+	+	+	+	+	+
16	*Ipomoea cairica* (L.) (Leaves)	+	–	+	+	+	+	+	+	–	–	–
17	*Spathodea campanulata* P.Beauv. ssp. nilotica (Seem) (Stem bark)	–	–	+	+	+	+	+	+	–	+	–
18	*Abrus precatorius L. ssp africanus* Verdc. (Roots and seeds)	+	+	+	+	+	+	+	+	+	+	+
19	*Triumfetta rhomboidea* Jacq. (Leaves)	+	+	+	+	+	+	+	+	+	+	+
20	*Psydrax schimperiana* (A. Rich) (Stem bark)	+	+	+	+	+	+	+	+	+	+	+
21	*Ficus thonningii* (Leaves and stem bark)	+	+	+	+	+	–	+	+	–	+	–
22	*Rotheca myricoides* (Hochst.steane and maab.) (Whole plant)	+	+	+	+	+	+	–	+	+	+	+
23	*Croton macrostachyus*D	–	–	+	+	+	–	+	+	+	–	–

	elile (Stem bark)											
24	*Vernonia lasiopus* O Hoffin (Stem bark)	–	–	+	–	–	+	–	–	–	–	–
25	*Albizia gummifera* (J.F. Gmel.) (Leaves and Stem bark)	+	–	+	+	+	+	+	+	+	+	+
26	*Zanthoxylum gilletii* (De Wild.) P.G. Waterman (Stem bark)	+	+	+	+	+	+	+	+	+	+	+
27	*Microglossa pyrifolia* (Lam.) Kuntze (Leaves)	–	+	+	+	+	+	+	+	+	+	+
28	*Senna didymobotyra* (Fresen.) Irwin and Barneby (Leaves)	+	+	+	+	+	+	+	+	+	+	+
29	*Trichilia emetica* Vahl (Stem bark)	–	+	+	+	+	+	+	+	+	+	+
30	*Entada abyssinica* Steud.ex A.Rich. (Stem bark)	+	+	+	+	+	+	+	+	+	+	+
31	*Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus)Pax (Stem bark)	–	+	+	+	+	+	–	+	+	+	+
32	*Ocimum gratissimum* L. Suave wiild O. tomentosum oliv. (Leaves)	+	+	+	+	+	+	+	+	+	+	+
33	*Prunus africana* (Hook.f.)	–	–	+	+	–	–	+	+	–	–	–

	kalkman (Stem bark)											
34	*Phyllanthus fischeri* Pax (Leaves and roots)	++	++	++++	–	+++	++++	–	++	–	++++	–
35	*Olea hotch spp. Hochstetteri* (Stem bark)	–	+	++++	–	+	+	–	–	+	+++	+

KEY:

- Symbolizes absence of phytochemical compound

+ Symbolizes presence of phytochemical compound

4.3.2 ALKALOIDS

The fluorescence of alkaloids predominantly light blue is intensified by treatment of 10% methanolic sulphuric acid. Other alkaloids e.g cinchonine show a deep violet fluorescence (hardly visible in photos) with others showing bright yellow and yellow green florescence.

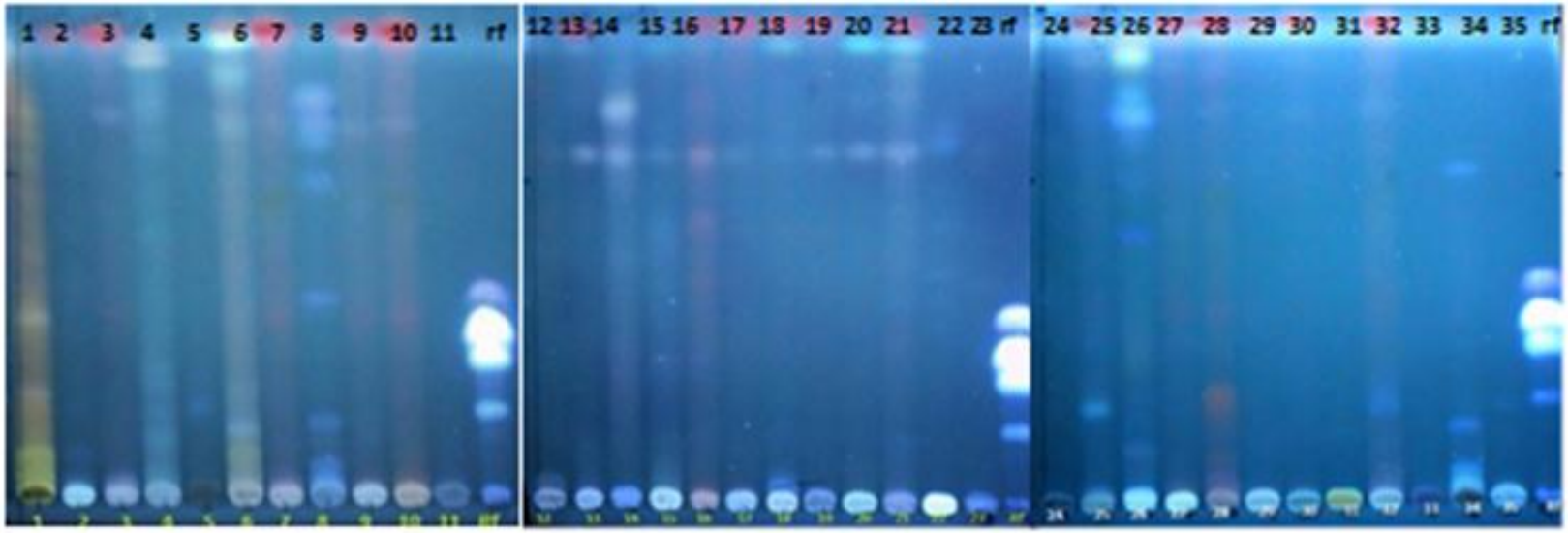

Figure 4.1: TLC chromatogram showing possible **Alkaloids** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Cinchonidin, Cinchonin and Chinidium for the phytochemical class.

4.3.3 ANTHRAQUINONES

Bands show characteristic yellow and blue fluorescence. All major compounds such as aloins or aloinosides show quenching in UV 254.Treatment with KOH reagent intensifies the yellow fluorescence of aloin and aloinosides as well as the blue fluorescence of aloe resins.

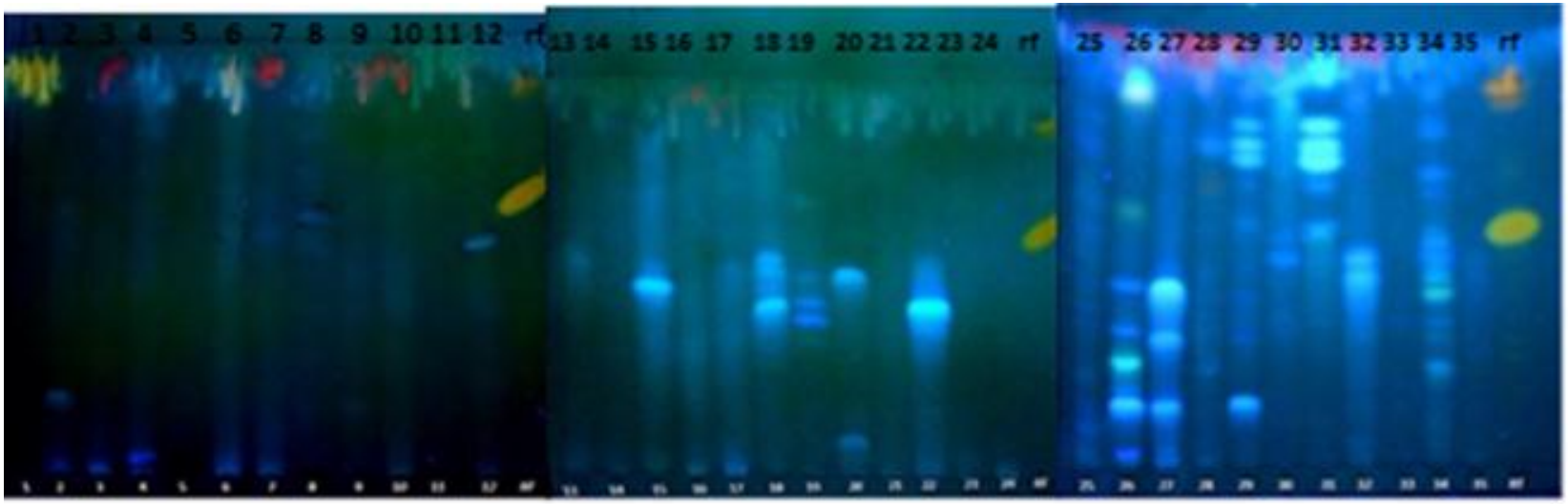

Figure 4.2 TLC chromatogram showing possible **Anthraquinones** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard aloin and franguline for the phytochemical class.

4.3.4 XANTHINES

Show red, blue and green fluorescence at UV 360nm.

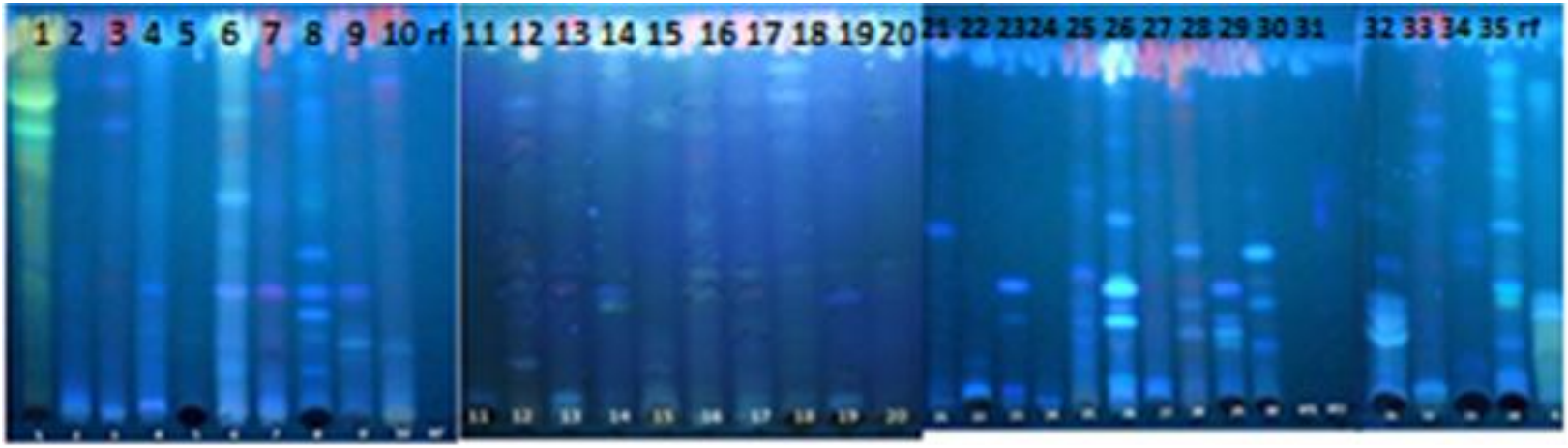

Figure 4.3 TLC chromatogram showing possible **Xanthines** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Caffein, theophylline and theobromine for the phytochemical class.

4.3.5 VALEPOTRIATES

The reference solution's ingredients of Valmane® showed the following Rf-values:

Valtrate/Isovaltrate 0.70, Didrovaltrate 0.60, Acevaltrate 0.55 and IVHD 0.40.Show blue/brown fluorescence.

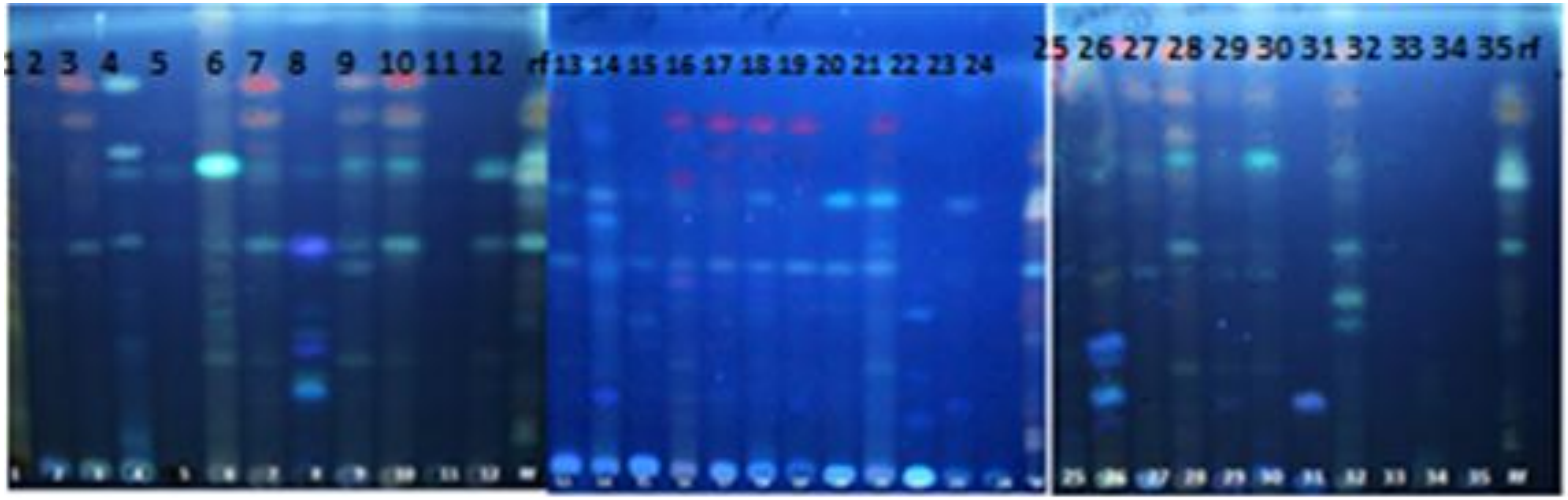

Figure 4.4. TLC chromatogram showing possible **Valepotriates** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Valmane for the phytochemical class.

4.3.6 CARDIOACTIVE GLYCOSIDES

Gitoxin has the smallest Rf-value and Digitoxin has the biggest Rf-value. After spraying with conc.sulphuric acid and heating for 1-3 minutes at

100 degrees celcius, Blue, brown, green and yellowish fluorescence appear at 365nm and appear brown or blue in normal daylight.

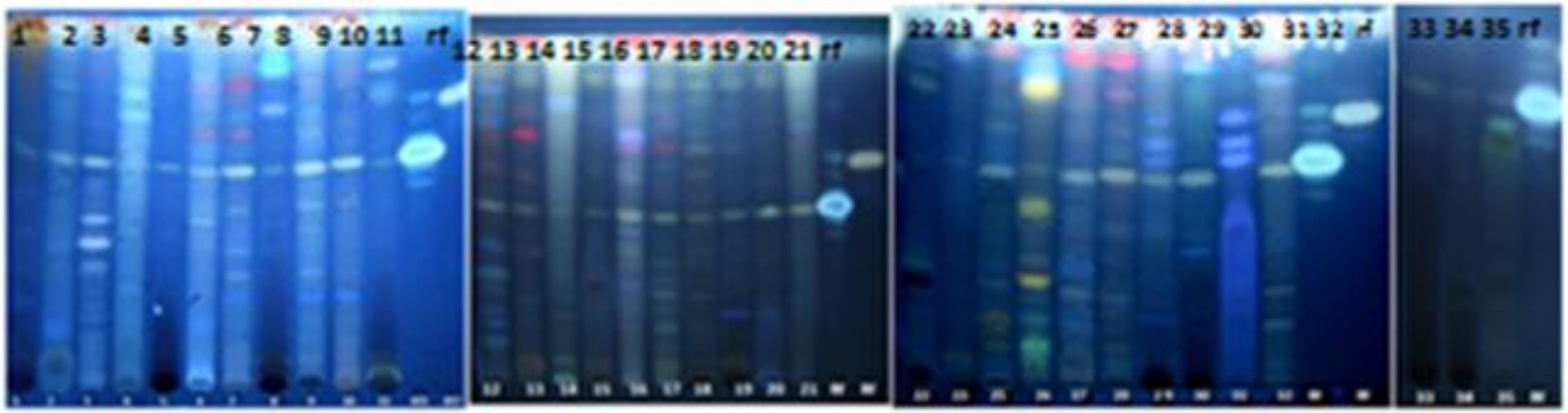

Figure 4.5 TLC chromatogram showing possible **Cardioactive Glycosides** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Digitoxin and Gitoxin for the phytochemical class.

4.3.7 FLAVONOIDS

Show yellow-orange or yellow-green and blue fluorescence. Spraying with PEG reagent strengthens the fluorescence.

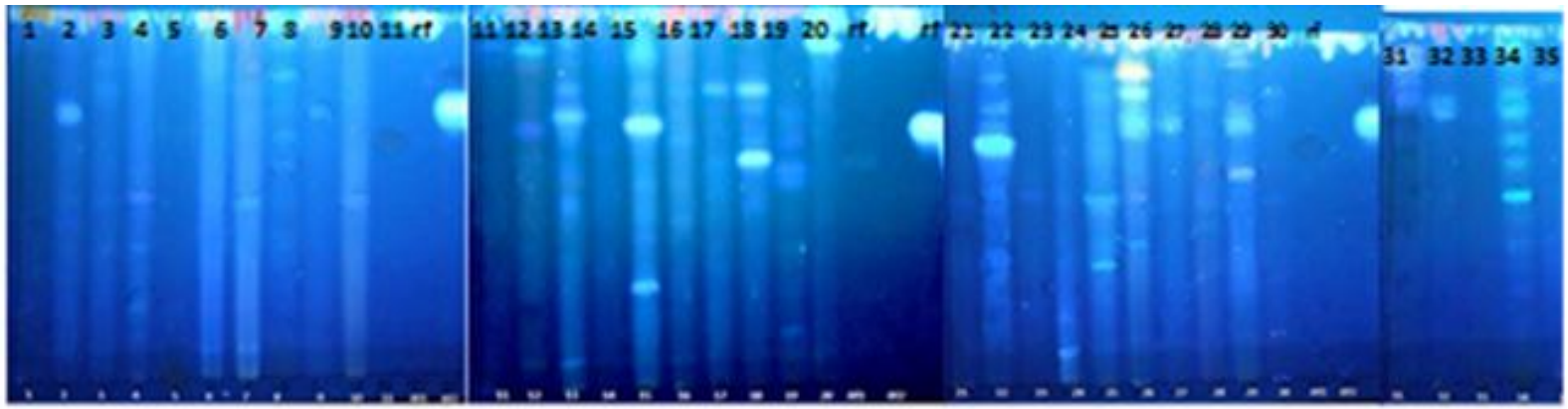

Figure 4.6 TLC chromatogram showing possible **Flavonoids** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard rutin, chlorogenic acid and hyperoside for the phytochemical class.

4.3.8 ESSENTIAL OILS

At UV 254 fluorescence is quenched and bands appear as dark zones against a light green fluorescent background of the TLC plate. After spraying with Vanillin-sulphuric acid essential oil compounds show strong blue, green, red and brown colouration.

Figure 4.7 TLC chromatogram showing possible **Essential oils** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard anethole, carvone, 1, 8-cineol (eucalyptol), eugenol, menthol and thymol for the phytochemical class.

4.3.10 COUMARIN DRUGS

Characteristic bright blue, blue-green, yellow green and Violet-blue fluorescence of Coumarins at UV-365 nm.

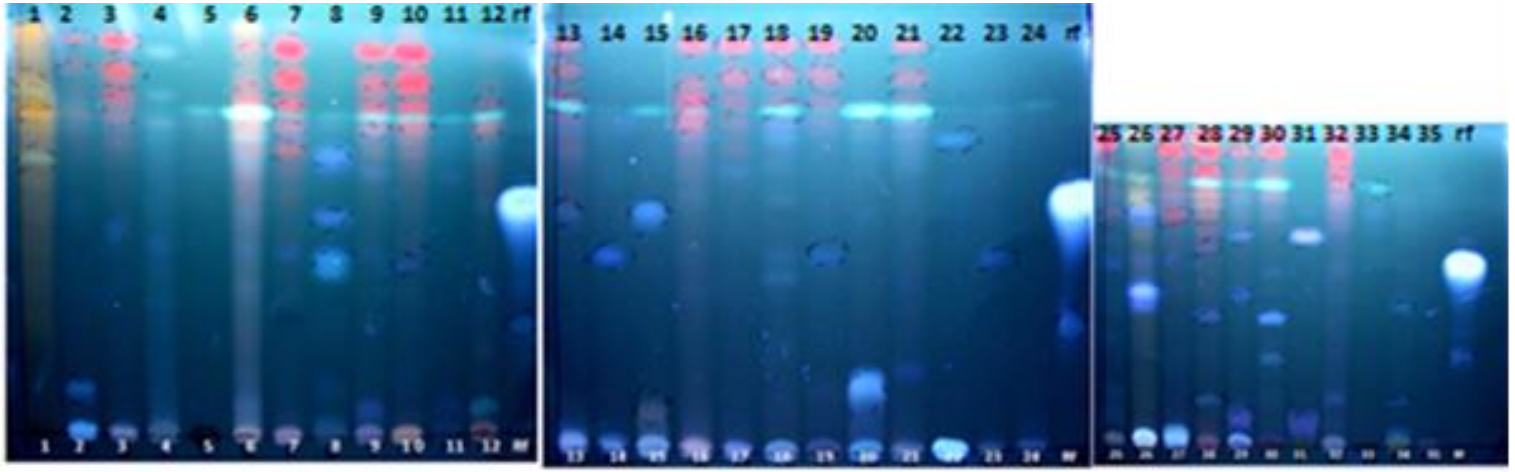

Figure 4.8 TLC chromatogram showing possible **Coumarins** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Caffeic acid for the phytochemical class.

4.3.11 LIGNANS

Blue and blue-green fluorescence zones are seen under UV 365.The blue fluorescence strengthens after spraying with PEG reagent.

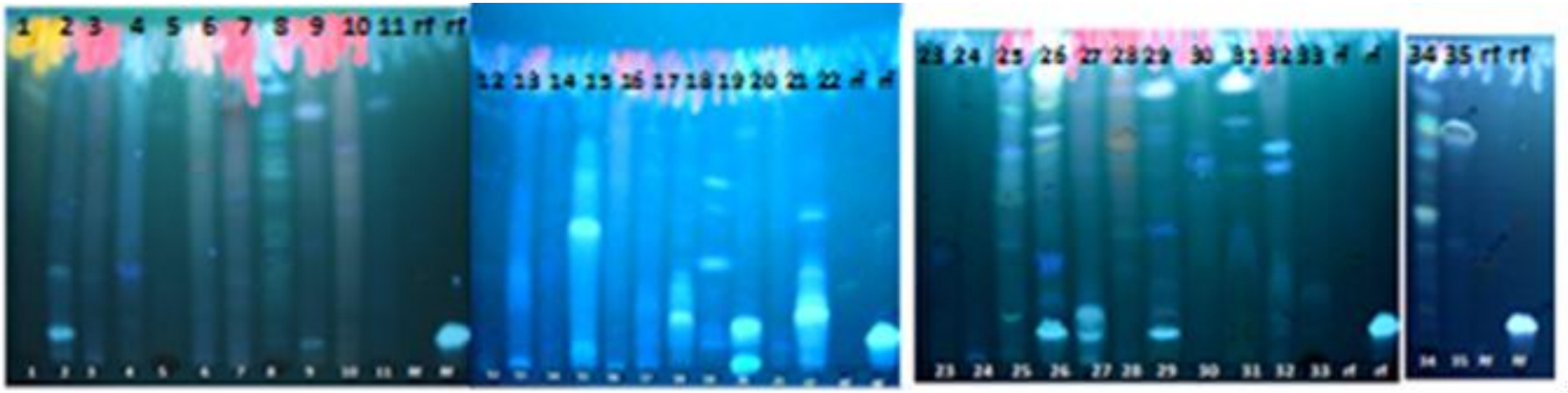

Figure 4.9 TLC chromatogram showing possible **Lignans** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Rutin; chlorogenic acid and hyperoside for the phytochemical class.

4.3.12 SAPONINS

Show grey blue, yellow brown and red fluorescence bands at UV 365 and after spraying with Anisaldeyde-sulphuric acid reagent.

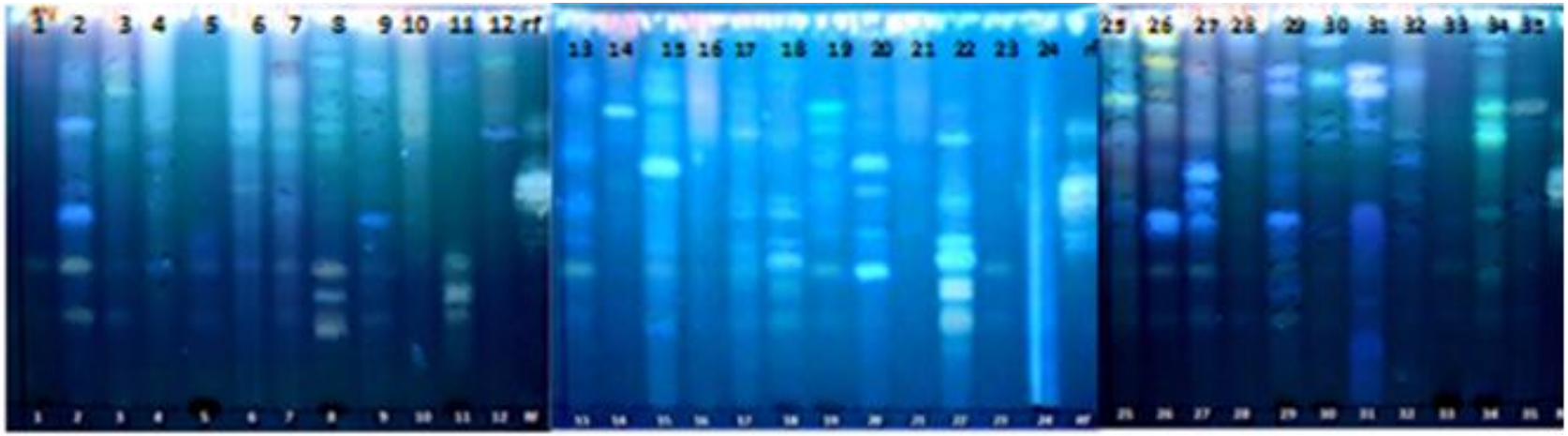

Figure 4.10 TLC chromatogram showing possible **Saponins** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Aescin for the phytochemical class.

4.3.13 ARBUTIN DRUGS

Distinct grey and blue zones are seen.

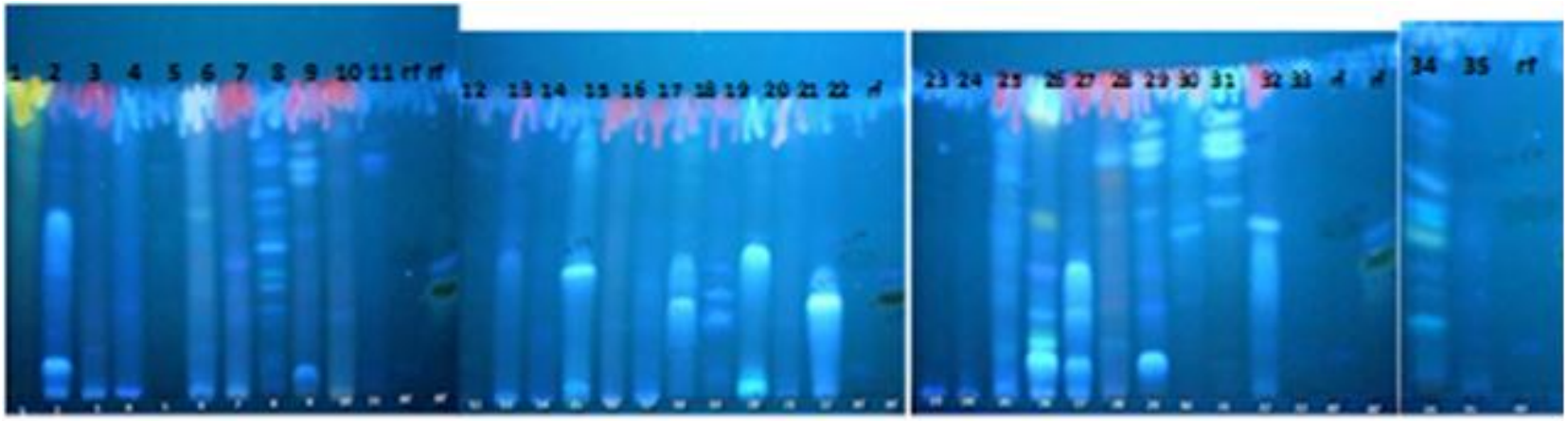

Figure 4.11 TLC chromatogram showing possible **Arbutin Drugs** as seen under UV.Lane labeled 1-35 represents the different extracts as described in Table 4.1 with the label rf representing the reference standard Arbutin and Rutin for the phytochemical class.

4.4 DISCUSSION

Thirty five selected medicinal plants used in the study area for treatment and management of cancer were investigated for their phytochemical constituents. The phytochemicals present included alkaloids, anthraquinones, xanthines, valepotriates, cardioactive glycosides, flavonoids, essential oils, coumarins, lignans, saponins and arbutin drugs. The different reference standards used were a guide to the phytochemical class constituents in the medicinal plants. 71.4% of the medicinal plants contained alkaloids, 57.1% had anthraquinones, and 94.2% had xanthines, and 82.8% contained valepotriates, 94.2% cardioactive glycosides, 82.8% flavonoids, 77.1% essential oils, 85.7% coumarin drugs, 68.5% lignans, 80% saponins and 62.85% arbutin drugs. This results show that majority of the selected anti-cancer medicinal plants have a wide range of biologically important phytochemicals the highest being cardioactive glycosides and xanthines.

"The phenolic compounds such as flavonoids possess biological properties such as antiapoptosis, antiaging, anticarcinogenic, anti-inflammatory, ant atherosclerotic, cardiovascular protective and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities", (Han *et al*, 2007). "Flavonoids comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine for thousands of years. Of the many actions of flavonoids, antioxidant and antiproliferative effects stand out. Moreover, the inhibitory action on inflammatory cells, especially mast cells, appears to surpass any other clinically available compound. Given that certain substituents are known to be required or increase their actions, the therapeutic potential of select flavonoids is fairly obvious. The areas that hold most promise are chronic inflammatory and allergic diseases, as well as coronary artery disease and breast cancer",

(Middleton *et al*., 2000). Mutagenic studies in bacteria, Quercetin showed antimutagenicity of BP a PAH carcinogen (Ogawa *et al.,* 1985). Flavone-8-acetic acid was also shown to have antitumor effects (Thomsen *et al.,* 1991). "Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall", (Marjorie, 1996). They also are effective antioxidant and show strong anticancer activities (Salah, *et al*, 1995).

Essential oils are not a chemical consistent substance class. They are all characterized by high volatility and an aromatic smell. Most of the molecules consist of mono- and sesquiterpenes. Phenylpropan base units and sulfur and nitrogen containing substances (for example anthranilic acid esters) are also found. A plant's essential oil is composed of many individual components (Wagner and Bladt, 1996).

Coumarin has been shown to possess anti-cancer activity in vivo, possibly due to its metabolites 7-hydroxycoumarin which is known to inhibit the release of cyclin D1, which is overexpressed in many types of cancer (Aoife *et al*, 2004). "Coumarin derivatives have been found to have numerous therapeutic applications including photochemotherapy, antitumor and anti-HIV therapy, and as central nervous system (CNS) stimulants, antibacterial, anti-inflammatory, anti-coagulants and dyes", (Musa *et al*, 2008).

Various lignans are known to have anti-tumor, antimitotic and antiviral activity and to specifically inhibit certain enzymes (MacRae *et al*, 1984). "The lignans and genistein were strongly suppressive against incorporations of [^{3}H] thymidine, [^{3}H] uridine, and [^{3}H] leucine into HL-60 cells. These results showed that some of the naturally occurring flavonoids and

lignans inhibited HL-60 cell growth with a non-toxic mechanism, possibly via cessation of DNA, RNA, and/or protein synthesis of the leukemic cells" (Hirano *et al,* 1994).

"Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins", (Francis *et al.,* 2002). "They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methyl pentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature", (Francis *et al.,* 2002). Saponins have also been known to be anti-inflammatory (Just *et al,* 1998). "Classical methods are used to ascertain the presence of saponins in a crude plant extract, and to elucidate their composition throughout purification steps. TLC and staining with dehydrating reagents containing aromatic aldehydes (such as anisyl aldehyde in sulfuric acid) are commonly used. The pure saponins may also be hydrolyzed to verify the nature of its glycosidic moieties", (Francis *et al.,* 2002). "Saponins isolated from different plants and animals have been shown to specifically inhibit the growth of cancer cells in vitro", (Kuznetzova et al. 1982; Rao & Sung, 1995; Konoshima et al. 1998; Marino *et al.* 1998; Mimaki et al. 1998a; Podolak *et al.* 1998). "Triterpenoid saponins (avicins from Acacia victoriae) selectively inhibited growth of tumour cell lines by cell cycle arrest in human breast cancer cell line and apoptosis in leukemia cell line", (Mujoo *et al.* 2001).There was

also reduction in incidence and multiplication of cancer in a murine skin carcinogenesis model (Hanausek *et al.* 2001).

Alkaloids have been associated with various medicinal uses especially their biological property of cytotoxicity (Okwu, 2001). Alkaloids are also known to be analgesic, antispasmodic and antibacterial (Stray, 1998). The biogenesis principle of amino acid (biogenetic amine) and non-amine component is basis of every alkaloid. The classification of alkaloids is based on the heterocycle's structure or on the biogenetic origin. A greater percentage of alkaloids are derived from the amino acids lysine, tryptophan, tyrosine and arginine (Wagner and Bladt, 1996). Two complex indole alkaloids, vinblastine and vincristine, used in the clinical treatment of a variety of cancers have been extracted from leaf extracts of *Catharanthus roseus* (L.) G. Don (Noble, 1990). "*Vinca* alkaloids inhibit cell proliferation by altering the dynamics of tubulin addition and loss at the ends of mitotic spindle microtubules rather than by depolymerizing the microtubules. The specific alterations of spindle microtubule dynamics appear to differ among the five *Vinca* congeners, and such differences may be responsible for differences in the antitumor specificities of the drugs", (Jordan *et al*, 1991).

Cardioactive glycosides have been reported to be hypotensive (Nyarko and Addy, 1990). A group of glycosidic plant ingredients is called cardioactive steroid glycosides and cause specific myocardial effects owing to special structural characteristics. "Cardiac glycosides (CGs) are natural compounds sharing the ability to operate as potent inhibitors of the plasma membrane Na^+/K^+-ATPase, hence promoting - via an indirect mechanism - the intracellular accumulation of Ca^{2+} ions. In cardiomyocytes, increased intracellular Ca^{2+} concentrations exert prominent positive inotropic effects, that is, they increase myocardial contractility. CGs

have been suggested to exert potent antineoplastic effects, notably as they appear to increase the immunogenicity of dying cancer cells", (Menger *et al*, 2013). Digitoxigenin can be classified as a cardioactive steroid aglycone's prototype. Especially *the cis-trans-cis* connection of the steroid skeletal structure's rings A-B-C, which is derived from the pregnan's C21-frame (hydroxylated at C3 and C14, wearing an unsaturated lactone ring at C17), causes the cardiac effects (Wagner and Bladt, 1996). The ability of digitalis to block cell proliferation has been well established for some time. Digitalis has been shown to induce programmed cell death in therapeutic concentrations to different malignant cell lines (Haux J., 1999).

Anthracene derivatives are the characteristic ingredients of hydroxyanthraquinone drugs. They usually contain C- and O- bound sugar moieties. The sugar's chemical bonding to the aglycone's different C- atoms leads to more variations (Wagner and Bladt, 1996). The most abundant anthraquinone of rhubarb, a traditional Chinese medicine; emodin which has been demonstrated to have excellent antiproliferative properties (Huang *et al*, 2006). "Valepotriates are triesters of a terpenoid, trihydric alcohol. This alcohol has a structure of an iridoid cyclopenta-c-pyran with an attached epoxide ring", (Wagner, 1996). Valepotriates have been shown to be cytotoxic against the human metastatic prostate cancer cell line, PC-3M (Xu *et al* , 2007).

Plant-derived methylxanthines that include caffeine (from the coffee bean), theophylline (from the tea leaf), and theobromine (from the cocoa bean) are among the most widely consumed stimulant substances in the world with Americans consuming an average of 200 mg of caffeine per day (Daly, 2007). These effects are mediated primarily via blockade of adenosine receptors. Xanthine analogs with improved properties have been developed as

potential treatments for diseases such as Parkinson's disease. Behavioral stimulant effects of methylxanthines were mediated by blockade of adenosine receptors (Snyder et al., 1981), although at higher concentrations methylxanthines also have effects on several other target proteins such as phosphodiesterases (Daly, 2007). The naturally occurring methylxanthines such as caffeine are nonselective and have micromolar affinities at adenosine receptors (Müller and Jacobson, 2011). A large number of derivatives and analogs of these compounds have been made, with the aim of obtaining higher affinity and more selective ligands as research tools to characterize the function of adenosine receptors as well as for therapeutic purposes. Xanthine-based drugs have been evaluated clinically in diseases including asthma, Parkinson's disease, and pain (Daly, 2007).

Arbutin is a glycoside; a glycosylated hydroquinone extracted from the bearberry plant in the genus *Arctostaphylos*. It inhibits tyrosinase and thus prevents the formation of melanin (Dusková *et al*., 2015). In vitro studies of human melanocytes exposed to arbutin at concentrations below 300 μg/mL reported decreased tyrosinase activity and melanin content with little evidence of cytotoxicity. The German Institute of Food Research in Potsdam found that intestinal bacteria can transform arbutin into hydroquinone, which creates an environment favorable for intestinal cancer (Blaut *et al*., 2006).

The present study evidences the presence of various phytochemical bioactive compounds that forms an important reservoir that can be exploited for therapeutic use.

4.5 CONCLUSION

The present study shows the presence of medicinally important constituents in the plants studied could be seen as a good source for useful drugs. We postulate that the anticancer activity is linked to the various constituents of the plants as cross-referenced with published

data. Due to the enormous amount of phytochemicals present traditional medicine practice is recommended strongly for the plants under study and further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

CHAPTER FIVE

5.0 CYTOTOXIC ACTIVITY OF ANTI-CANCER MEDICINAL PLANTS OF KAKAMEGA COUNTY IN KENYA AGAINST DRUG SENSITIVE AND MULTIDRUG RESISTANT CANCER CELLS

5.1 INTRODUCTION

Cancer ranks highly as one of the single largest causes of death worldwide (Jemal *et al*., 2008). "There is a growing trend in cancer morbidity indicating deficiency in the present cancer therapies which include surgical operation, radiotherapy and chemotherapy. Since the average survival rates have remained essentially unchanged despite such aggressive treatments, there is a critical need for anti-cancer agents with higher efficacy, and less side effects that can be acquired at an affordable cost with plants providing the best alternative" (Fadeyi *et al.*, 2013). Some of the most effective cancer drugs that have been derived from natural products (Lee, 2010). Several drugs have been derived from plants forming a great percentage of current chemotherapeutics (Cragg *et al,* 2005; Newman *et al,* 2003). Currently, herbal medicine plays a major role in the health systems of many developing nations because of their availability and affordability and rich existing traditional knowledge (Taylor *et al*., 2001). The medicinal plants under consideration here have been reported elsewhere in this study to have potential anticancer properties (Ochwang'i *et al,* 2014). "Accounting for

chemotherapy failure in over 90% of patients with metastatic cancer, multidrug resistance (MDR) is quite a challenge for cancer treatment worldwide", (Kuete *et al.,* 2013). The various chemical compounds from these medicinal plants makes them important candidates in the discovery of therapeutics against sensitive and resistant phenotypes (Newman and Cragg, 2007). The present study explored the *in vitro* anti-cancer cytotoxic potential of thirty five organic and nineteen aqueous extracts of medicinal plants against drug sensitive and MDR cancer cell lines which depended on the availability of the extract and ease of extraction. The degrees of resistance were calculated by dividing the IC50 value of the resistant cell line by the corresponding parental sensitive cell line. When the degree of resistance on the tested cell line toward the control drug doxorubicin is high, then the studied cell lines can be considered as suitable cell models to study drug resistance. Extracts with lower degrees of resistance than those of doxorubicin can be exploited in a possible fight against cancer diseases involving MDR phenotypes. In addition, collateral sensitivity (sample more active on resistant cells than on sensitive cells) highlights its good antiproliferative activity (Kuete *et al.,* 2013).

5.2 MATERIALS AND METHODS

5.2.1 Extract preparation

Organic and aqueous extracts were prepared using dichlomethane: methanol and water respectively as ealier stated. Simulating what is done in the field by TMPs (Ochwang'i *et al,* 2014), a variety of plant combinations that has shown additive effects according to the TMPs in equal weight ratios were prepared.

5.2.2 Human cell lines

In this study both sensitive and multi-drug resistant cancer cell lines were used to be able to compare the effects of the candidate herbal extracts on each. "Drug sensitive acute

lymphoblastic leukaemia CCRF-CEM (ATCC® CCL-119™) and multidrug resistant acute lymphoblastic leukaemia CEM/ADR5000, cell lines were cultured and grown in Roswell Park Memorial Institute (RPMI)-1640 Medium (Invitrogen) supplemented with 10% foetal bovine serum (FBS), 100 units/ml penicillin and 100 micrograms/ml streptomycin. Human wild-type HCT116 (p53+/+) colon cancer cells as well as knockout clones without tumor suppressor p53 HCT116 (p53−/−) derived by homologous recombination (ATCC® CCL-247™), Human embryonic kidney cells [HEK-293] (ATCC® CRL-1573™), Breast cancer cells, transduced with control vector (MDA-MB-231-pcDNA3) and those with cDNA for the breast cancer resistance protein BCRP (MDA-MB-231-BCRP clone 23), (HTB-26™), Human glioblastoma multiforme U87MG cells (non-transduced) and U87MG cell line transduced with an expression vector harboring an epidermal growth factor receptor (EGFR) gene with a genomic deletion of exons 2 through 7 (U87MG.ΔEGFR) (ATCC® HTB-14™) were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) (Invitrogen) and 1% penicillin (100 U/mL)-streptomycin (100 μg/mL) (Invitrogen). MDA-MB-231-BCRP, U87MG.ΔEGFR and HCT116 (p53−/−) were continuously treated with 800 ng/mL and 400 μg/mL geneticin, respectively", (Kuete *et al*, 2013). All the cells were purchased from American Type Culture Collection (ATCC, USA) and cultured at 37 degrees in a 5% CO_2 atmosphere with 95% humidity (Mahavorasirikul et al, 2010; Machana *et al*, 2011). The cells were passaged twice weekly and experiments with candidate medicinal plant extracts performed with cells in the logarithmic growth phase. Combinational therapy screening was done drug sensitive acute lymphoblastic leukaemia CCRF-CEM (ATCC® CCL-119™) for both aqueous and organic extracts.

5.2.3 Resazurin reduction assay

Viability of the cells was used as an indicator for the cytotoxicity of the plant extracts. Cell viability was determined according to O'Brien *et al* 2000 and Kuete *et al*., 2013). The lower the IC_{50} the more effective the sample's anti-cancer activity.

5.3 RESULTS

5. 3.1 Screening results

In all the results presented, the medicinal plants under investigation are abbreviated as (A) for Organic extracts and (B) for Aqueous extracts of the same plant: Comparisons for cytotoxic activity was done between aqueous and organic extracts. **1A**.*Harungana madagascariensis* Lam.ex poir, **2A**.*Fuerstia africana* T.C.E. Fr., **3A**. *Sida rhombifolia* L., **4A**. *Zanthoxylum rubescens* Hook. f, 5A. *Bridelia micrantha* (Hochst.) Baill, **7A.** *Tragia brevipes* Pax, **8A**. *Phyllanthus sapialis*, **9A.** *Conyza sumatrensis* (Retz.)E.H Walker, **10A**. *Momordica foetida* Schumach., **11A.***Synsepalum cerasiferum* Synonym: *Afrosersalisia cerasifera* (Welw.) Aubrev. **12A.***Aloe volkensii* Engl., **13A.** *Aeschynomene abyssinica* (A.Rich.) Vatke, **14A**. Futumia *africana* Benth. **15A.** *Cyphostemma serpens* (A. Rich), **16A.** *Ipomoea cairica* (L.), **17A.** *Spathodea campanulata* P.Beauv. ssp. nilotica (Seem),**18A.** *Abrus precatorius* L. ssp africanus Verdc., **19A.** *Triumfetta rhomboidea* Jacq., **20A.** *Psydrax schimperiana* (A. Rich) , **21A.**Ficus thonningii,**22A.***Rotheca myricoides* (Hochst.steane and maab.),**23A**.*Croton macrostachyus* Delile, **24A.***Vernonia lasiopus* O Hoffin ,**25A.***Albizia gummifera* (J.F. Gmel.), **26A.***Zanthoxylum gilletii* (De Wild.) P.G. Waterman, **27A.***Microglossa pyrifolia* (Lam.) Kuntze , **28A.***Senna didymobotyra* (Fresen.) Irwin and Barneby ,**29A.** *Trichilia emetica* Vahl ,**30A**. *Entada abyssinica* Steud.ex A.Rich. ,**31A.** *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax

,**32A**.*Ocimum gratissimum* L. Suave wiild O. tomentosum oliv. , **33A**. *Prunus africana* (Hook.f.) kalkman, **34A.** *Phyllanthus fischeri* Pax, **35A.** Olea hotch spp. Hochstetteri.

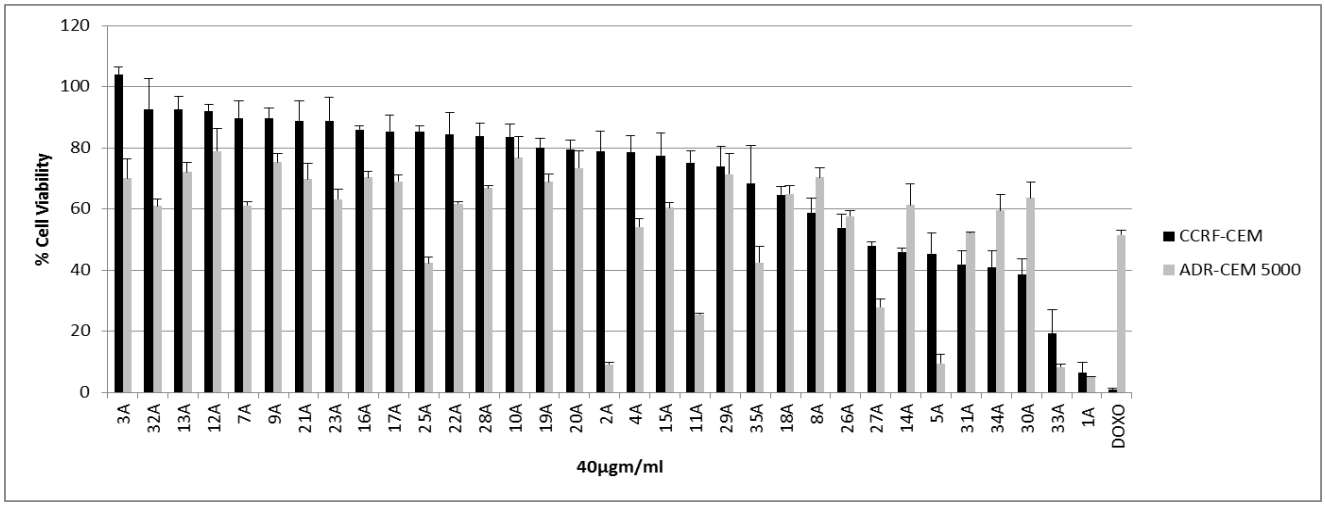

Figure 5.1 Cell viability (%) of sensitive leukemia CCRF-CEM and resistant ADR 5000-CEM cancer cell line treated with organic plant extracts at 40 µg/mL (Kuete *et al*., 2013) and doxorubicin 10 µM.

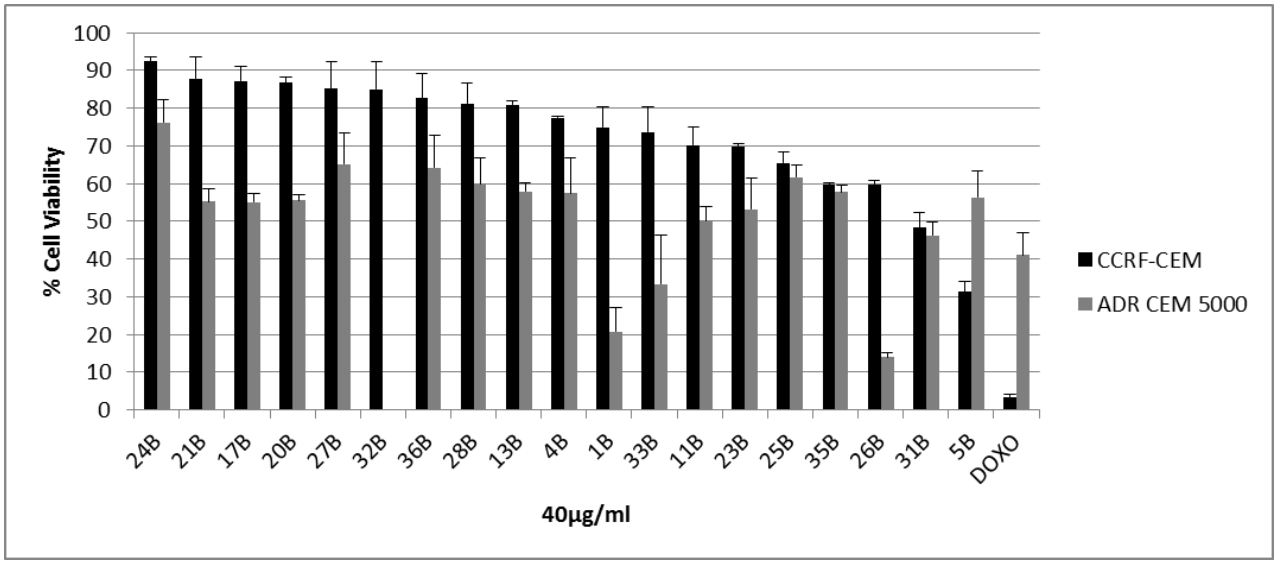

Figure 5.2 Cell viability (%) of leukemia CCRF-CEM and ADR 5000-CEM cancer cell line treated with aqueous plant extracts at 40 µg/mL and doxorubicin 10 µM.

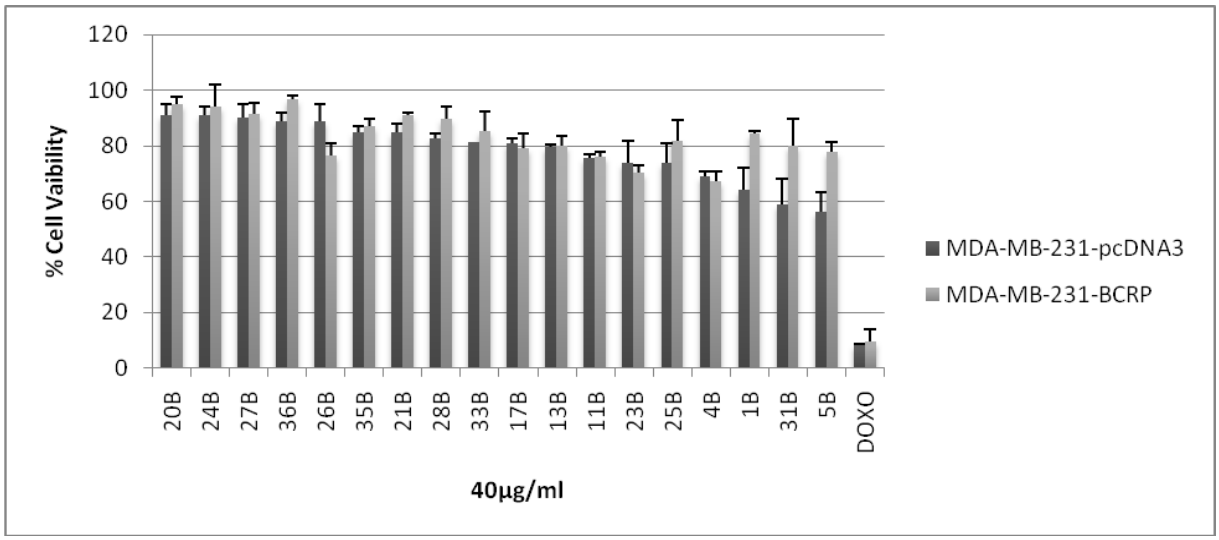

Figure 5.3 Cell viability (%) of breast cancer cells, transduced with control vector (MDA-MB-231-pcDNA3 and breast cancer resistance protein BCRP (MDA-MB-231-BCRP clone 23) treated with aqueous plant extracts at 40 µg/mL and doxorubicin 10 µM.

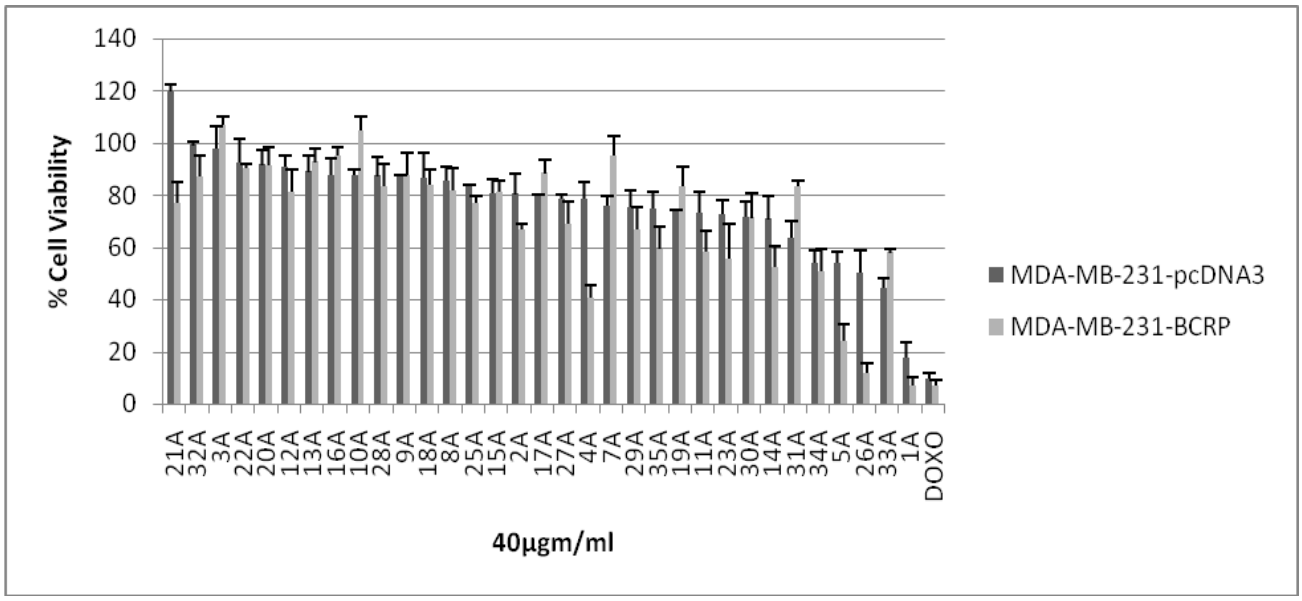

Figure 5.4 Cell viability (%) of breast cancer cells, transduced with control vector (MDA-MB-231-pcDNA3 and breast cancer resistance protein BCRP (MDA-MB-231-BCRP clone 23) treated with organic plant extracts at 40 µg/mL and doxorubicin 10 µM.

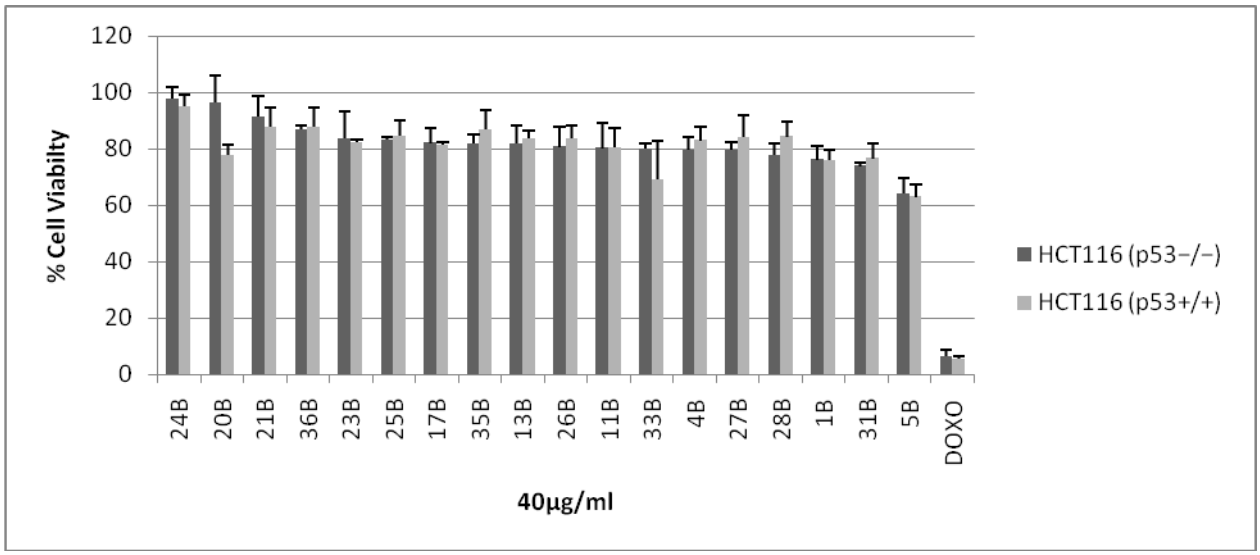

Figure 5.5 Cell viability (%) Human wild-type HCT116 (p53+/+) and clones of colon cancer cells HCT116 (p53−/−) colon cancer cells treated with aqueous plant extracts at 40 µg/mL and doxorubicin 10 µM.

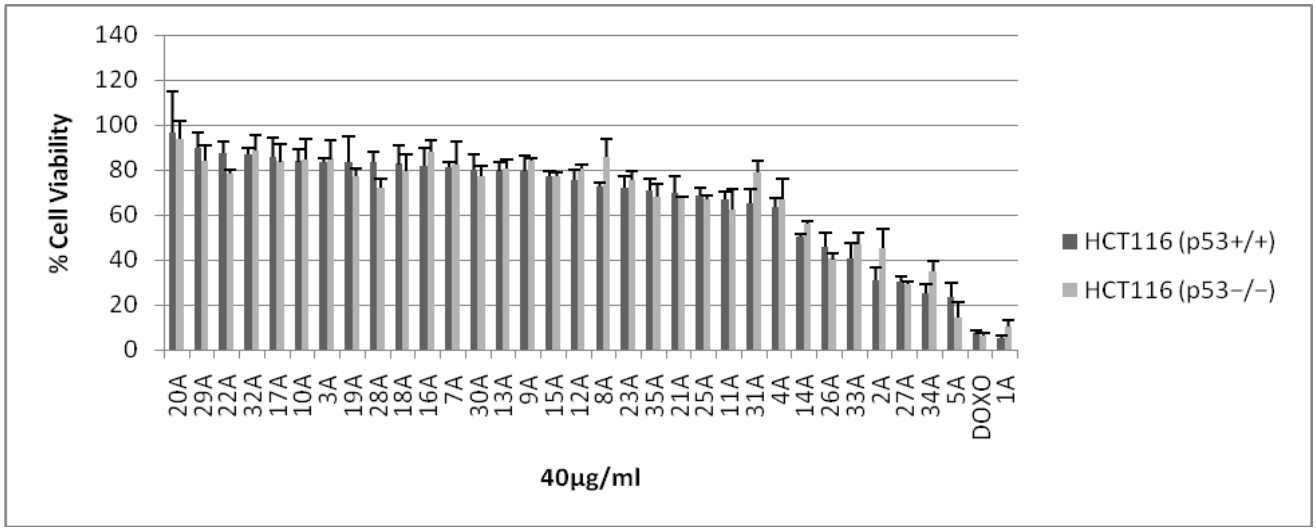

Figure 5.6 Cell viability (%) Human wild-type HCT116 (p53+/+) and clones of colon cancer cells HCT116 (p53−/−) colon cancer cells treated with organic plant extracts at 40 µg/mL and doxorubicin 10 µM.

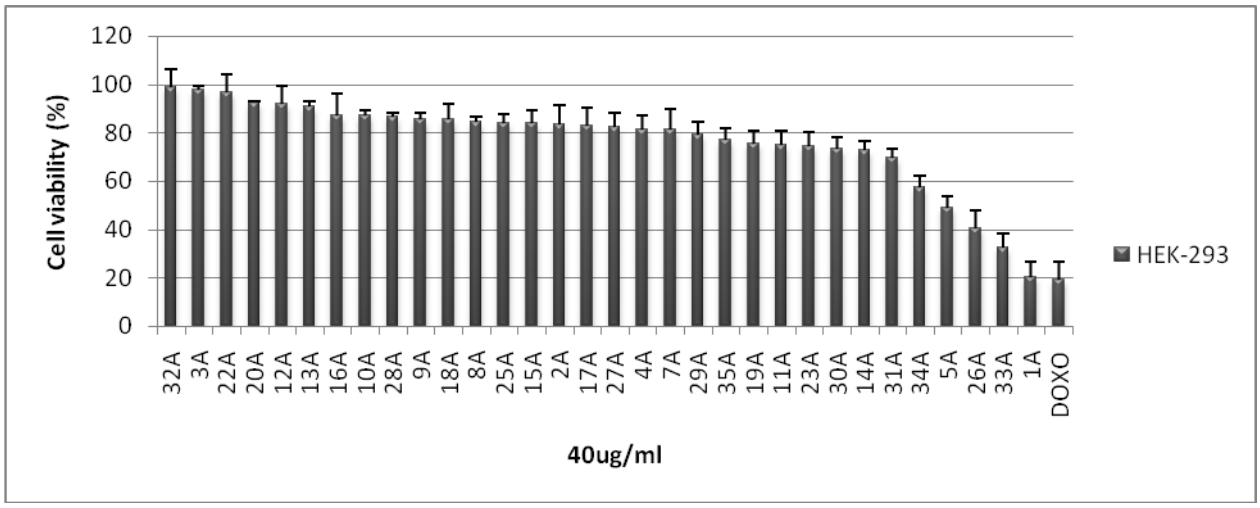

Figure 5.7 Cell viability (%) Human embryonic kidney cells HEK-293 treated with organic plant extracts at 40 µg/mL and doxorubicin 10 µM.

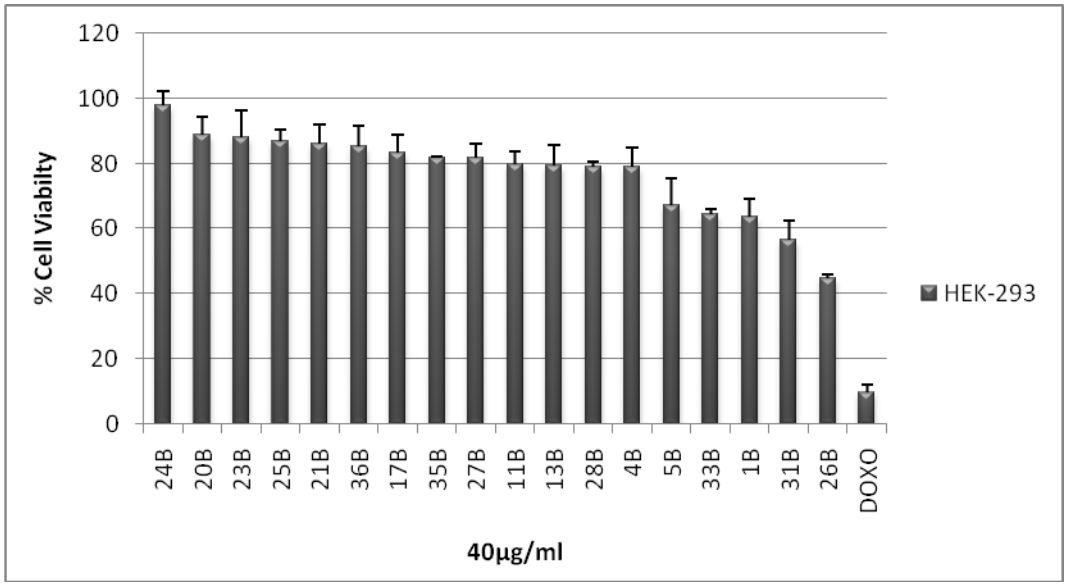

Figure 5.8 Cell viability (%) Human embryonic kidney cells HEK-293 treated with aqueous plant extracts at 40 µg/mL and doxorubicin 10 µM.

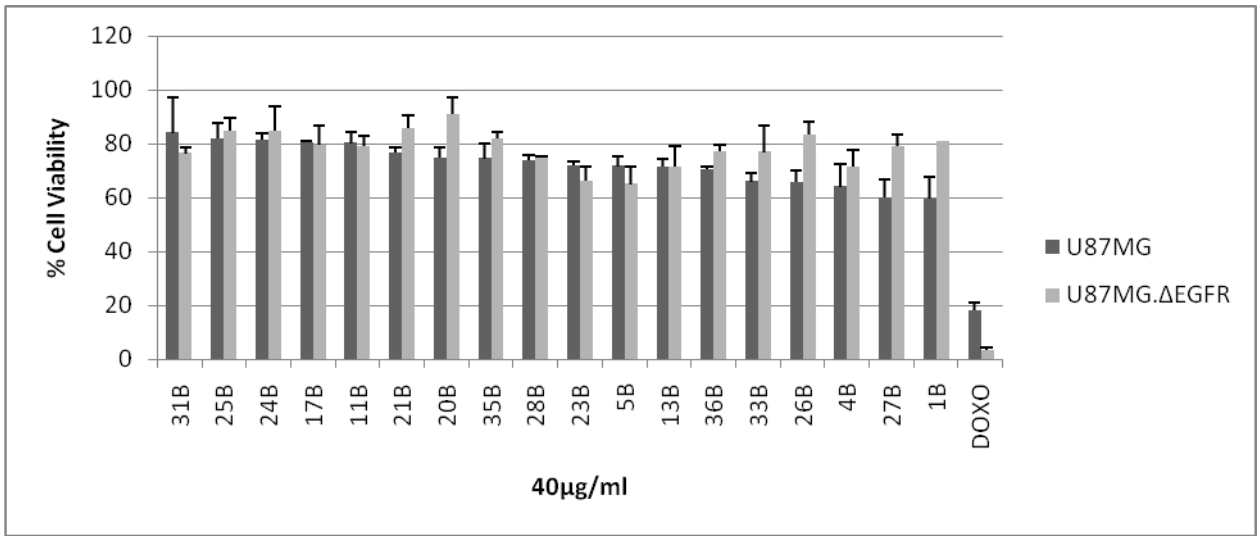

Figure 5.9 Cell viability (%) Human glioblastoma multiforme U87MG and Human glioblastoma cancer cell line (U87MGΔEGFR) cancer cells treated with aqueous plant extracts at 40 µg/mL and doxorubicin 10 µM.

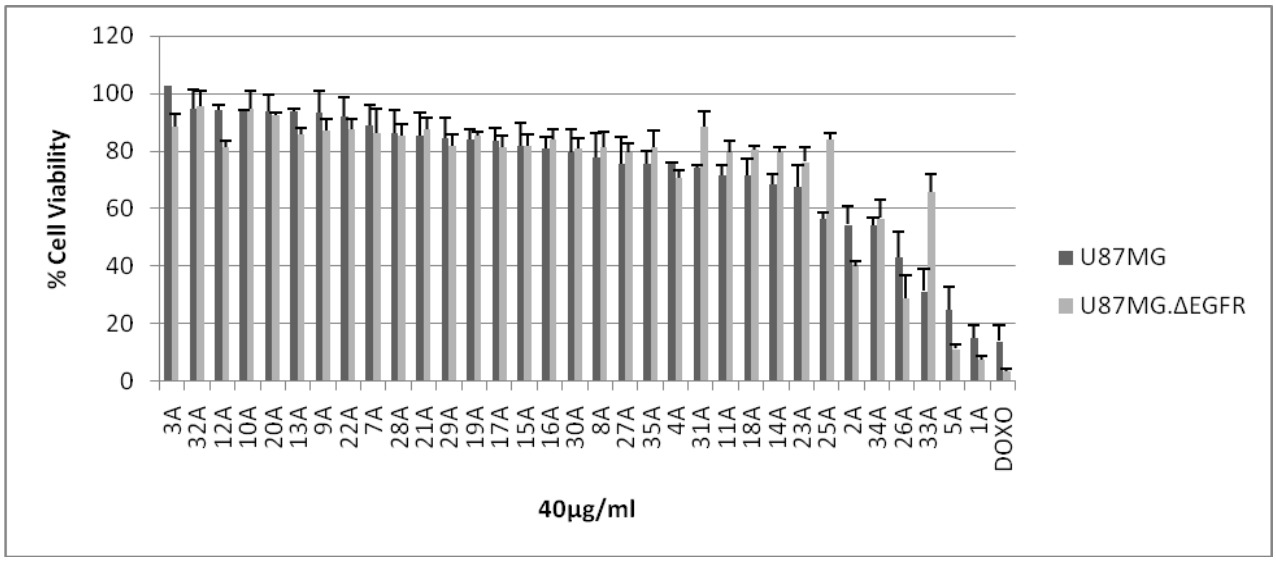

Figure 5.10 Cell viability (%) Human glioblastoma multiforme U87MG and Human glioblastoma cancer cell line (U87MGΔEGFR) cancer cells treated with organic plant extracts at 40 µg/mL and doxorubicin 10 µM.

5. 3.2 Combinational Screening Results

5.3.2.1 Aqueous Extract combinational screening results

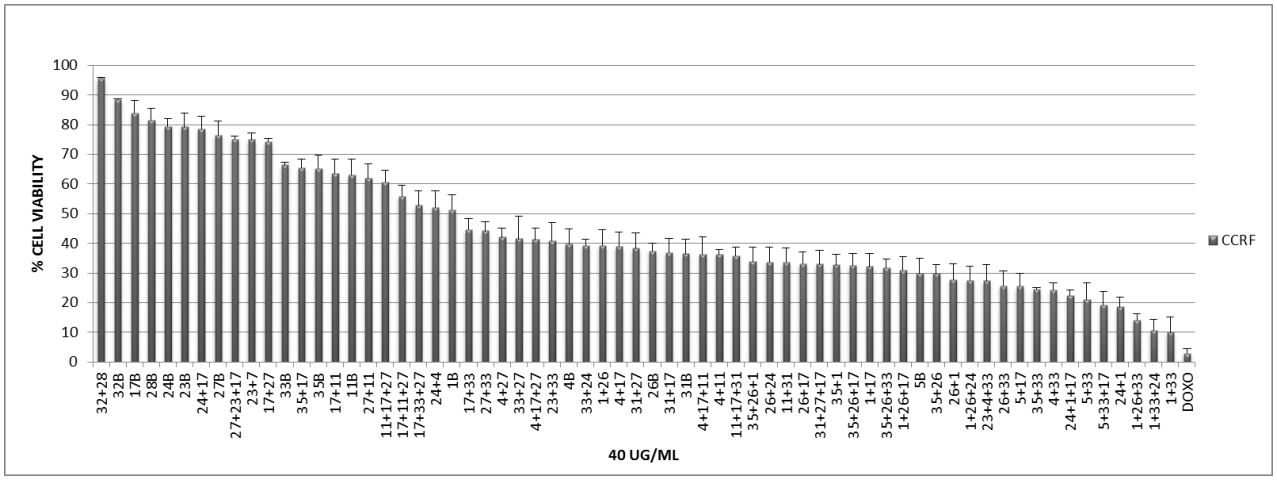

Figure 5.11 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with aqueous plant extracts at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

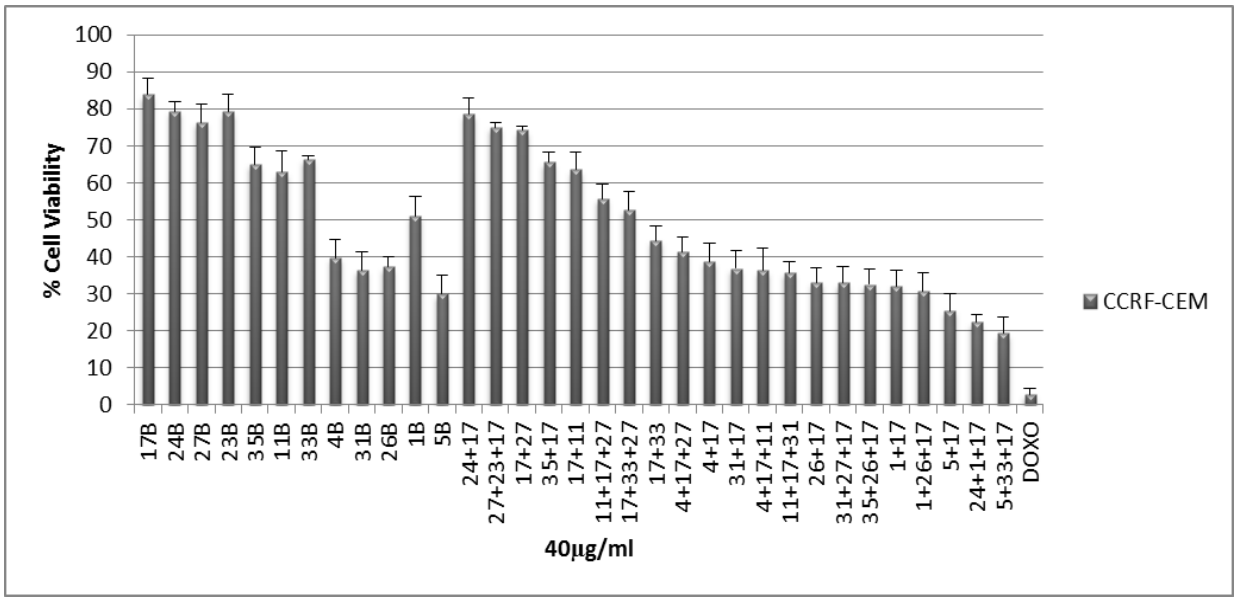

Figure 5.12 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with aqueous plant extract 17B (*Spathodea campanulata* P.Beauv. ssp. nilotica (Seem)) combinations at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

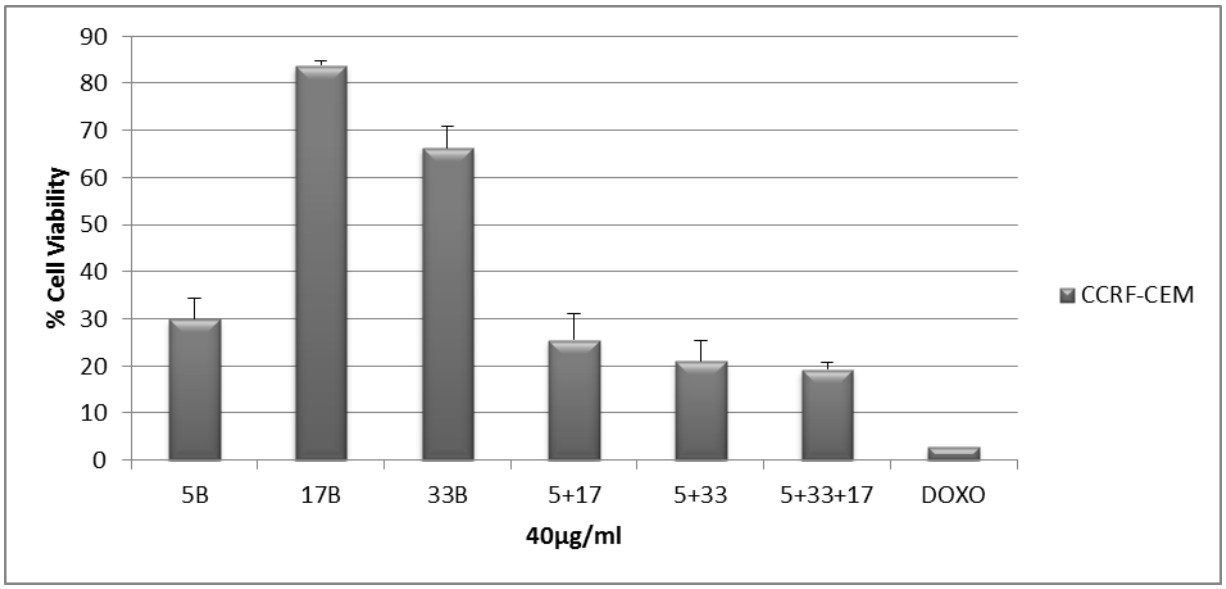

Figure 5.13 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with aqueous plant extract 5B (*Bridelia micrantha* (Hochst.) Baill) combinations at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

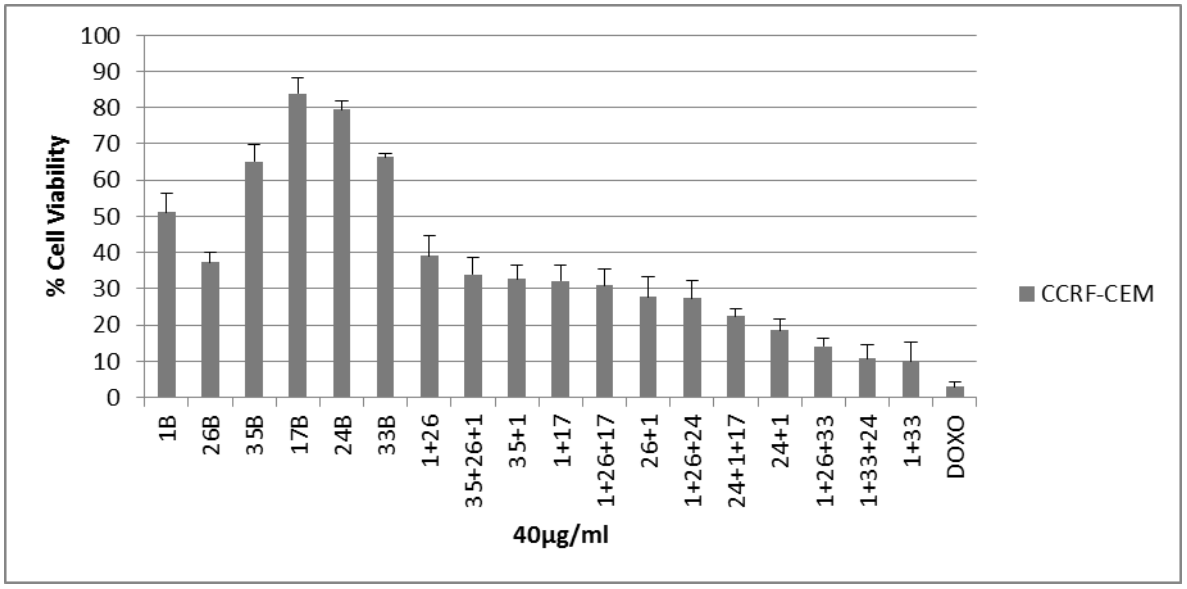

Figure 5.14 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with aqueous plant extract 1B (*Harungana madagascariensis* Lam.ex poir) combinations at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

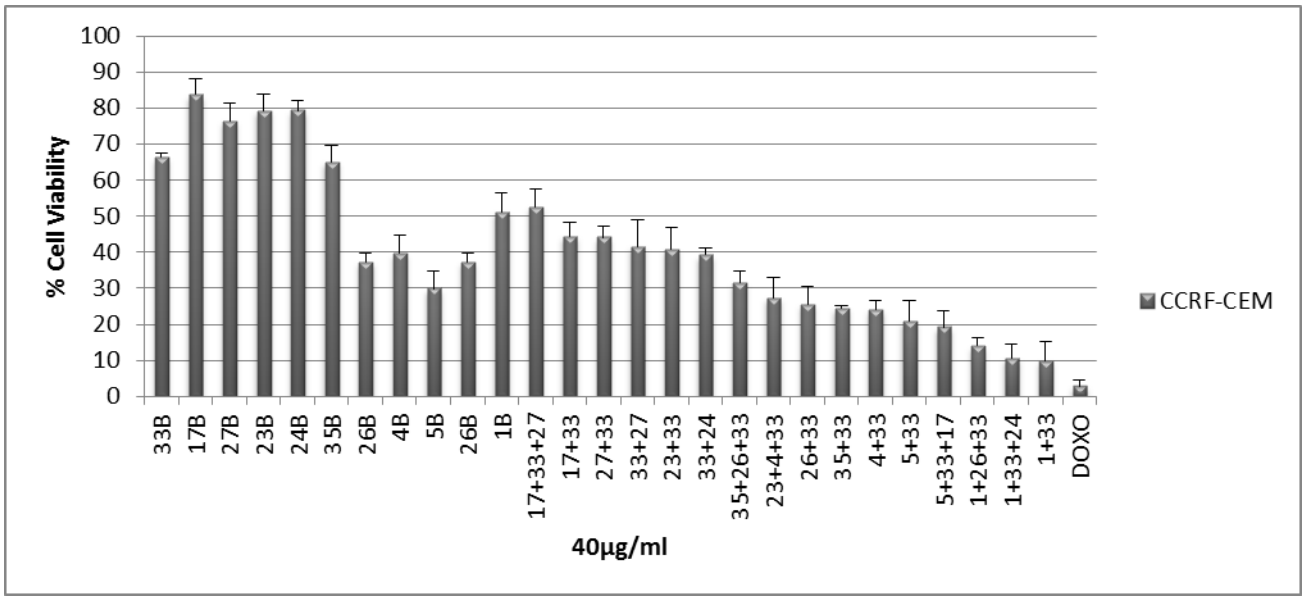

Figure 5.15 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with aqueous plant extract 33B (*Prunus africana* (Hook.f.) kalkman) combinations at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

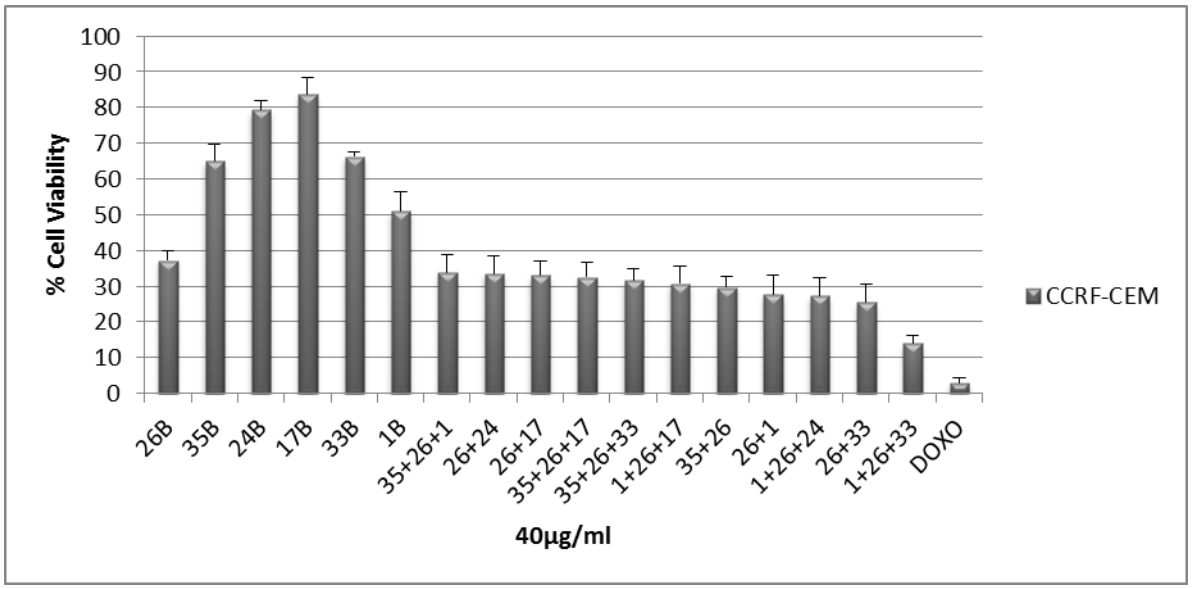

Figure 5.16 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with aqueous plant extract 26B (*Zanthoxylum gilletii* (De Wild.) P.G. Waterman) combinations at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

5.3.2.2 Organic Extract combinational screening results

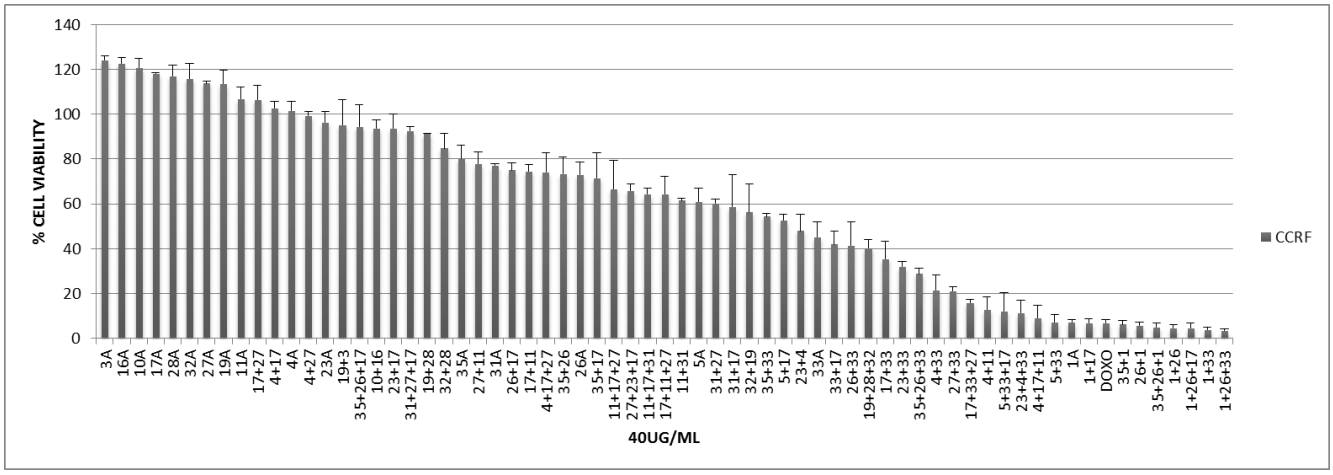

Figure 5.17 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with organic plant extracts combinations at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM

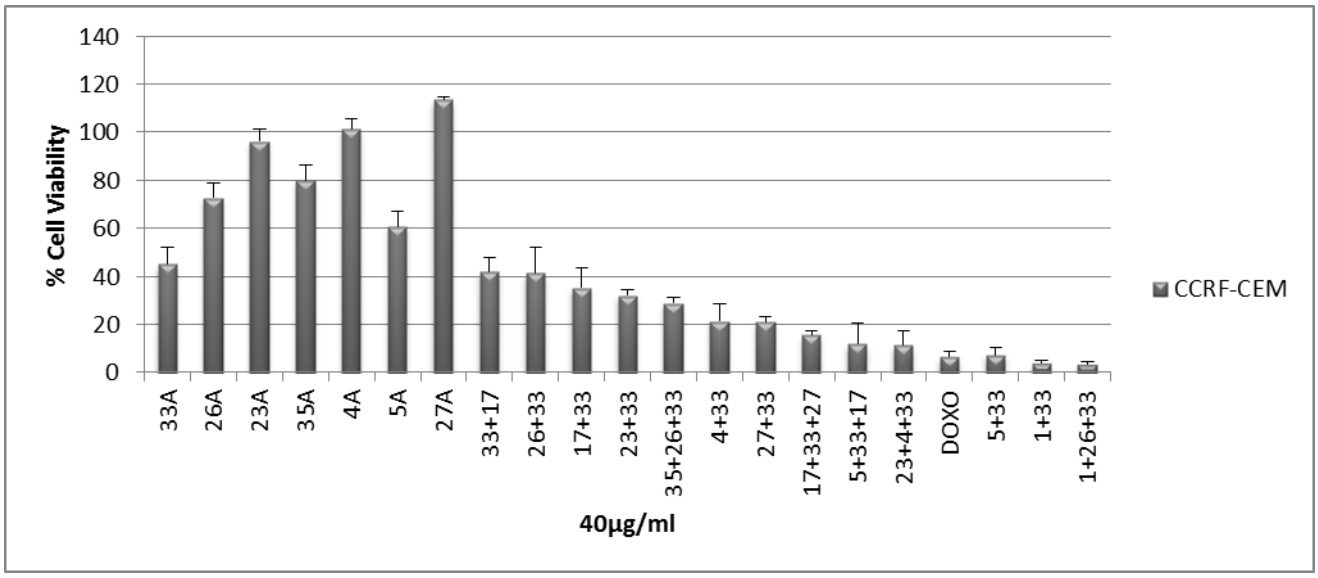

Figure 5.18. Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with organic plant extract 33A (*Prunus africana* (Hook.f.) kalkman) combinations with extracts 26A,23A,35A,4A,5A and 27A at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

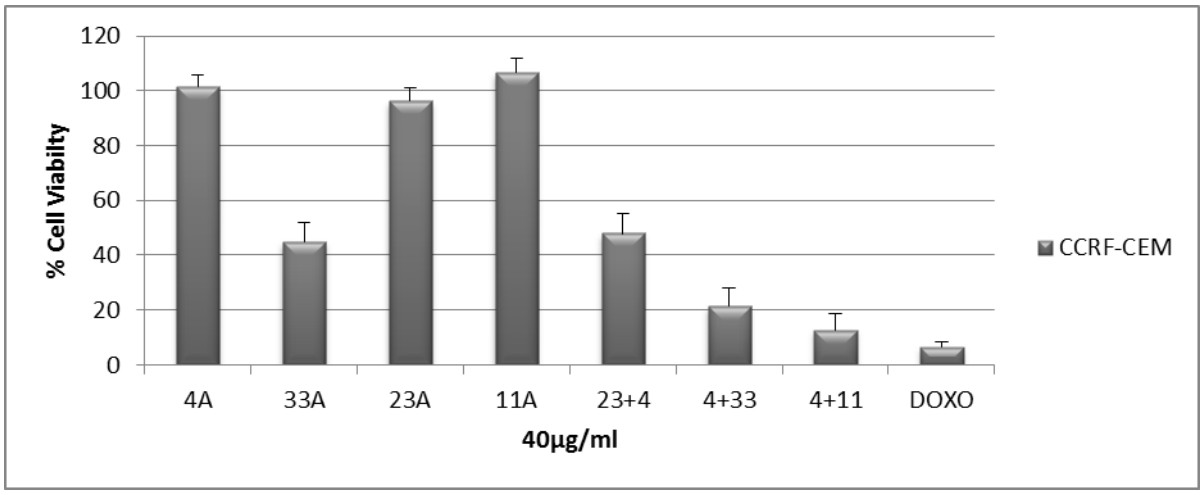

Figure 5.19 Cell viability (%) of leukemia CCRF-CEM cancer cell line treated with organic plant extract 4A (*Zanthoxylum rubescens* Hook. f.) combinations with extract 33A,23A and 11A at 40 µg/mL at a ratio of 1:1 and doxorubicin 10 µM.

5. 3.3 Dose response IC_{50} results

Dose response IC50 values; the concentration of the extract at which only 50% of the cells are viable ,were obtained for extracts with the best cytotoxic anticancer activity with any of the sensitive and multidrug resistant cell lines.This included organic extracts **26A (***Zanthoxylum gilletii* (De Wild.) P.G. Waterman),**33A(***Prunus africana* (Hook.f.) kalkman) ,**1A(***Harungana madagascariensis* Lam.ex poir) ,**5A (***Bridelia micrantha* (Hochst.) Baill) ,**27A (***Microglossa pyrifolia* (Lam.) Kuntze),**30A (***Entada abyssinica* Steud.ex A.Rich.),**34A (***Phyllanthus fischeri* Pax) ,**2A (***Fuerstia africana* T.C.E. Fr.) and aqueous extract **5B (***Bridelia micrantha* (Hochst.) Baill).

Table 5. 4 IC50 values (μg/mL) and degree of resistance (in brakets) of selected medicinal plants."The degree of resistance (in brakets) was determined as the ratio of IC50 value of the resistant/IC50 sensitive cell line; (−): >40 μg/mL which determines the sensitivity of the cancer cell line to the candidate herbal extract",(Kuete et al ,2013).

Cell lines	**Plant extracts IC_{50}**									
	1A	**5A**	**5B**	**27A**	**34A**	**33A**	**26A**	**30A**	**34A**	**2A**
CCRF-CEM	9.11±0.63	23.35±399	38.62±9.95	_	_	34.93±4.48	34.6±5.42	_	_	32.47±525
CEM/ADR5000	32.64±1.16(3.58)	39.05±3.54(1.67)	21.16±0.09 (0.55)	_	_	31.17±1.71(0.89)	_	28.03±1.63(0.22)	23.45±2.59(0.255)	24.70±0.97(0.76)
MDA-MB231	28.91±3.12	_	_	_	_	39.38±1.95	_	_	_	_
MDA-MB231/ BCRP	29.08±0.41 (0.99)	_	_	_	_	39.28±5.11(0.997)	_	_	_	_

U87MG	22.42±5.94	_	_	_	_	29.68± 0.17	_	_	_	_
U87MG.ΔEGFR	26.36±1.079 (1.18)	_	_	_	_	_	_	_	_	_
HCT116 (p53−/−)	_	_	37.18±0.42(1.09)	39.69±3.3 (1.10)	40.00±2.26 (1.11)	32.94±3.54(1.06)	_	_	40.00±2.26(1.11)	_
HCT116 (p53+/+)	_	_	33.97±0.41	36.12±2.63	35.74±1.59	31.07±0.78	_	_	35.74±1.59	-

5.4 DISCUSSION

Thirty four organic and nineteen aqueous plant extracts were screened against sensitive and multi-drug resistant cancer cell lines with a single concentration of 40 μg/mL for each extract. Screening results (Figure 5.1 and figure 5.2) indicated that eight of the thirty four organic plant extracts and two of the aqueous plant extracts showed less than 50% growth proliferation of CCRF-CEM cells. The organic extracts include *Harungana madagascariensis* Lam.ex poir (6.56%), *Prunus africana* (Hook.f.) kalkman (19.43%), *Entada abyssinica* Steud.ex A.Rich. (38.69%), *Phyllanthus fischeri* Pax, (40.78%), *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax (41.84%), *Bridelia micrantha* (Hochst.) Baill (45.41%), Futumia *africana* Benth. (45.88%) and *Microglossa pyrifolia* (Lam.) Kuntze (48.06%).The aqueous extracts include *Bridelia micrantha* (Hochst.) Baill (31.32%) and *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax (48.29%).

Four aqueous extracts and eight organic extracts showed less than 50% growth proliferation of CEM/ADR5000 cells (Figure 5.1 and Figure 5.2). These include aqueous extracts of *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax (46.19%),. *Prunus africana* (Hook.f.) kalkman (33.29%), *Harungana madagascariensis* Lam.ex poir (20.74%) and *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (13.93%).Organic extracts include those of Olea hotch spp. Hochstetteri (42.45%), *Albizia gummifera* (J.F. Gmel.) (42.35%), *Microglossa pyrifolia* (Lam.) Kuntze (27.90%), *Synsepalum cerasiferum* Synonym: *Afrosersalisia cerasifera* (Welw.) Aubrev. (25.52%), *Bridelia micrantha* (Hochst.) Baill (9.38%), *Fuerstia africana* T.C.E. Fr. (9.18%), *Prunus africana* (Hook.f.) kalkman (8.32%), *Harungana madagascariensis* Lam.ex poir (5.09%).

Screening results (Figure 5.3 and Figure 5.4) indicated that two organic plants extracts and none of the aqueous plant extracts showed less than 50% growth proliferation of MDA-MB231 cells. The organic extracts include *Prunus africana* (Hook.f.) kalkman (44.19%) and *Harungana madagascariensis* Lam.ex poir (17.89%). Four of the thirty four organic plant extracts and none of the aqueous plant extracts showed less than 50% growth proliferation of MDA-MB231/ BCRP cells (Figure 5.3 and Figure 5.4).This are extracts of *Zanthoxylum rubescens* Hook. f (40.55%), *Bridelia micrantha* (Hochst.) Baill (23.98%), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (11.83%), *Harungana madagascariensis* Lam.ex poir (6.93%).

Screening results (Figure 5.7 and Figure 5.8) indicated that four of the thirty four organic plant extracts and one of the aqueous plant extracts showed less than 50% growth proliferation of HEK-293 cells. This was observed with organic extracts of *Microglossa pyrifolia* (Lam.) Kuntze (49.16%), *Bridelia micrantha* (Hochst.) Baill (40.69%), *Prunus africana* (Hook.f.) kalkman (32.63%) and *Harungana madagascariensis* Lam.ex poir (19.54%). This was shown by only one aqueous extract of *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (44.87%).

Four of the thirty four organic plant extracts and none of the aqueous plant extracts showed less than 50% growth proliferation of U87MG cells (Figure 5.9 and Figure 5.10). *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (43.23%), *Prunus africana* (Hook.f.) kalkman (31.29%), *Bridelia micrantha* (Hochst.) Baill (24.91%) and *Harungana madagascariensis* Lam.ex poir (15.15%). Screening results (Figure 5.9 and Figure 5.10) indicated that four of the thirty four organic plant extracts and none of the aqueous plant extracts showed less than 50% growth proliferation of U87MG.ΔEGFR cells. The organic

extracts include; *Fuerstia africana* T.C.E. Fr. (39.84%), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (28.84%), *Bridelia micrantha* (Hochst.) Baill (11.31%) and *Harungana madagascariensis* Lam.ex poir (7.48%).

Seven of the thirty four organic plant extracts and none of the aqueous plant extracts showed less than 50% growth proliferation of HCT116 (p53−/−) cells (Figure 5.3 and Figure 5.4). Extracts of *Prunus africana* (Hook.f.) kalkman (46.73%), *Fuerstia africana* T.C.E. Fr. (45.04%), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (40.32%), *Phyllanthus fischeri* Pax (34.79%), *Microglossa pyrifolia* (Lam.) Kuntze (29.09%), *Bridelia micrantha* (Hochst.) Baill (14.40%) and *Harungana madagascariensis* Lam.ex poir (10.56%). Screening results (Figure 5.3 and Figure 5.4) indicated that seven of the thirty four organic plant extracts and none of the aqueous plant extracts showed less than 50% growth proliferation of HCT116 (p53+/+). *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (45.62%), *Prunus africana* (Hook.f.) kalkman (40.41%), *Fuerstia africana* T.C.E. Fr. (30.86%), *Microglossa pyrifolia* (Lam.) Kuntze (30.11%), *Phyllanthus fischeri* Pax (25.42%), *Bridelia micrantha* (Hochst.) Baill (23.56%) and *Harungana madagascariensis* Lam.ex poir (5.22%).

The IC_{50} values of samples showing best cytotoxic activity were determined on a set of eight cancer cell lines, including both sensitive and MDR phenotypes as shown in Table 5.1. *Prunus africana* (Hook.f.) kalkman extract inhibited the proliferation of seven out of eight tested cancer cell lines, with IC_{50} values below 40 μg/mL while *Harungana madagascariensis* Lam.ex poir extract inhibited 6/8 of the cells tested. Other extracts showed selective activities with the IC_{50} values being obtained on 4/8 tested cells lines for the aqueous extract of *Bridelia micrantha* (Hochst.) Baill, 2/8 for its organic part; 2/8 for *Microglossa pyrifolia* (Lam.) Kuntze and *Fuerstia africana* T.C.E. Fr.,3/8 for *Phyllanthus fischeri* Pax and 1/8 for

Zanthoxylum gilletii (De Wild.) P.G. Waterman and *Entada abyssinica* Steud.ex A.Rich (Table 5.1)

"American National Cancer Institute sets the criteria of 30 μg/mL as the upper IC_{50} limit considered promising for purification of a crude extract" (Suffness and Pezzuto,1990). "The highest concentration tested (40 μg/mL) in our screening was slightly above this limit. Considering this cutoff point, the IC50 values below or around 30 μg/mL were recorded with a number of extracts", (Kuete *et al*, 2013).These included organic extracts of *Zanthoxylum gilletii* (De Wild.) P.G. Waterman, *Prunus africana* (Hook.f.) kalkman, *Harungana madagascariensis* Lam.ex poir ,*Bridelia micrantha* (Hochst.) Baill , *Microglossa pyrifolia* (Lam.) Kuntze, *Entada abyssinica* Steud.ex A.Rich., *Phyllanthus fischeri* Pax , *Fuerstia africana* T.C.E. Fr. and aqueous extract of *Bridelia micrantha* (Hochst.) Baill. (Table 1). From these results, some of the tested medicinal plants have a lot of potential as anticancer agents. All the screening results were compared to Doxorubicin, a standard anticancer drug.

MDR is a quite a challenge in cancer treatment worldwideand has been seen to cause chemotherapy failure in over 90% of patients with metastatic cancer (Longley and Johnston, 2005; Liu *et al* 2012). According to Kuete *et al* 2013;This study investigated both sensitive and MDR cell lines and calculated the degrees of resistance by dividing the IC_{50} value of the resistant cell line by the corresponding parental sensitive cell line to determine how sensitive the cells were to the candidate herbal extracts. The present study tested cell lines overexpressing two ATP-binding cassette transporters, i.e. P-glycoprotein (ABCB1/MDR1) or breast cancer resistance protein (ABCG2/BCRP). Furthermore, we tested a p53 knockout cell line and a transfectant cell line harboring a mutation- activated EGFR gene (ΔEGFR) as examples for resistance- inducing tumor suppressors and oncogenes. Collateral sensitivity

(sample more active on resistant cells than on sensitive cells) was observed with the aqueous extract of *Bridelia micrantha* (Hochst.) Baill**,** organic extracts of *Prunus africana* (Hook.f.) kalkman and *Fuerstia africana* T.C.E. Fr. against CEM/ADR5000 showing their good antiproliferative activity (Figure 5.1 and Figure 5.2).

This study reports the cytotoxicity of the plant extracts in the study area for the first time. *Harungana madagascariensis* Lam.ex poir cytotoxic activity shows great potential against sensitive and MDR cell lines. "*H. madagascariensis* has also been previously reported to have excellent analgesic and anti-inflammatory activities. Similarly, the plant inhibits the activity of G-glucosidase and was found to have antioxidant properties (Kouam et al., 2006a, 2006b). Kouam et al. (2007) have isolated a prenylated 1, 4-anthraquinone from the hexane extract of the stem-bark of *H. madagascariensis* and have shown it to possess G-glucosidase inhibition and antioxidant activities", (Iwalewa *et al*, 2009). However cytotoxic anticancer activity of *H. madagascariensis* has not been documented especially from the specific region of Kakamega County, Kenya.

Prunus africana bark was discovered for management of benign prostatic hyperplasia 35 years ago. The extract is formulated and sold as capsules (Pygeum africanum) by pharmaceutical companies mainly in Europe. This has led to increased harvesting to the extent that it was declared endangered by the Convention of International Trade in Endangered Species (CITES) in 1995.In the present study, *Prunus africana* stem bark extract showed good cytotoxicity activity against various cancer cell lines (Table 5.1).

As mentioned elsewhere in this study, TMPs rarely use medicinal plants as single entities for treatment and management of cancer but use combinations in equal ratios of the extracts

probably taking advantage of traditionally recognized synergistic effects from indeginous knowledge. To test this, the present study simulated the potential field situation by combining the extracts in equal ratios; weight: volume and screening them against sensitive cancer cells CCRF-CEM. The present study showed excellent results and great potential of combinational therapy, some exceeding the standard anti-cancer drug Doxorubicin. Marked synergistic cytotoxicity were seen with combinations of aqueous extracts of *Harungana madagascariensis* Lam.ex poir and *Prunus africana* (Hook.f.) kalkman (9.95%) compared to their single activity of 51.14% and 66.31% respectively (Figure 5.14 and Figure 5.15). Different combinations of extracts of *Harungana madagascariensis* Lam.ex poir, *Spathodea campanulata* P.Beauv. ssp. nilotica (Seem),, *Zanthoxylum gilletii* (De Wild.) P.G. Waterman, *Prunus africana* (Hook.f.) kalkman, and *Bridelia micrantha* (Hochst.) Baill.; showed cytotoxic synergy compared to single entities. These results validate the use of these plants by TMPs as combination therapy. Organic extract combinations were even more effective, especially with *Harungana madagascariensis* Lam.ex poir and *Prunus africana* (Hook.f.) kalkman) (Figure 5.18) that showed better cytotoxicity compared to the standard anticancer drug Doxorubicin showing cell viability of less than 6%. This provides an excellent candidate for therapeutic development. These findings further support the practice by TMPs. However, this study shows that organic extracts are more efficacious, indicating a need to guide the TMPs to modify their extraction methods as appropriate. This study recommends the use of combined plants extracts for the TMPs as they portend to have the most effective anticancer therapeutic potential of the medicinal plants studied.

5.5 CONCLUSION

The present study shows the *in vitro* cytotoxic anticancer potential of thirty five organic and nineteen aqueous extracts of medicinal plants used in Kakamega County, Kenya. Some of the aqueous and organic extracts have shown good activity against both sensitive and MDR cancer cell lines. This forms a potential pool for further exploration as anticancer agents. Synergistic studies using Bliss model and checker board assay is highly recommended to ascertain the additive effects of the medicinal plants. The use of these medicinal plants in traditional practice is recommended strongly and the active constituents responsible for the activity of these plants especially key candidate plants *Harungana madagascariensis* Lam.ex poir, *Prunus africana (Hook.f.) kalkman and Bridelia micrantha* (Hochst.) Baill should be investigated.

CHAPTER SIX

6.0 BIOACTIVITY-GUIDED FRACTIONATION AND MECHANISM ANALYSIS OF KEY CANDIDATE MEDICINAL PLANTS; *Harungana madagascariensis* Lam.ex poir, *Prunus africana (Hook.f.) kalkman AND Bridelia micrantha* (Hochst.) Baill .

6.1 INTRODUCTION

"There is a growing trend in cancer morbidity indicating deficiency in the present cancer therapies which include surgical operation, radiotherapy and chemotherapy. Since the average survival rates have remained essentially unchanged despite such aggressive treatments, there is a critical need for anti-cancer agents with higher efficacy, and less side effects that can be acquired at an affordable cost with plants providing the best alternative", (Fadeyi *et al.*, 2013). "Natural product drugs play a dominant role in pharmaceutical care. This is especially

obvious in the case of antitumor drugs, as exemplified by paclitaxel (Taxol®), vincristine (Oncovin®), vinorelbine (Navelbine®), teniposide (Vumon®), and various water-soluble analogs of camptothecin (e.g., Hycamtin®)", (Pezzuto,1997). The diversity of phytochemicals from the medicinal plants makes them an important reseivour in the search for potentially active drugs on sensitive and resistant phenotypes (Newman and Cragg, 2007). Here, biochemical components and potential mechanisms of action of *Harungana madagascariensis Lam.ex poir* , *Bridelia micrantha (Hochst.) Baill.* and *Prunus africana (Hook.f.) kalkman* extracts and tested against sensitive and Multi Drug Resistant (MDR).We employed HPLC-MS for fractionation purposes and measured Reactive Oxygen Species by Flow Cytometry.

Reactive Oxygen Species generating drugs have a direct antiproliferative effect on cancer cell lines (Chen *et al*; 2010). "The increase in intracellular ROS levels and the mitochondrial injury cause other cellular effects such as oxidative DNA damage andinhibition of cancer cell migration", (Wiench *et al,* 2012).Therefore we postulate that any herbal candidate that is able to elicit increased ROS production is then effectively able to cause apoptosis of the cancer cell lines.

"High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used

in the separation", (Gerber *et al*., 2004).We employed HPLC to try and separate constituent individual fractions that maybe responsible for the bioactivity of the candidate herbal extracts.

6.2 MATERIALS AND METHODS

6.2.1 Extract preparation

Organic and aqueous extracts were prepared using dichlomethane: methanol and water respectively as per protocol stated earlier.

6.2.2 Human cell lines

Drug sensitive CCRF-CEM (ATCC® CCL-119™) and multidrug resistant CEM/ADR5000, were cultured and grown according to Kuete *et al*, 2013.

6.2.3 High Performance Liquid Chromatography (HPLC)

The aqueous extracts of *Bridelia micrantha* (Hochst.) Baill. and *Harungana madagascariensis* Lam.ex poir were dissolved in MeOH as a solvent. (The extract is not soluble in DMSO or Acetonitrile (MeCN) at 20 mg mL^{-1}). DMSO was used as a solvent for the organic extracts of *Harungana madagascariensis* Lam.ex poir, *Bridelia micrantha* (Hochst.) Baill and *Prunus africana* (Hook.f.) kalkman. The extract was analyzed by HPLC (Agilent 1100 Series) equipped with a LiChrospher RP 18 (3 × 125 mm; 5 µm, Merck KGaA, Darmstadt, Germany). The column was used at 40 °C and a flow rate of 1 mL min^{-1} with a linear elution gradient composed of H_2O and acetonitrile (20 min; minute 20 to minute 25: 100% acetonitrile). For every run between 2.5 and 20 µL of a sample at a concentration of 20 mg mL^{-1} was injected. The fractions were detected via a diode array detector. For separation of the crude extract for biological assays a subsequent located fraction collector (Agilent 1100 Series) was used. Four fractionations were dispensed into four 96-well plates each (50µg, 100µg, 200µg and 400µg) and tested against CCRF-CEM cancer cells.

The solvent proportions included:

Minute **0**: **99%** H_2O+0.1% (v/v) tfa (trifluoroacetic acid) **1%** acetinitrile

Minute **1**: **90%** H_2O+0.1% (v/v) tfa (trifluoroacetic acid) **10%** acetinitrile

Minute **20**: **70%** H_2O+0.1% (v/v) tfa (trifluoroacetic acid) **30%** acetinitrile

Minute **22**: **0%** H_2O+0.1% (v/v) tfa (trifluoroacetic acid) **100%** acetinitrile

Minute **25**: **0%** H_2O+0.1% (v/v) tfa (trifluoroacetic acid) **100%** acetinitrile

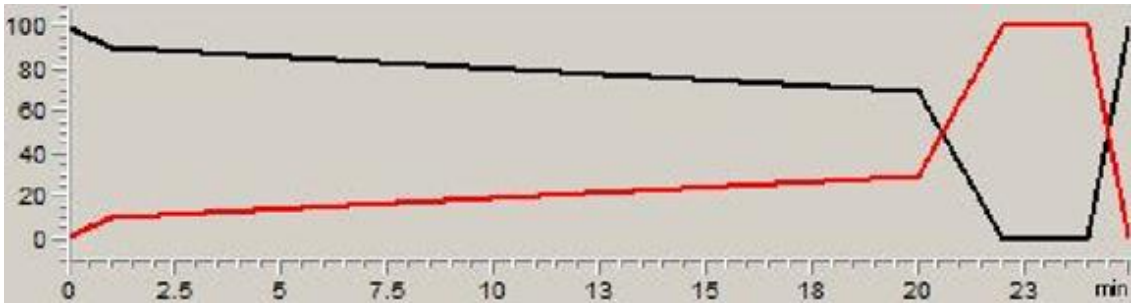

Figure 6.1: HPLC solvent proportions showing linear elution gradient composed of H_2O and acetonitrile.

6.2.4 HPLC-Mass Spectrometry

The molecular weights of selected peaks of the organic extract of *Harungana madagascariensis* Lam.ex poir were determined using an HPLC-MS (Agilent 1260 Series LC and 6130 Series Quadrupole MS System). The mass spectra were recorded using electro spray ionization (ESI) with positive and negative polarization. A Superspher RP 18 (125 × 2 mm; 4 µm, Merck KGaA, Darmstadt, Germany) column was used at 40 °C. For every run between 0.5 and 5 µL of a sample at a concentration of 1-20 mg mL−1 was injected. The elution was performed with a gradient of H_2O + 0.1% (v/v) formic acid and acetonitrile and a flow rate of 0.45 mL min−1.

6.2.5 Fraction Bioactivity assay using Resazurin reduction assay

Resazurin reduction assay was performed according to Kuete *et al* , 2013 to assess the cytotoxicity of the eluted fractions toward various sensitive CCRF-CEM cancer cell line.

6.2.6 Measurement of Reactive Oxygen Species (ROS) by Flow Cytometry.

As reported by Wiench et al, 2012;2, 7-Dichlorodihydrofluorescein diacetate (H2DCFHDA) (Sigma-Aldrich, Germany) is a probe used for the highly sensitive and quantifiable detection of reactive oxygen species (ROS). The nonfluorescent H2DCFH-DA diffuses into the cells and is cleaved by cytoplasmic esterases into 2, 7-dichlorodihydrofluorescein (H2DCF) which is unable to diffuse back out of the cells. In the presence of hydrogen peroxide, H2DCF is oxidized to the fluorescent molecule dichlorofluorescein (DCF) by peroxidases. The fluorescent signal emanating from DCF can be measured and quantified by flow cytometry, thus providing an indication of intracellular ROS concentration (Bass *et al* 1983, Cossarizza *et al* 2009). Briefly, 2×10^6 leukemia CCRF-CEM and CEM-ADR 5000 cells were resuspended in PBS and incubated with 2 μM H2DCFH-DA for 20 min in the dark. Subsequently, cells were washed with PBS and resuspended in RPMI 1640 culture medium containing different concentrations (1/2xIC50, 1xIC50 and 2xIC50) of organic extracts of *Prunus africana (Hook.f.)* kalkman, *Harungana madagascariensis* Lam.ex poir (1A) and *Bridelia micrantha* (Hochst.) Baill (5A) or DMSO (solvent control), H_2O_2 (Sigma-Aldrich, Germany) as a positive control and unstained cells as a negative control in 12 well plates. After 24 hours of incubation, cells were washed and suspended in PBS. Subsequently cells were measured in a FACS Calibur flow cytometer (Becton-Dickinson, Germany) as per Wiench *et al*, 2012.

6.2 RESULTS

6.2.1 HPLC CHROMATOGRAMS

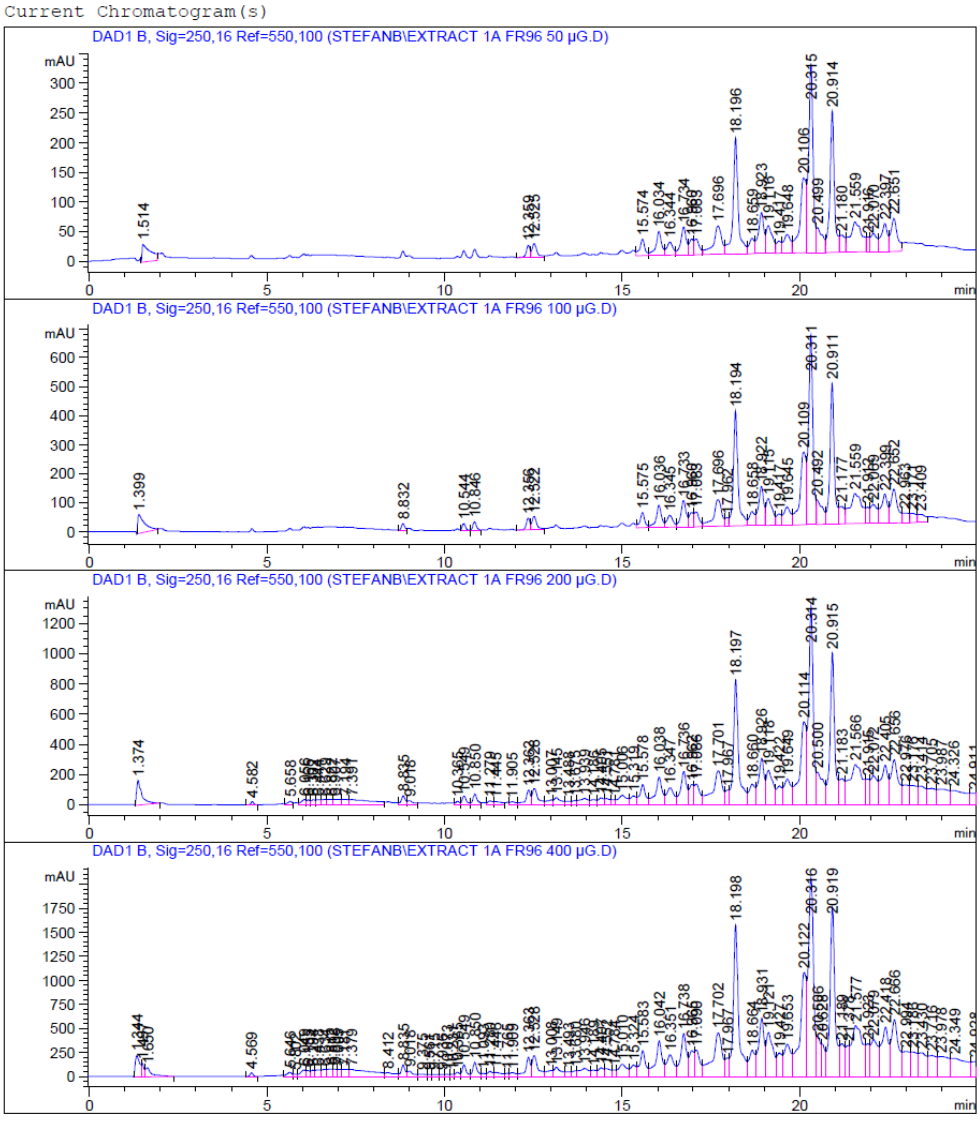

Figure 6. 1 HPLC Chromatograms for the organic extract of *Harungana madagascariensis* Lam.ex poir showing its different fractions peaks of the possible bioactive compounds.

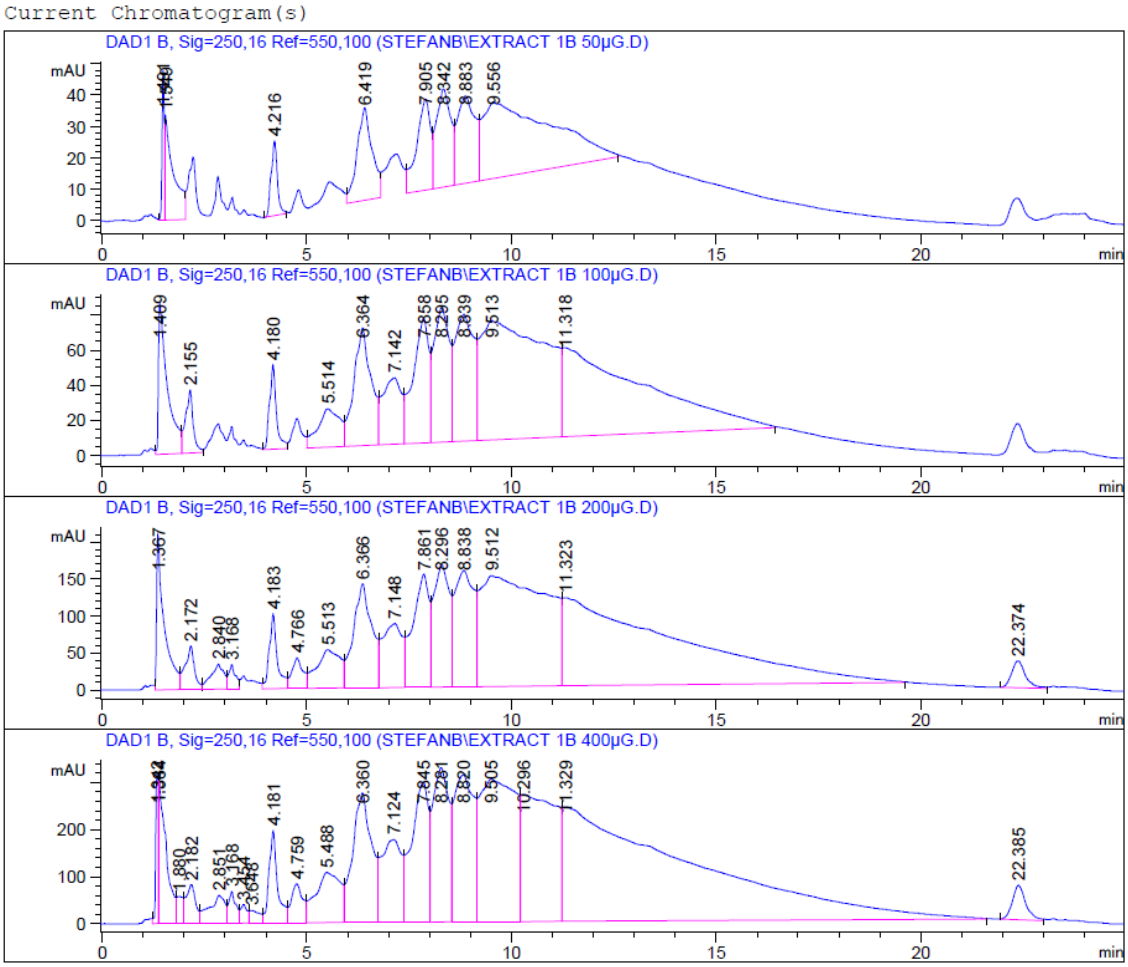

Figure 6.2 HPLC Chromatograms for the aqueous extract of *Harungana madagascariensis* Lam.ex poir showing its different fractions peaks of the possible bioactive compounds

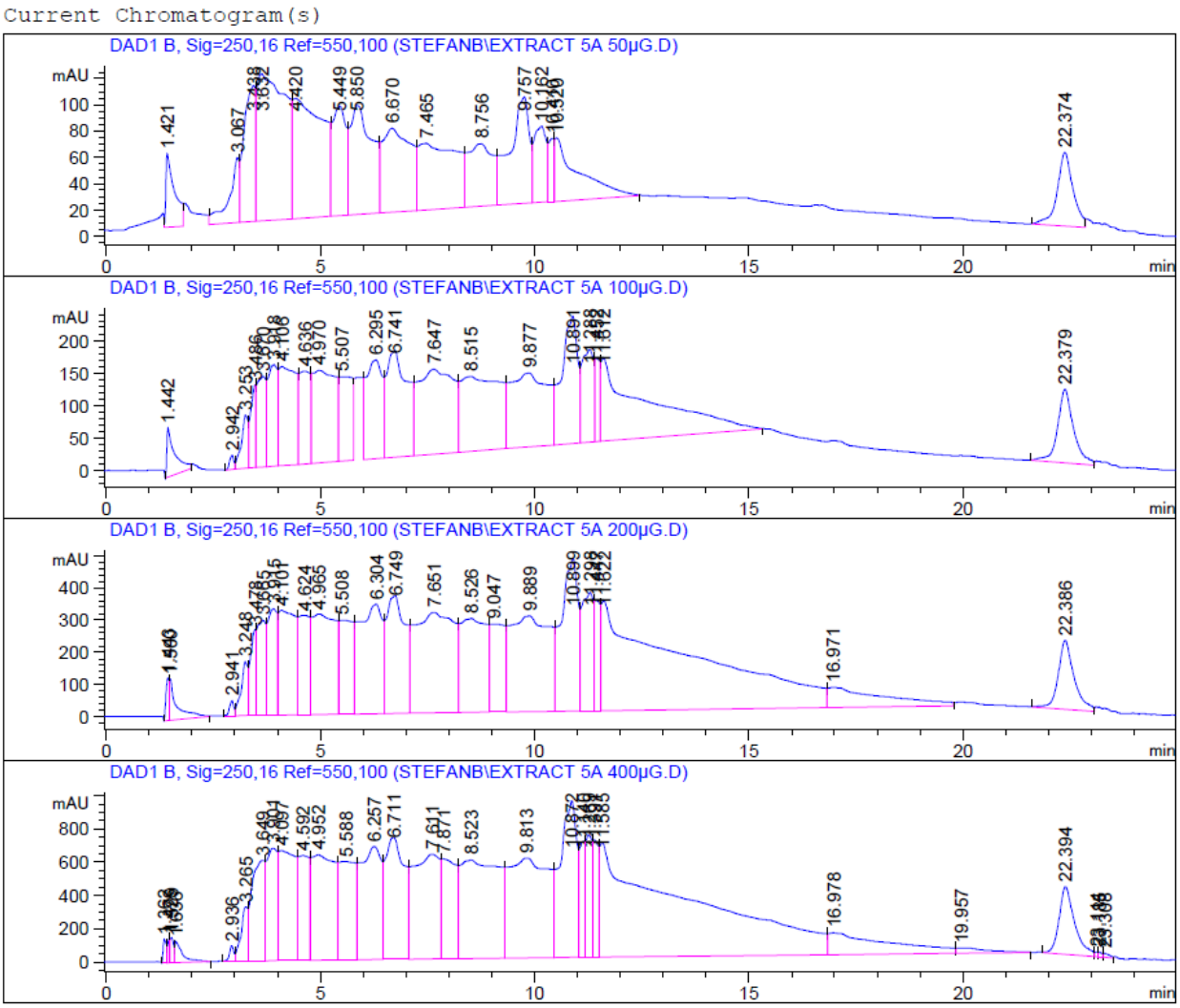

Figure 6.3 HPLC Chromatograms for the organic extract of *Bridelia micrantha (Hochst.) Baill*. showing its different fractions peaks of the possible bioactive compounds

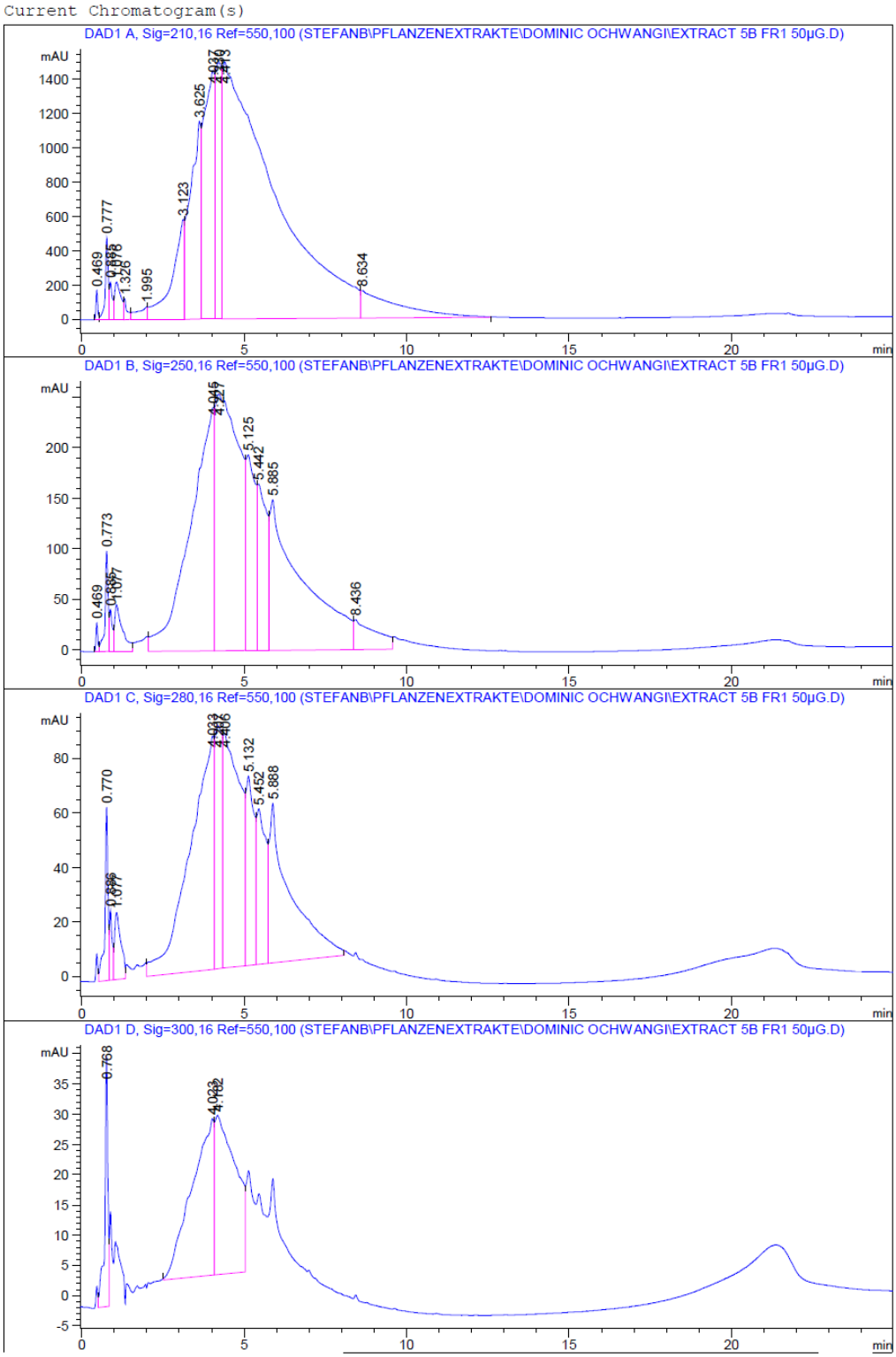

Figure 6.4 HPLC Chromatograms for the aqueous extract of *Bridelia micrantha (Hochst.) Baill*. showing its different fractions peaks of the possible bioactive compounds

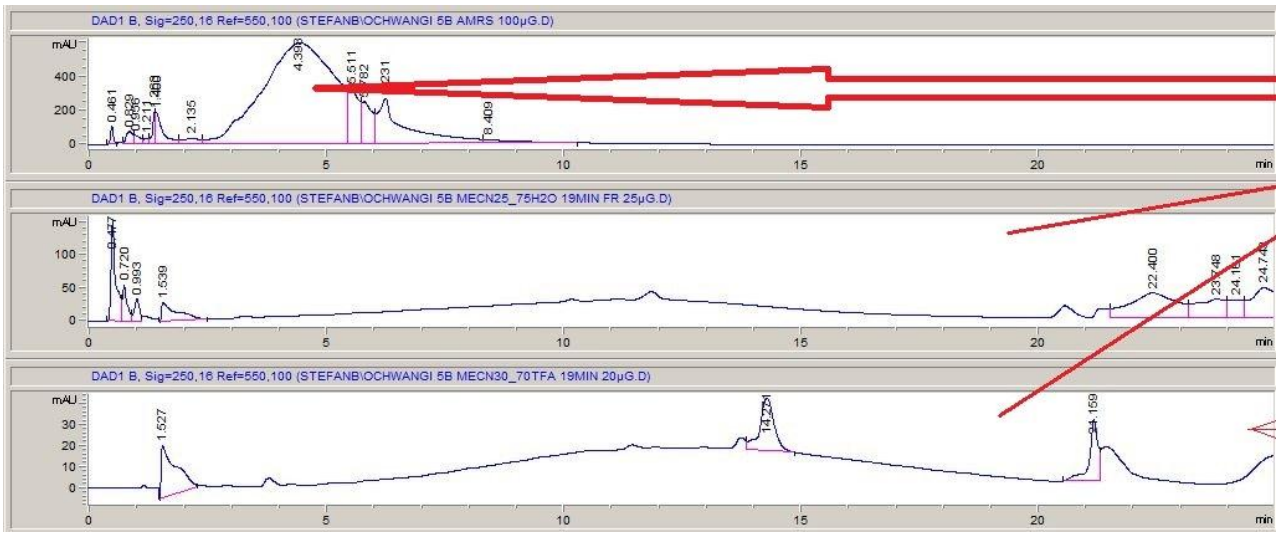

Figure 6.5 HPLC Chromatograms for the aqueous extract of *Bridelia micrantha (Hochst.) Baill*. showing better separation showing its different fractions peaks of the possible bioactive compounds

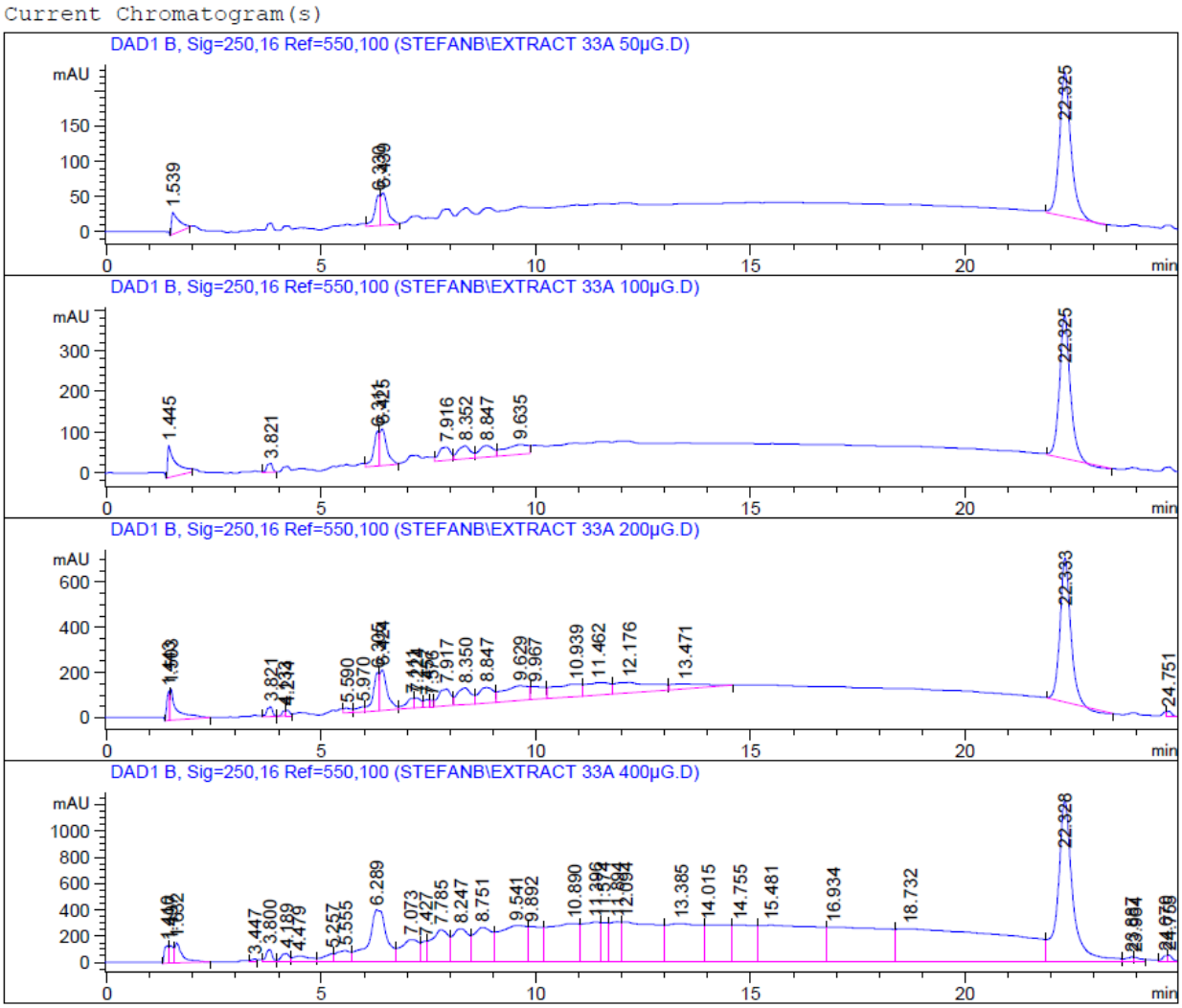

Figure 6.6 HPLC Chromatograms for the organic extract of *Prunus africana (Hook.f.) kalkman* showing its different fractions peaks of the possible bioactive compounds

6.2.2 RESAZURIN ASSAY RESULTS FOR HPLC FRACTIONS

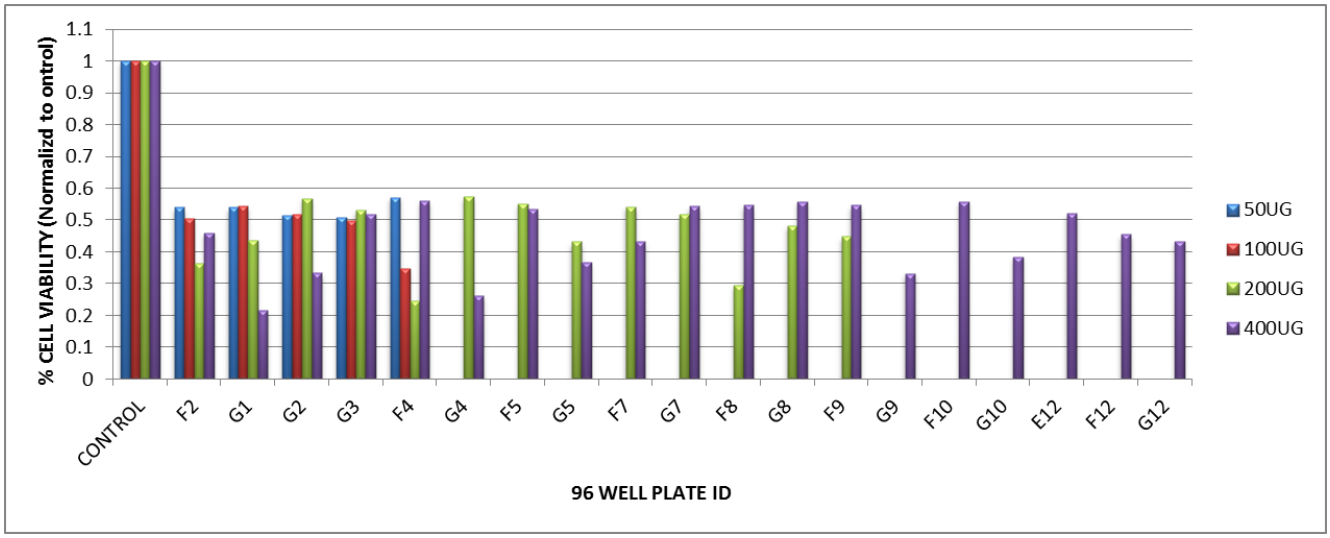

Figure 6.7 Bioactivity resazurin assay results for 96 well plate HPLC fractions of organic extract of *Harungana madagascariensis* Lam.ex poir against CCRF cells.

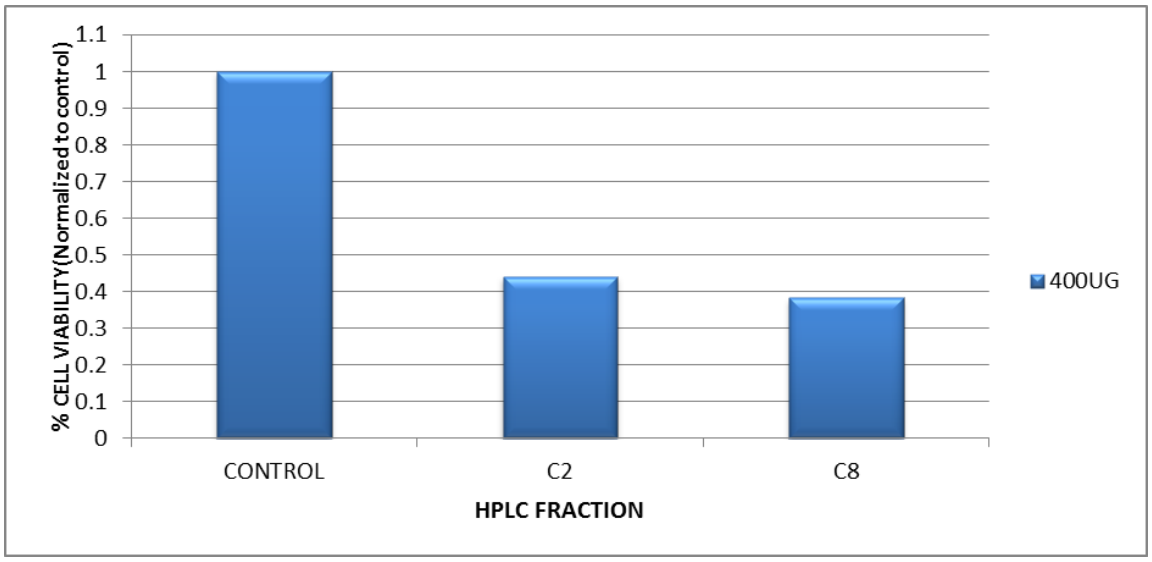

Figure 6.8 Bioactivity resazurin assay results for 96 well plate HPLC fractions of aqueous extract of *Harungana madagascariensis* Lam.ex poir against CCRF cells showing fractions with % cell viability less than 50%.

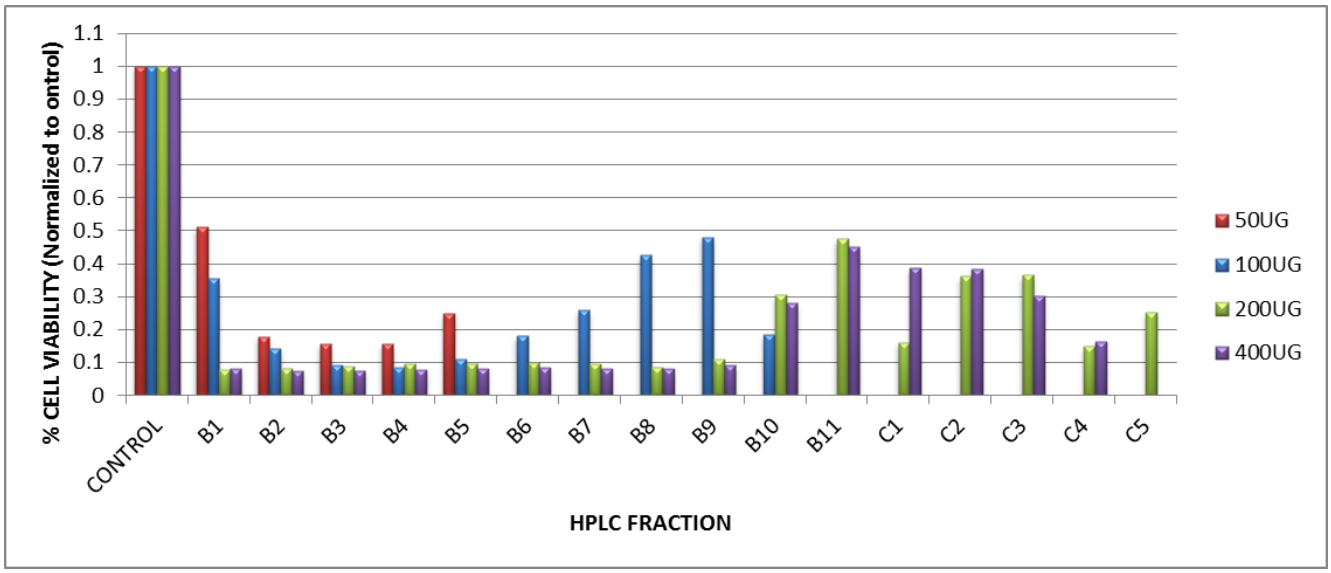

Figure 6.9 Bioactivity resazurin assay results for 96 well plate HPLC fractions of aqueous extract of *Bridelia micrantha (Hochst.) Baill.* against CCRF cells showing fractions with % cell viability less than 50%.

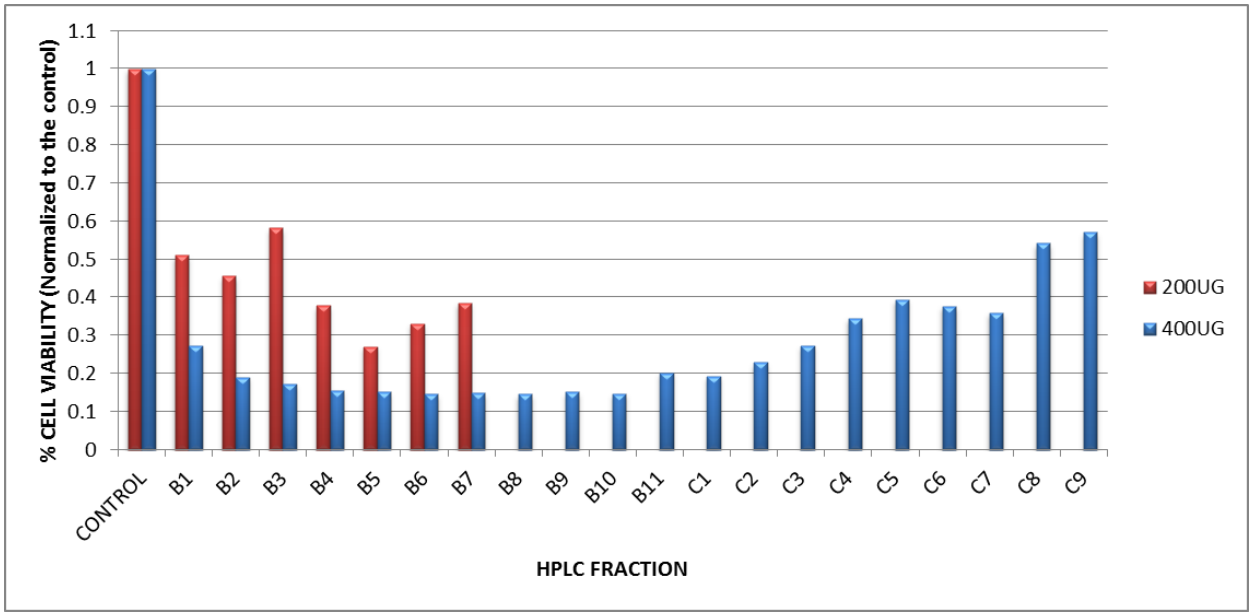

Figure 6.10 Bioactivity resazurin assay results for 96 well plate HPLC fractions of organic extract of *Bridelia micrantha (Hochst.) Baill.* against CCRF.

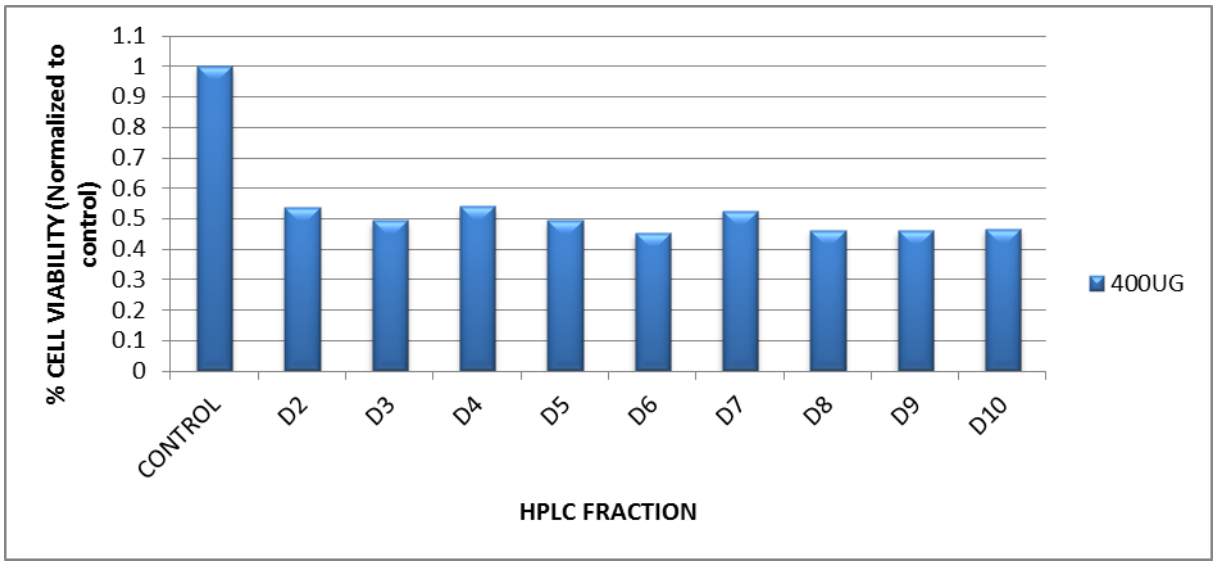

Figure 6.11 Bioactivity resazurin assay results for 96 well plate HPLC fractions of organic extract of *Prunus africana (Hook.f.) kalkman* against CCRF cells showing % cell viability.

6.2.3 HPLC-MS RESULTS

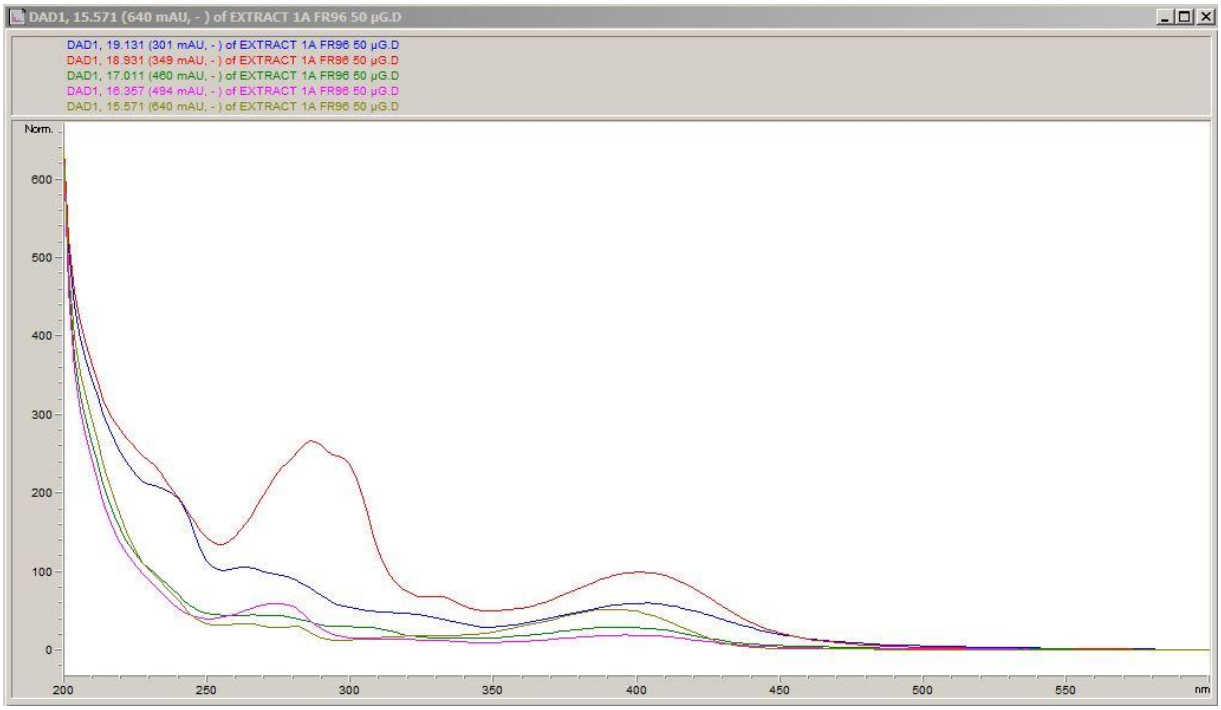

Figure 6.12 UV spectra of compounds of 5 most active fractions of Extract 1A (*Harungana madagascariensis* Lam.ex poir) (Fraction chemical formula identities haven't been established yet)

Table 6. 5 HPLC-MS results of five of the most active fractions of the organic extract of *Harungana madagascariensis* Lam.ex poir (The chemical identity of the fractions hasn't been established yet.)

	Retention time	Mass
Fraction F10	15.57 min	424
Fraction F7	16.35 min	Not detectable
Fraction F4	17.01 min	356
Fraction G4	18.93 min	Not detectable
Fraction G5	19.13 min	438 or 456

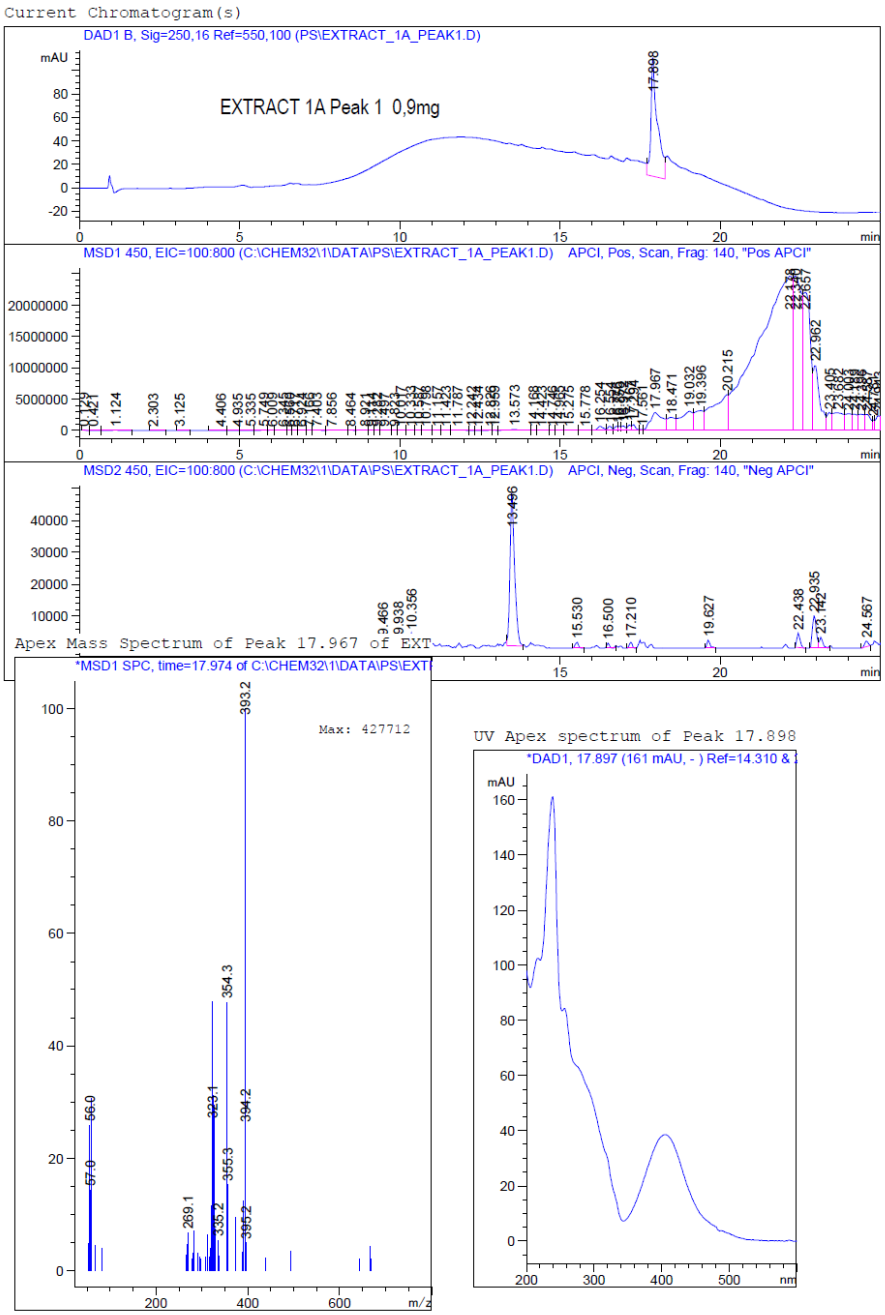

Figure 6.13 Mass spectra of the organic extract of *Harungana madagascariensis* Lam.ex poir showing peak 1 with retention time of 17.898 of the possible bioactive compounds of fraction F1of the 96 well assignment (appendix 2) in extract 1A.

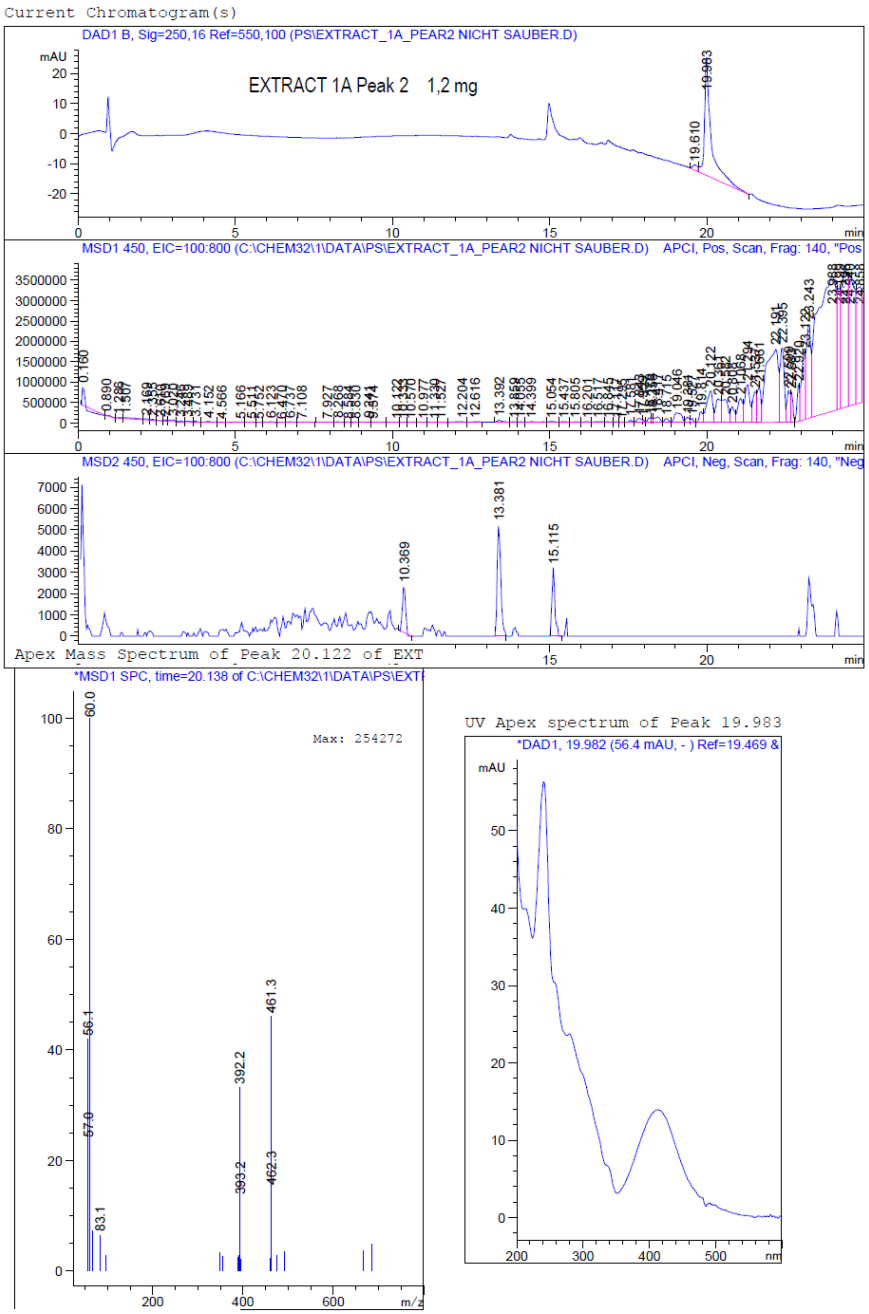

Figure 6.14 Mass spectra of the organic extract of *Harungana madagascariensis* Lam.ex poir showing peak 2 with a retention time of 19.983 of the possible bioactive compound of fraction G8 of the 96 well assignments (appendix 2) in extract 1A

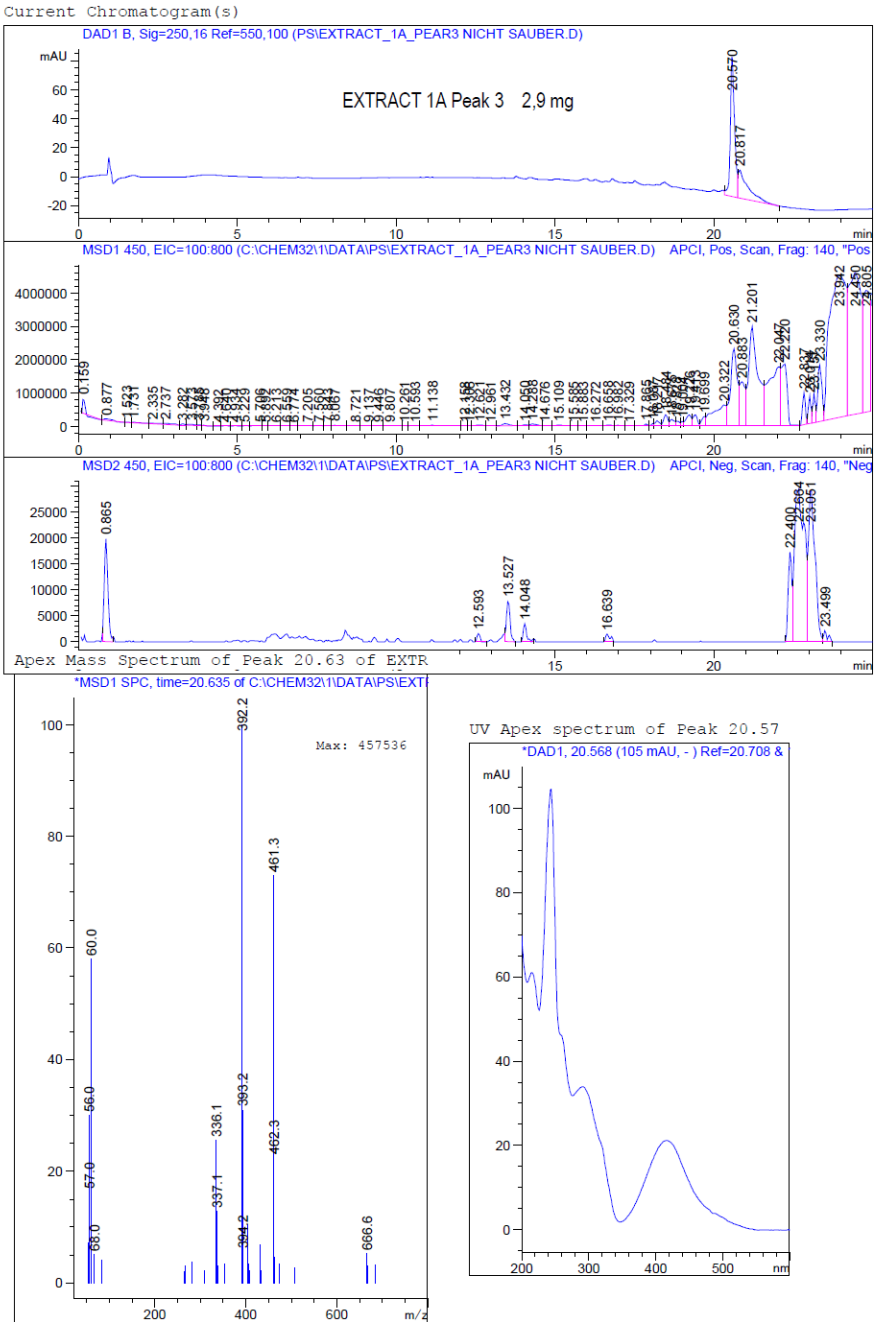

Figure 6.15 Mass spectra of the organic extract of *Harungana madagascariensis* Lam.ex poir showing peak 3 of retention time 20.57 of fraction H10 in the 96 well assignments (appendix 2) of extract 1A of the possible bioactive compound.

6.2.4 ROS RESULTS

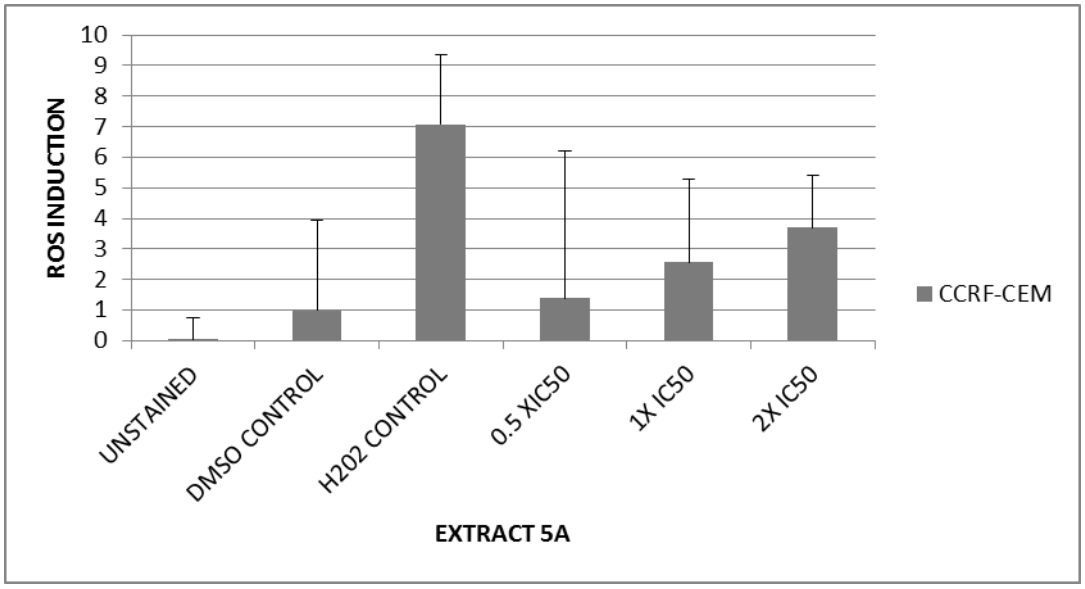

Figure 6.16. Statistical quantification of ROS induction after *Bridelia micrantha (Hochst.) Baill*. (5A) treatment in CCRF-CEM cells. Data points represent mean (fold change) ± SEM of at least three independent experiments. Measurements normalized to the control.

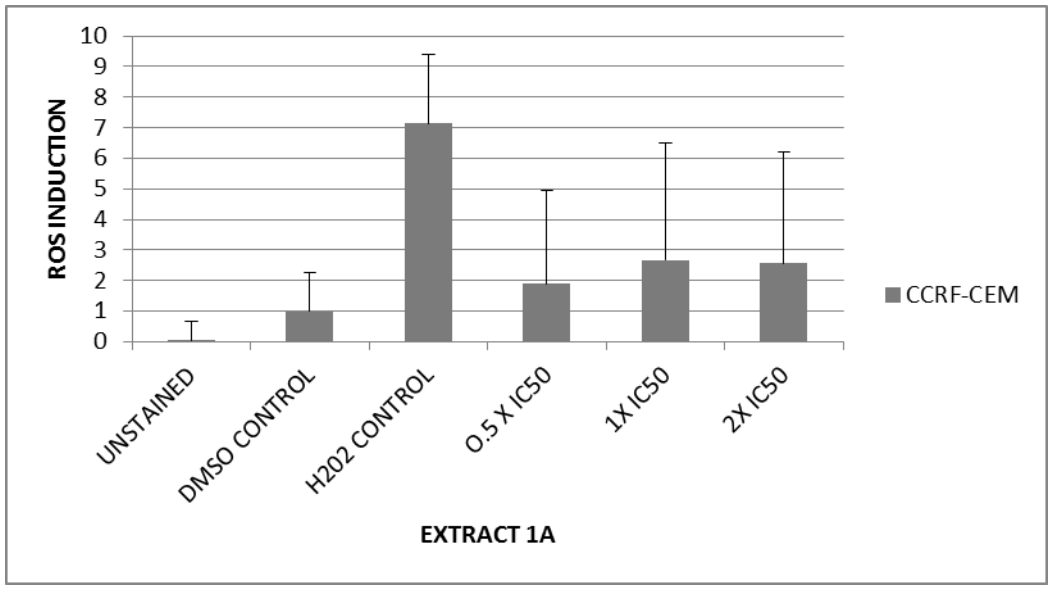

Figure 6.17 Statistical quantification of ROS induction after *Harungana madagascariensis* Lam.ex poir (1A) treatment in CCRF-CEM cells. Data points represent mean (fold change) ± SEM of at least three independent experiments. Measurements normalized to the control.

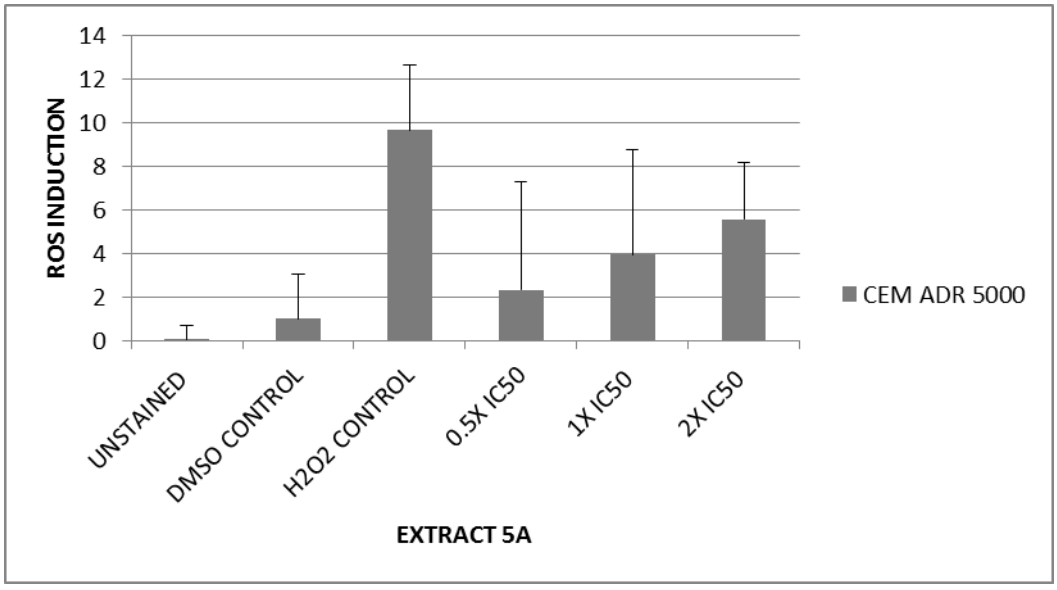

Figure 6.18 Statistical quantification of ROS induction after *Bridelia micrantha (Hochst.) Baill.*(5A) treatment in CEM ADR 5000 cells. Data points represent mean (fold change) ± SEM of at least three independent experiments. Measurements normalized to the control.

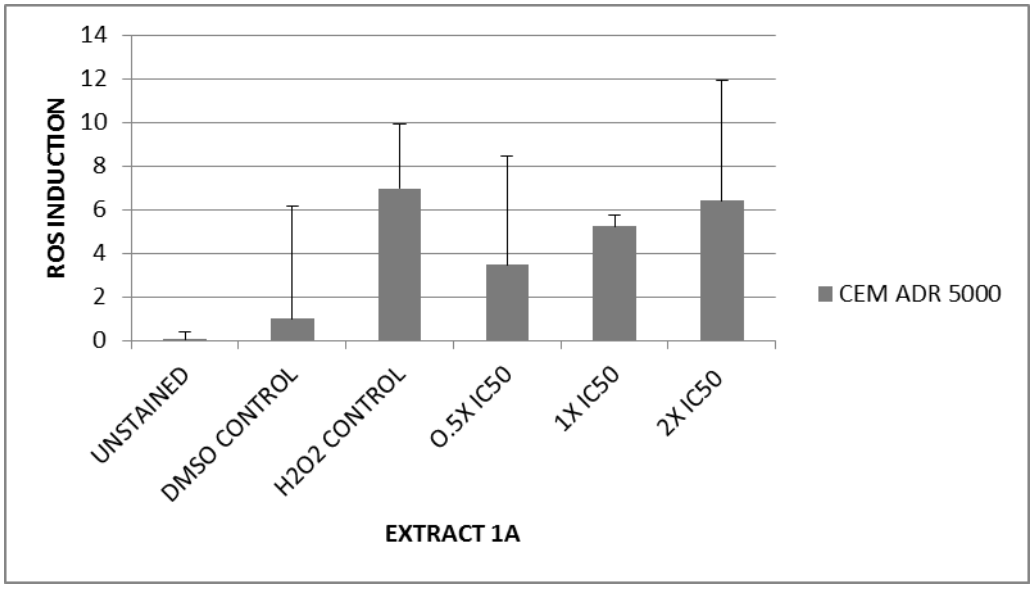

Figure 6.19 Statistical quantification of ROS induction after *Harungana madagascariensis* Lam.ex poir (1A) treatment in CEM ADR 5000 cells. Data points represent mean (fold change) ± SEM of at least three independent experiments. Measurements normalized to the control.

6.4 DISCUSSION

We were able to isolate three different compounds from the extract *Harungana madagascariensis* Lam.ex poir however all the active peaks/region had the same UV/vis spectrum (Figure 6.3 and 6.4). Bioassay results of aqueous extract of *Bridelia micrantha (Hochst.) Baill.* implied that there are many bioactive compounds within the extract that we fractionated with a new method (Figure 6.5 and Figure 6.6) that gave better results (Figure 6.7).We tried to separate the accumulation of different peaks with and without acid as the mobile phase however some peaks disappeared behind the smeary long peak (from minute 5 to minute 20) (Figure 6.7).This can however be re-extracted with something more lipophilic in order to hopefully get rid of the long smeary peak and then test the fractionated new extract for biological activity. From the UV chromatograms, the peak/substance responsible for bioactivity in the assay was determined e.g. well F10 had activity and from the 96 well assignment we can cross-reference which retention time it is and compare it with *Harungana madagascariensis* Lam.ex poir fractionations. The UV spectrum of each for bioactivity responsible peak within the corresponding fractions/wells can be determined. The present study determined that the "active peaks" (compounds) within the extract are very small unfortunately not the big ones and there are many other peaks directly next to them, which meant that a purification seems to will be very difficult. However results at 400µg showed a little bit weaker but also a bioactivity in the wells G2, G10 and G12 (Table 6.1) with the compounds of the big peaks. It would be easier to purify them also because they have more

characteristic UV spectra (Figure 6.3). HPLC-MS results showed the masses of the main compounds in the 5 most active; 50µg fractions (Table 6.6) wells F4, F7, F10, G4 and G5).

Resazurin assay results for 96 well plate HPLC fractions of organic extract of *Harungana madagascariensis* Lam.ex poir against CCRF cells showed less than 50% cell viability from 200 µg fractions of wells G1(36%),G2(43%),G4 (24%),F8 (43%), G9 (29%), F10 (48%) and G10 (44%).Significant cytotoxicity was also seen with 400 µg fractions F2 (45%),G1 (21%),G2 (33%),G4 (26%),G5 (36%),F7 (43%),G9 (33%),G10 (38%),F12 (45%) and G12 (43%) (Figure 6.7).Aqueous extract of *Harungana madagascariensis* Lam.ex poir showed cytotoxic activity against CCRF cells in 400 µg fractions of C2 (44%) and C8 (38%) (Figure 6.8). Rezasurin assay results of aqueous HPLC fractions of *Bridelia micrantha* (Hochst.)Baill showed less than 50% CCRF cell viability in 50 µg fractions B7 (17%), B8 (15%), B9 (15%) and B10 (24%).100 µg fractions showed activity in B2 (35%),B3 (14%),B4 (9%),B5(8%),B6(11%),B7(18%),B8(25%),B9 (42%),B10 (47%) and B11 (18%).200 µg fractions showed activity in B1(7%),B2(8%),B3 (8%),B4 (9%),B5 (9.6%),B6(9.9%),B7 (9.4%),B8(8.6%),B9(10%),B10 (30%),B11(47%),C1 (15%),C2 (36%),C3 (36%),C4(14%) and C5(25%).400 µg fractions showed activity in HPLC fractions B1(8.2%),B2 (7.4%),B3 (7.5%),B4(7.8%),B5(8%),B6(8.3%),B7(8.1%),B8(8%),B9(9.1%),B10(28%),B11(45%),B12 (38%)C1(38%),C2 (30%) and C3 (16%) (Figure 6.9) Rezasurin assay results of organic HPLC fractions of *Bridelia micrantha* (Hochst.)Baill showed less than 50% CCRF cell viability in 200 µg of B5 (45%), B7 (37%), B9(27%) and B10(38).400 µg fractions showed less than 50% in fractions of B1(27%),B2(19%),B3(17%),B4(15),B5(15%),B6 (14%),B7(14%),B8(14%),B9(15%),B10(14%),B11(20%),C1(19%),C2 (22%),C3(27%),C4 (34%),C5 (39%),C6 (37%) and C7 (36%) (Figure 6.10).

Rezasurin assay results of HPLC fractions of organic extracts of *Prunus africana* (Hook.f.) showed less than 50% CCRF cell viability in 400 µg of D3 (49%),D5(49%),D6(45%),D8(46%),D9 (46%) and D10 (46%) (Figure 6.11).

The present study also investigated the possible primary cellular mechanism of *Harungana madagascariensis* Lam.ex poir and *Bridelia micrantha (Hochst.) Baill.* cytotoxicity. Excessive ROS accumulation, leads to induction of apoptosis (Criddle *et al*, 2006). "The elevated levels of ROS strain the mitochondria, leading to a breakdown of the mitochondrial membrane potential and finally to the release of proapoptotic compounds and thus the activation of caspases involved in the intrinsic pathway of apoptosis. The oxidative DNA damage detected is also a consequence of the elevated ROS production and could likely be the trigger a cell-cycle arrest", (Jackson and Bartek, 2009).There was a fold-increase of ROS induction by extract of *Bridelia micrantha (Hochst.) Baill.* and *Harungana madagascariensis Lam.ex poir* against CCRF-CEM cancer cells with $2xIC_{50}$ having an elevated induction comparatively (Figure 6.15 and Figure 6.16).Results also show a fold-increase of ROS of the two extracts against MDR CEM ADR 5000 with $2xIC_{50}$ recording one comparable to the positive control H_2O_2.This gives a good indicator of the potential of the candidate herbal extract.For ROS studies the whole extacts was used an not the fractions from HPLC-MS.

6.5 CONCLUSION

The present study provided an analysis of the various biochemical fractions of extracts of *Harungana madagascariensis* Lam.ex poir, *Bridelia micrantha (Hochst.) Baill and Prunus africana (Hook.f.) kalkman* and their activity against sensitive CCRF-CEM cancer cells and provides an insight for further exploration of these medicinal plants for therapeutic discovery. ROS results explain the possible primary cellular mechanism of the extracts of *Harungana*

madagascariensis Lam.ex poir and *Bridelia micrantha (Hochst.) Baill.* leading to cytotoxicity.More assays to elucidate the primary functions is also encouraged. Further work using Nuclear magnetic resonance (NMR) needs to be done to determine the possible molecular formula of the bioactive compounds in the extracts.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION AND CONCLUSION

The present study investigated the pharmacophysiological potential of medicinal plants used for management and treatment of cancer in Kakamega County, Kenya. It identified 65 plants belonging to 59 genera and 34 families from the ethnobotanical survey.The study area was selected due to the high biodiversity capacity of the Kakamega Tropical Rainforest and leading to availability of diverse flora and subsequent use in traditional ethnomedicine. Several previous *in vitro* and *in vivo* studies reported that some of these plants possessed strong anticancer and cytotoxic effects.Fleshy or dried leaves were used most frequently by a majority of TMPs interviewed suggesting that these components of the plant contained the highest concentration of the active phytochemical compounds.Sustainable methods of harvesting were used by the THMPs to protect the plants from destruction and overutilization.It is important to note that the oral route was the most commonly used route of administration for the plant formulations of which were used as a combinational therapy.Thirty five medicinal plants that were mentioned by a majority of respondents were air died,ground and extracted using dichlomethane,methanol and water.Phytochemical analysis of the organic extracts was conducted using TLC revealing the presence of

phytochemicals such as alkaloids, anthraquinones, xanthines, valepotriates, cardioactive glycosides,flavonoids,essential oils,coumarins,lignans,saponins and arbutin drugs.Most of this compounds have been shown in other studies to be responsible for different biological properties including anticancer ((Han *et al*, 2007). The present study identified phytochemical compounds that form a valuable pool of bioactive constituents that should be isolated, purified and characterized.Cytotoxicity screening of thirty four organic and nineteen aqueous plant extracts was done against sensitive and multi-drug resistant cancer cell lines with a single concentration of 40 μg/mL for each plant sample.

23% of the medicinal plants showed less than 50% growth proliferation of sensitive CCRF-CEM leukemia cancer cells. The organic extracts include *Harungana madagascariensis* Lam.ex poir (6.56%), *Prunus africana* (Hook.f.) kalkman (19.43%), *Entada abyssinica* Steud.ex A.Rich. (38.69%), *Phyllanthus fischeri* Pax, (40.78%), *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax (41.84%), *Bridelia micrantha* (Hochst.) Baill (45.41%), Futumia *africana* Benth. (45.88%) and *Microglossa pyrifolia* (Lam.) Kuntze (48.06%).The aqueous extracts include *Bridelia micrantha* (Hochst.) Baill (31.32%) and *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax (48.29%).Four aqueous extracts and eight organic extracts showed less than 50% growth proliferation of CEM/ADR5000 cells .These include aqueous extracts of *Shirakiopsis elliptica* (Hochst.) Esser Synonym: *Sapium ellipticum* (Hochst.kraus) Pax (46.19%), *Prunus africana* (Hook.f.) kalkman (33.29%), *Harungana madagascariensis* Lam.ex poir (20.74%) and *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (13.93%).Organic extracts include those of Olea hotch spp. Hochstetteri (42.45%), *Albizia gummifera* (J.F. Gmel.) (42.35%), *Microglossa pyrifolia* (Lam.) Kuntze (27.90%), *Synsepalum cerasiferum*

Synonym: *Afrosersalisia cerasifera* (Welw.) Aubrev. (25.52%), *Bridelia micrantha* (Hochst.) Baill (9.38%), *Fuerstia africana* T.C.E. Fr. (9.18%), *Prunus africana* (Hook.f.) kalkman (8.32%), *Harungana madagascariensis* Lam.ex poir (5.09%) which showed a dramatic cytotoxicity. Organic extracts of *Prunus africana* (Hook.f.) kalkman (44.19%) and *Harungana madagascariensis* Lam.ex poir (17.89%) showed less than 50% growth proliferation of MDA-MB231 cells with *Zanthoxylum rubescens* Hook. f (40.55%), *Bridelia micrantha* (Hochst.) Baill (23.98%), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (11.83%), *Harungana madagascariensis* Lam.ex poir (6.93%) showing less than 50% growth proliferation of MDA-MB231/ BCRP cells. Organic extracts of *Microglossa pyrifolia* (Lam.) Kuntze (49.16%), *Bridelia micrantha* (Hochst.) Baill (40.69%), *Prunus africana* (Hook.f.) kalkman (32.63%) and *Harungana madagascariensis* Lam.ex poir (19.54%) and aqueous extract of *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (44.87%) showed less than 50% growth proliferation of HEK-293 cells. Organic extracts of *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (43.23%), *Prunus africana* (Hook.f.) kalkman (31.29%), *Bridelia micrantha* (Hochst.) Baill (24.91%) and *Harungana madagascariensis* Lam.ex poir (15.15%) showed less than 50% growth proliferation of U87MG cells. Organic extracts of *Fuerstia africana* T.C.E. Fr.(39.84%), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (28.84%), *Bridelia micrantha* (Hochst.) Baill (11.31%) and *Harungana madagascariensis* Lam.ex poir (7.48%) showed less than 50% growth proliferation of U87MG.ΔEGFR cells.Organic extracts of *Prunus africana* (Hook.f.) kalkman (46.73%), *Fuerstia africana* T.C.E. Fr. (45.04%), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (40.32%), *Phyllanthus fischeri* Pax (34.79%), *Microglossa pyrifolia* (Lam.) Kuntze (29.09%), *Bridelia micrantha* (Hochst.) Baill (14.40%) and *Harungana madagascariensis* Lam.ex poir (10.56%) showed less than 50% growth

proliferation of HCT116 (p53−/−) cells. Organic extracts of *Zanthoxylum gilletii* (De Wild.) P.G. Waterman (45.62%), *Prunus africana* (Hook.f.) kalkman (40.41%), *Fuerstia africana* T.C.E. Fr. (30.86%), *Microglossa pyrifolia* (Lam.) Kuntze (30.11%), *Phyllanthus fischeri* Pax (25.42%), *Bridelia micrantha* (Hochst.) Baill (23.56%) and *Harungana madagascariensis* Lam.ex poir (5.22%) showed less than 50% growth proliferation of HCT116 (p53+/+).

The IC_{50} values of samples showing most effective cytotoxic activity were determined on a set of eight cancer cell lines, including both sensitive and MDR phenotypes. *Prunus africana* (Hook.f.) kalkman extract inhibited the proliferation of seven out of eight tested cancer cell lines, with IC_{50} values below 40 μg/mL while *Harungana madagascariensis* Lam.ex poir extract inhibited 6/8 of the cells tested. IC_{50} values were obtained on 4/8 tested cells lines for the aqueous extract of *Bridelia micrantha* (Hochst.) Baill, 2/8 for its organic part; 2/8 for *Microglossa pyrifolia* (Lam.) Kuntze and *Fuerstia africana* T.C.E. Fr.,3/8 for *Phyllanthus fischeri* Pax and 1/8 for *Zanthoxylum gilletii* (De Wild.) P.G. Waterman and *Entada abyssinica* Steud.ex A.Rich. All the screening results were compared to Doxorubicin, a standard anticancer drug.

The present study indicates that some of the tested medicinal plants have a lot of potential as anticancer agents. We investigated both sensitive and MDR cell lines and calculated the degrees of resistanceindicative of the sensitivity of cancer cell lines to the candidate herbal extracts. The present study tested cell lines overexpressing two ATP-binding cassette transporters, i.e. P-glycoprotein (ABCB1/MDR1) or breast cancer resistance protein (ABCG2/BCRP). Furthermore, we tested a p53 knockout cell line and a transfectant cell line harboring a mutation- activated EGFR gene (ΔEGFR) as examples for resistance- inducing tumor suppressors and oncogenes. Collateral sensitivity (sample more active on resistant cells

than on sensitive cells) was observed with the extract of aqueous extract of *Bridelia micrantha* (Hochst.) Baill**,** organic extracts of *Prunus africana* (Hook.f.) kalkman and *Fuerstia africana* T.C.E. Fr. against CEM/ADR5000 showing their good antiproliferative activity. The cytotoxicity of the extracts in the present study used in Kakamega County, Kenya is being reported for the first time. *Harungana madagascariensis* Lam.ex poir cytotoxic activity shows great potential against sensitive and MDR cell lines. *H. madagascariensis* has been shown to have both analgesic and anti-inflammatory properties (Nwodo, 1989). "Similarly, the plant inhibits the activity of G-glucosidase and was found to have antioxidant properties (Kouam et al., 2006a, 2006b). Kouam et al. (2007) have isolated a prenylated 1, 4-anthraquinone from the hexane extract of the stem-bark of *H. madagascariensis* and have shown it to possess G-glucosidase inhibition and antioxidant activities. However anticancer activity of *H. madagascariensis* has not been documented from the specific study region. Extracts from the stem of *H. madagascariensis* stimulates NO release and this may be a mechanism whereby the constituents of the plant elicit its therapeutic effects in herbal medicine. This study may have relevance in hemostasis, thrombosis and cancer chemotherapy", (Iwalewa *et al*; 2009). *Prunus africana* bark whose bark was discovered to be effective in the management of benign prostatic hyperplasia 35 years ago. The extract is formulated and sold as capsules (Pygeum africanum) by pharmaceutical companies mainly in Europe. This has led to increased harvesting to the extent that it was declared endangered by the Convention of International Trade in Endangered Species (CITES) in 1995.In the present study, *Prunus africana* stem bark extract showed good cytotoxicity activity against various cancer cell lines.The present study reported that TMPs never use the medicinal plants as single entities for treatment and management of

cancer but use combinations in equal ratios of the extracts to take advantage of the synergistic effects. The present study combined the extracts in equal ratios; weight: volume simulating what is done in the field and screened them against sensitive cancer cells CCRF-CEM. The present study showed excellent results and great potential of combinational screening, some of whose cytotoxicity was better that Doxorubicin which is a standard anticancer drug. Marked synergistic cytotoxicity were seen with combinations of aqueous extracts of *Harungana madagascariensis* Lam.ex poir and *Prunus africana* (Hook.f.) kalkman (9.95%) compared to their single activity of 51.14% and 66.31% respectively. Different combinations of extracts of *Harungana madagascariensis* Lam.ex poir, *Spathodea campanulata* P.Beauv. ssp. nilotica (Seem), *Zanthoxylum gilletii* (De Wild.) P.G. Waterman, *Prunus africana* (Hook.f.) kalkman and *Bridelia micrantha* (Hochst.) Baill. showed additive cytotoxic synergy compared to single entities. These results validate the use of these plants by TMPs who use most of them in combinations. Organic extract combinations even showed a more dramatic effect especially with *Harungana madagascariensis* Lam.ex poir that showed better cytotoxicity compared to the standard anticancer drug Doxorubicin showing cell viability of less than 6%. This provides an excellent candidature for therapeutic development. These findings further validates the culture of TMPs but advices the use of better extraction methods because the organic extracts have better activity compared to aqueous extracts of the same plant. Organic plant extracts combinations of *Spathodea campanulata* P.Beauv. ssp. nilotica (Seem),*Conyza sumatrensis* (Retz.) E.H Walker, *Microglossa pyrifolia* (Lam.) Kuntze and *Juniperus procera* Endl. extracts showed less than 50% growth proliferation of MDA-MB-231-pcDNA3 (49.59%), MDA-MB-231-BCRP clone 23 (35-69%), HCT116 (p53−/−) (33.46%), ADR-CEM 5000 (30.45%) and HCT116 (p53+/+) (27.37%) cancer cell lines. The

present study encourages the use of pulverized extracts that have been combined as the best way forward for TMPs. These combinations have a great potential in therapeutic studies of anticancer potential of medicinal plants of Kakamega County, Kenya.The present study shows the *in vitro* cytotoxic anticancer potential of thirty four organic and nineteen aqueous extracts of medicinal plants used in Kakamega County, Kenya. Some of the aqueous and organic extracts have shown good activity against both sensitive and MDR cancer cell lines. This forms a potential pool for further exploration. HPLC and Mass Spectrometry of organic extract of *Harungana madagascariensis* Lam.ex poir and aqueous extract of *Bridelia micrantha (Hochst.) Baill.* showed that there are many bioactive compounds within the extracts. The present study also investigated the possible primary cellular mechanism of the two extracts by ROS induction assay. There was a fold-increase of ROS induction by extract of *Bridelia micrantha (Hochst.) Baill.* and *Harungana madagascariensis Lam.ex poir* against CCRF-CEM and MDR CEM ADR 5000 cancer cells. This evidence based analysis of the various biochemical fractions of extracts of *Harungana madagascariensis* Lam.ex poir, *Bridelia micrantha (Hochst.) Baill and Prunus africana (Hook.f.) kalkman* and their activity against sensitive CCRF-CEM cancer cells provides an insight for further exploration of these medicinal plants for therapeutic discovery. ROS results explain the possible primary cellular mechanism of the extracts of *Harungana madagascariensis* Lam.ex poir and *Bridelia micrantha (Hochst.) Baill.* leading to cytotoxicity. Further work using Nuclear magnetic resonance (NMR) needs to be done to determine the possible molecular formula of the bioactive compounds in the extracts.It is recommended that such validation should be communicated to TMPs who should improve on their practices to make herbal medicine practice effective.

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7.2 PUBLICATIONS

The following manuscripts for publications have been extracted from the different chapters of this thesis:

A paper derived from chapter three has been published; *Dominic O. Ochwang'i ,*, Charles N. Kimwele, Jemimah A. Oduma , Peter K. Gathumbi ,James M. Mbaria ,Stephen G. Kiama .Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya.* ***Journal of Ethnopharmacology****, Vol. 151 Issue 3, Pages 1040-1055, 2014.*

A paper derived from chapter four has been published: Ochwang'i DO, Kimwele CN, Oduma JA, Gathumbi PK, Kiama SG, Thomas Efferth (2016) Phytochemical Screening of Medicinal Plants of the Kakamega Country, Kenya Commonly Used against Cancer. ***Med Aromat Plants (Los Angel)*** *5: 277.doi: 10.4172/2167-0412.1000277*

A paper has been extracted from chapter five and is under review for publication in the ***Journal of Ethnopharmacology***: *Cytotoxic activity of anti-cancer medicinal plants of Kakamega County in Kenya against drug sensitive and multidrug resistant cancer cells. Dominic O. Ochwang'i [a,*], Charles N. Kimwele [b], Jemimah A. Oduma [c], Peter K. Gathumbi [d], Stephen G. Kiama [e], Thomas Efferth [f]*

A paper has been extracted from chapter six and is under consideration for publication: *Bioactivity-guided fractionation and mechanism analysis of Harungana madagascariensis lam.ex poir, Prunus africana (hook.f.) kalkman and Bridelia micrantha (hochst.) baill. Dominic O. Ochwang'i [a,*], Charles N. Kimwele [b], Jemimah A. Oduma [c], Peter K. Gathumbi [d], Stephen G. Kiama [e], Thomas Efferth [f]*

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APPENDICES

APPENDIX 1: DATA ACQUISITION QUESTIONNAIRE

A collaborative RISE AFFNET Natural Product Project by the University of Nairobi in Kenya

A QUESTIONNAIRE FOR HERBAL PLANTS USED FOR TREATMENT OF CANCER IN KAKAMEGA COUNTY, KENYA.

PART ONE: CONSENT

A.RESEARCHER'S DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual property rights of herbal practitioners.

2. We will at no time initiate or conduct practices that are deemed to obtain information from respondents by intimidation, coercion or false pretence.

3. We will be under no obligation to edit or tamper with the information provided by the respondents.

4. The information collected will be used for the described research purpose and not any undisclosed intentions.

Researchers:

1) Dr. Dominic Ochwang'i……..

2) Prof. Charles Kimwele……..

3) Prof. Dr.Jemimah Oduma……..

4) Prof. P.K. Gathumbi……..

5) Prof. Stephen Kiama……

B: RESPONDENTS CONSENT AGREEMENT

I……...hereby agree to participate in this study with my full consent and conscience and declare that to the best of my knowledge the information that I have provided is true, accurate and complete.

Signature/Thumb print.....

PART TWO

A: HERBALIST BIODATA

Enumerator (name)…… Date of interview……

Serial No…….. Name of Respondent……

Division……..Location……Sub location……

Village…… Telephone…… Gender……

(Answer by ticking {√} in the appropriate Box)

1) What is your age?

a) Below 18 years { } b) 18-25 { } c) 26-35 years { } d) 36-45 years { }

e) 46-55 years { } f) Over 55years { }

2) What is your current marital status?

a) Single { } b) Married { } c) Divorced { } d) Widowed { }

3) What is your highest level of education?

a) None { } b) Primary { } c) Secondary { } d) University { } e) other (Please

specify)……

4) What is your religion? ……

5) What is your local language……..

6) What is your professional training? .....

7) Are you employed? a) Yes { } b) No { }

8) If yes what is the nature of employment?.....

9) What is your major source of income? ……..

B. INFORMATION ON TRADITIONAL HERBAL PRACTICE

10) For how long have you practiced as a traditional herbalist?.....

11) Where do you practice as a traditional herbalist (Location)?.....

12) How did you acquire your skills as a herbalist?.....

13) Do you belong to any registered group of herbalists?

a) Yes { } b) No { }

14) If yes what is the name of your group?.....

15) Have you ever treated any cancer condition?

Yes { } No { }

C.KNOWLEDGE OF TRADITIONAL HERBAL PRACTICE

	16. What kind of cancers have you treated? Please list the different cancers you have treated.	17) What symptoms do the patients show?	18) How many cases in the last one year?

1			
2			

19. How do you diagnose cancer traditionally?

……
……
……

20. Which is the first advice you give to the patients after diagnosis? Do they prefer to go to hospital?

……
……

21. Do you use medicinal plants to manage /treat cancer?

Yes { } b) No { }

22) If yes,

i) Was the patient referred to you?.....

ii) Did the cancer reoccur after treatment?.....

iii) If so which one? …….

iv) How long did it take before re-occurrence?.....

D: MEDICINAL PLANTS USED

23) Which plant(s) do you use to treat the cancers you have mentioned? **Please list in order of preference of the plants used.**

Plants treatin	Plant	Form in	Method of	Habitat (Boundar	Route of admin	Mixture	Dosage	Side effects	Availability

g Cancer (Condition one)	part(s) used (roots, stem, leaves, bark, tuber, fruit etc.)	which it is used (powder, boiled etc)	preparation (powder, boiled single, boiled mixture, soaked in water) How much of the plant in how much water?	y marker,bush,crop field,compound	(oral,topical, rectal,inhalation,bathed etc)	(concoction) or single	(how much is given, after how long and for how long	reported/ remedy	of plant(readily or not)
Vernacular name									
1									
2									

i) How often do patients return to you after failure of treatment?.....

ii) How long does it take after consumption of the herbs for the anticancer effect to occur? **Please mention for each plant.**

a) ……..

b) ……

c) ……

d) ……..

iii) If the treatment does not work what do you do?

a) Repeat the treatment……

b) Change the herb used…….

c) Refer to hospital……

iv) How long can you keep the medicine before it goes bad? ……

v) How long does the patient take the medicine before feeling well?.....

vi) Have you had any case of toxicity for any of the plants used? Which plants?……

……..……

……..……

vii) What were the signs of toxicity? How did you attend to the toxicity?……

……

……

……

xii) What problems do you encounter in herbal medicine practice?

……

APPENDIX II: HPLC FRACTION ASSIGNMENT OF 96 WELL PLATES

Position	Fraktionsstart	Fraktionsende
A1	0	0,25
A2	0,26	0,5
A3	0,51	0,75
A4	0,76	1
A5	1,01	1,25
A6	1,26	1,5
A7	1,51	1,75
A8	1,76	2
A9	2,01	2,25
A10	2,26	2,5
A11	2,51	2,75
A12	2,76	3
B12	3,01	3,25
B11	3,26	3,5
B10	3,51	3,75
B9	3,76	4
B8	4,01	4,25
B7	4,26	4,5
B6	4,51	4,75
B5	4,76	5
B4	5,01	5,25
B3	5,26	5,5
B2	5,51	5,75
B1	5,76	6
C1	6,01	6,25
C2	6,26	6,5
C3	6,51	6,75
C4	6,76	7
C5	7,01	7,25
C6	7,26	7,5
C7	7,51	7,75
C8	7,76	8
C9	8,01	8,25
C10	8,26	8,5
C11	8,51	8,75
C12	8,76	9
D12	9,01	9,25
D11	9,26	9,5
D10	9,51	9,75
D9	9,76	10
D8	10,01	10,25
D7	10,26	10,5
D6	10,51	10,75
D5	10,76	11
D4	11,01	11,25
D3	11,26	11,5
D2	11,51	11,75
D1	11,76	12

Position	Fraktionsstart	Fraktionsende
E1	12,01	12,25
E2	12,26	12,5
E3	12,51	12,75
E4	12,76	13
E5	13,01	13,25
E6	13,26	13,5
E7	13,51	13,75
E8	13,76	14
E9	14,01	14,25
E10	14,26	14,5
E11	14,51	14,75
E12	14,76	15
F12	15,01	15,25
F11	15,26	15,5
F10	15,51	15,75
F9	15,76	16
F8	16,01	16,25
F7	16,26	16,5
F6	16,51	16,75
F5	16,76	17
F4	17,01	17,25
F3	17,26	17,5
F2	17,51	17,75
F1	17,76	18
G1	18,01	18,25
G2	18,26	18,5
G3	18,51	18,75
G4	18,76	19
G5	19,01	19,25
G6	19,26	19,5
G7	19,51	19,75
G8	19,76	20
G9	20,01	20,25
G10	20,26	20,5
G11	20,51	20,75
G12	20,76	21
H12	21,01	21,25
H11	21,26	21,5
H10	21,51	21,75
H9	21,76	22
H8	22,01	22,25
H7	22,26	22,5
H6	22,51	22,75
H5	22,76	23
H4	leer	
H3	leer	
H2	leer	
H1	leer	