European Journal of Medicinal Plants
5(4): 366-376, 2015, Article no.EJMP.2015.035
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

P. N. Nabende[1*], S. M. Karanja[1], J. K. Mwatha[2] and S. W. Wachira[3]

[1]College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya.
[2]Centre for Biotechnology Research and Development, Kenya Medical Research Institute, Nairobi, Kenya.
[3]Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, Nairobi, Kenya.

Authors' contributions

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

Article Information

DOI: 10.9734/EJMP/2015/14081
Editor(s):
(1) Thomas Efferth, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Germany.
(2) Marcello Iriti, Faculty of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Fathilah Abdul Razak, Department of Oral Biology & Biomedical Sciences, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia.
(2) Mondher Boulaaba, Centre de Biotechnologie de Borj-Cédria, Laboratoire des Plantes Extremophiles, Tunisie.
(3) Mariano Bizzarri, Department of Experimental medicine, University La Sapienza, Roma, Italy.
(4) Anonymous, Morocco.
(5) Anonymous, Quaid-i-Azam University, Pakistan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=792&id=13&aid=7250

Original Research Article

Received 18th September 2014
Accepted 14th November 2014
Published 15th December 2014

ABSTRACT

Aims: To determine the anti-proliferative activity of *Prunus africana, Warburgia stuhlmannii* and *Maytenus senegalensis* in breast and colon cancer cell lines and to assess their toxicity levels based on responses against Vero cells and the Swiss albino mice.
Study Design: Experimental laboratory-based study.

**Corresponding author: E-mail: simiyu.patricia@gmail.com, swachira2013@gmail.com;*

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

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

1. INTRODUCTION

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

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

For thousands of years, plants and other natural products have been used to treat a variety of diseases and as a result, a number of modern drugs have been developed from them [6]. So far, about 30 compounds derived from plants have been proven to be clinically active against various types of cancer cells [7], this is a very small portion as it is anticipated that plants can provide potential bioactive compounds for the development of new methods to combat cancer diseases [8]. The discovery of vinca alkaloids, vinblastine, vincristine and cytotoxic podophyllotoxins in 1950s in plants began the extensive research of anti-cancer drugs from plant sources [9]. A combination of vinblastine and vincristine with other cancer chemotherapeutic drugs have been used in the treatment of cancers like leukemias, lymphomas, advanced testicular cancer, breast and lung cancer as well as Kaposi's sarcoma [10]. An isomer of podophyllotoxin, epiphyllotoxin was isolated as having active anti-tumor activities from the roots of *Podophyllum* species, *Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich [11].

In Africa, 90% of the population relies on traditional medicine for primary healthcare [12]. The conventional health system in Kenya provides for only 30% of the population, this means that more than two-thirds of Kenyans rely

on traditional medicine for their healthcare needs [13]. The benefit of using the drug extracts from medicinal plants is that they are usually safer than their synthetic alternatives and are also more affordable [14]. Most cancer drugs available in the market are toxic as they kill both the normal and cancer cells, there is therefore need to look for a safer drug with high specificity to target cancer cells only.

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

Warburgia stuhlmannii belongs to the *canellaceae* family. The species is only found in Kenya and Tanzania. For medicinal purposes, the bark has been used as remedy for both tooth aches and rheumatism [18] while the stem bark is used in the treatment of both anti-tumor and anti-inflammatory diseases [19]. The extract was reported to have a Lethality value (LC_{50}) of 8 µg/ml [20], which was consistent with existing phytochemical information that the plant to possess anti-tumor and cytotoxic compounds [21].

Maytenus senegalensis, commonly known as the 'spike thorn' belongs to the *celastraceae* family [22]. It is found in Arabia, Afghanistan, India and widespread in the savannah regions of Africa [23]. Ethanolic extracts from its stem bark showed cytotoxic effects against carcinoma in cell cultured and leukemia in mice [24]. In some African regions, the roots and bark of *M. senegalensis* are used in traditional medicine for treatment of several illnesses including chest pain, rheumatism, snake bites, diarrhea, eye infection, dyspepsia and wounds [25,26]. In Sudan, the aqueous extract of the stem bark is commonly used in the treatment of tumors, dysentery and snake bites [27,28]. A study on the anti-inflammatory activities of *M. heterophyla* and *M. senegalensis* using carcigeenan-induced paw edema method on Winstar albino rats had portrayed significant anti-inflammatory activity that reduced edema by 51% and 35% respectively [29]. However, the many therapeutic claims over these three plants, their safe use have not been scientifically proven. It is these claims that this study has to verify.

2. MATERIALS AND METHODS

2.1 Plant Materials

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

2.2 Preparation of the Plant Extracts

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

2.3. Cell Culturing

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

2.4 MTT Assay for Cytotoxicity

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

2.5 Drug Administration

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

5 mice were used as negative control and these were given 0.2 ml of distilled water. Each mouse received only a single oral dose of the drug in the entire experiment. The mice were deprived of feeds 12 hours prior to introduction of the drug and 3 hours after. The animals were observed over a period of 24-48 hours for signs of acute oral toxicity, for example reduced activeness, convulsions, writhing, decreased motor activity, decreased body/limb tone, decreased respiration, mortality rate and survival period, in this study, none of these were noted.

2.6 LD_{50} Determination

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

2.7 Data Management and Analysis

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

3. RESULTS

3.1 Extraction

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

shows the percentage yields of the 12 plant extracts.

3.2 IC_{50} Results with the 4T1 Cells

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

3.3 IC_{50} Results with Colon Cancer Cells

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

3.4 CC_{50} Results with VERO Cells

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

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

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

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

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

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

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

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

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

3.5 Selectivity Index

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

3.6 Acute oral Toxicity with *Warburgia stuhlmannii*

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

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

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

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

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

** Mean difference is significant at the 0.05 level*

3.7 Acute Oral Toxicity with *Prunus africana*

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

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

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

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

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

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

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

4. DISCUSSION

4.1 Cytotoxicity Studies

Vero cells have been recommended for cytotoxicity studies and for the analysis of cell-substrate interactions in biomaterial research [36,37]. Our study shows investigations of anti-cancer potential of three plant species which has not been studied in Kenya, by screening for cytotoxic activity against healthy cells and two mouse model cancer cell lines, 6 out of the 12 extracts showed low or no toxicity against normal cell lines (Vero cells), this includes water and methanol extracts from the leaves of *P. africana,* water extracts of the leaf and stem bark of *M. senegalensis,* water extracts from the stem bark of *W. stuhlmannii,* whereas the other 6 showed toxicity ranging from 55.64 to 464.04µg/ml. Among the extracts that showed no toxicity on the normal cells (Vero) but showed the highest selective cytotoxicity against 4T1 and CT26 was

the methanol extract from the leaf of *P. africana*. Methanol extract of the stem bark of this plant (*P. africana*) had the highest selective cytotoxicity against 4T1 cells. There is possibility of the leaf and stem bark of *P. africana* extracts having similar phytochemicals and hence causing similar activities. The stem bark of this plant has been used traditionally for the treatment of Benign Prostate Hyperplasia (BPH) [15]. The selective cytotoxicity shown by this plant could be attributed to the summation effects of many compounds present in the extract. The pharmacology of some compounds from *Prunus africana* has been reported [38,39,40].

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

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

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

4.2 Acute Oral Toxicity

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

5. CONCLUSION

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

ACKNOWLEDGEMENTS

I am thankful to those who made the laboratory work in this study successful, especially Dr. James Kuria and Mr. Dalmas Odira from the Center for Traditional Medicine and Drug Research (CTMDR) and Mr. Julius Muchiri from the Center for Viruses Research (CVR).

CONSENT

Not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist

REFERENCES

1. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer. 2013;132(5):1133-1145. Available:http://dx.doi.org/10.1002/ijc.27711 PMID:22752881
2. South African Medical Journal. The cancer burden in Africa. 2007;97(10).

3. Silas F, Parkin DM, Chirenge M, Stein L, Abratt R, Wabinga H. Part I: Cancer in indigenous Africans-burden, distribution and trends, Lan Oncol. 2008;9:683-692.
4. Kenya Ministry of Public Health and Sanitation and Ministry of Medical Services. Draft of National Cancer Control Strategy 2010-2015. Nairobi; 2009. Available:http://www.ipcrc.net/pdfs/intl_programs/Final-Draft-of-the-Kenya-Cancer-Control-Strategy-April-2011.pdf
5. Mohamed El-Baradei. AIEA (International Atomic Energy Agency). A silent crisis: Cancer treatment in developing countries; 2003. IAEA/PI/A74E/03-01531.
6. Samuelsson G. Drugs of natural origin: A textbook of pharmacognosy. 4th ed. Stockholm, Swedish Pharmaceutical Press; 1999.
7. Joyce Nirmala MA, Samundeeswari, P Doepa Sankar. Natural plant sources in anti-cancer therapy- a review. RES PLANT BIOL. 2011;1(3):01.
8. Shoeb M. Cytotoxic compounds from the genus centauea. PhD thesis. Aberdeen, UK. The Robert Gordon University; 2005.
9. Cassady JM, Duoros JD (Eds). Anti-cancer agents based on natural product models. Academics press, New York. 1980;271-317.
10. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005;100:72-79.
11. Stahelin H. Activity of a new glycosidic lignin derivative (VP16-213) related to podophyllotoxin in experimental tumors. Eur J Can. 1973;9:215-21.
12. Miller NN. Traditional medicine in East Africa, American universities field staff report.1990;22:1-15.
13. National Coordinating Agency for Population Development (NCAPD). Draft policy on Traditional medicine and medicinal plants, Nairobi; 2007.
14. Iwu MM, Duncan AR, Okunji CO. New antimicrobials of Plant origin. In: Janis J. (Ed.). Perspectives in new crops and new uses. ASHS Press, Alexandra, V A. 1999;457-462.
15. Kokwaro JO. Medicinal plants of East Africa. East African Literature Bureau, Kampala, Nairobi, Dar-es-salaam; 1993.
16. Pojal J. Naturafrica-the herbalist handbook. Jean Pujol Natural Healers foundation, Durban, South Africa; 1990.
17. Iwu MM. Handbook of African medicinal plants. CRC Press, Boca Raton, Florida, USA; 1993.
18. Orwa C, Mutua A, Kindt R, Jamnadass R. and Simons A. Agroforestry database: A tree reference and selection guide version; 2009. Available:http://www.worldagroforestry.org/af/treedb/
19. Beentje J. Kenyan trees, shrubs and lianas. National Museums of Kenya, Nairobi; 1994.
20. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa JD Kiama SG. Biological screening of Kenyan medicinal plants using artemisia lina L. (*Artemiidae*). Pharmacol .2011;2:458-478.
21. Manguro LOA, Ugi I, Herman R, Lemmen P. Flavonol and drimane-type sesquiterpene glycosides of *Warburgia stuhlmannii* leaves. Phytochem 2003;63:497-502.
22. Farnsworth NR, Soejarto DD. Global importance of medicinal plants. In: Akerele O, Heywood V, Synge H, editors. The conservation of medicinal plants: Proceedings of an international consultation, 21-27 March, 1988, Chiang Mai, Thailand. Cambridge University Press, Cambridge. 1991;25-51.
23. Jansen PC, Mendes O. Plantas medicinais: Seu uso tradicional em Mozambique. Maputo: Imprensa do Partido; 1991.
24. Tin-wa M, Farnsworth NR, Fong HHS, Bloomster RN, Troja'nek J, Abraham DJ, Persinos GJ, Dakosi OB. Biological and phytochemical evaluation of plants IX. Anti-tumor activity of *Maytenus senegalensis (celastraceae)* and a preliminary phytochemical investigation. Lloydia 1971;34:79-87.
25. Matu EN, van Staden J. Anti-bacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J Ethnopharmacol. 2003;87:35-41.
26. Okine LKN, Nyarko AK, Osei-Kwabenam N, Oppongm IV, Barnes F, Ojosuhene M. The anti-diabetic activity of the herbal preparation ADD-199 in mice: A comparative study with two oral hypoglycemic adj. Hypoglycemic-of or relating to hypoglycemia; hypoglycemic agents. Hypoglycemic drugs. Journal of Ethnopharmacol. 2005;97:31-38.

27. Kupchan SM, Smith RM. Maytenolide, maytine, and maytolidine, novel nicotinoyl sesquiterpene alkaloids from *Maytenus serrata* (Hochst. ex A. Rich.) R. Wilezek, J Org Chem. 1977;42:115-118.
28. Shirota O, Tamemora T, Morita H, Takeya K, Hokawa H. Triterpenes from Brazilian medicinal plant "ChuChuhuasi" (*Maytenus akrukovii*). J Natural Prod. 1996;59:1072-1075.
29. Da Silva G, Tanica M, Rocha J, Serran R, Gomes ET, Sepodes B, Silva O. *In vivo* anti-inflammatory effect and toxicological screening of *Maytenus heterophyla* and *Maytenus senegalensis* extracts; 2010. Hum. Exp. (cited 10th May 2011). Available:http://www.ncbi.nlm.nih.gov/pubmed/20670987
30. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis, 2nd ed. Chapman and Hall, London. 1984;288.
31. Mosmann T. Rapid calorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. J immunol methods. 1983;16;65(1-2):55-63.
32. Masters RW. Animal cell culture, cytotoxicity and viability assays. 3rd Ed. 2000;202-203.
33. Wilson AP. Cytotoxicity and Viability assays in animal cell culture: A practical approach. 3rd Ed, Oxford University Press: Oxford. 2000;1.
34. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275-87.
35. Loomis TA, Hayes AW. Loomis's essentials of toxicology. 4th edition, California academic press. 1996;208-245.
36. ISO International Organization for Standardization. Biological evaluation of medical devices: Part 5. Tests for cytotoxicity: *in vitro* methods.10993-5:1992(E). 1st ed. ISO. Geneva. 1992;1-7.
37. Kirkpatrick CJ. Biological testing of materials and medical devices - A critical view of current and proposed methodologies for biocompatibility testing: Cytotoxicity *in vitro*. Regulatory Affairs. 1992;4(1):13-32.
38. Andro M, Riffaud J. Pyegum africanum extract for the treatment of patients with benign prostate hyperplasia: A review of 25 years published experience. Curr Ther Res. 1995;56(8):796-817.
39. Marandola P. Jallous H. Bombardelli E, et al. Fitoterapia. 1997;68:195-204.
40. Bombardelli E, Morazzoni P. *Prunus africana* (Hook.F) Kalkm. Fitoterapia. 1997;68:205-218.
41. Lawrence O, Arot Manguro, Ivan Ugi, Rudolf Hermann, Peter Lemmen. Flavonol and drimane-type sesquiterpene glycosides of *Warburgia stuhlmannii* leaves. Phytochem. 2003;63(4):497-502.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=792&id=13&aid=7250