South African Journal of Botany 88 (2013) 183–190

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Safety of *Prunus africana* and *Warburgia ugandensis* in asthma treatment

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ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form 5 July 2013
Accepted 8 July 2013
Available online 22 August 2013

Edited by L Verschaeve

Keywords:
Prunus africana
Warburgia ugandensis
Cytotoxicity
Vero cells
Acute toxicity
Lethal dose
Efficacy
Therapeutic dose
Traditional medicines

ABSTRACT

The aim of the study was to determine the possible cytotoxicity of the aqueous stem bark extracts of *Prunus africana* and *Warburgia ugandensis* to Vero E6 cells and acute toxicity in BALB/c mice. Despite being some of the most popular medicinal plants used in Africa, little is known about the safety. *In-vitro* cytotoxicity tests on Vero E6 cells were investigated using MTT assay to assess the safety of the two plant extracts. Vero E6 cells on growing to confluence were incubated with different drug concentrations for 48 h for the drug to take effect. Viability of the cells was measured by a scanning multiwell spectrophotometer, color intensity being equivalent to viable cells which reduce MTT to soluble formazan crystals. This was done by determining the CC_{50} of the extracts, CC_{50} being the concentration of the dose of the compound/extract that kills 50% of the cells. In acute toxicity a total of 55 mice were used. Mice were divided into eleven groups of 5 mice, one group served as negative control and ten groups received oral gavage doses at 500, 889.56, 1581.6, 2812.15 or 5000 mg/kg body weight once. Mortality and other signs of toxicity were recorded within 24 h and the weights of the surviving mice taken for 14 days thereafter. *P. africana* had CC_{50} of 104.08 μg/ml while *W. ugandensis* had $CC_{50} > 250$ μg/ml and both were classified as not cytotoxic. There was no mortality observed in groups of mice that received *P. africana* extracts at 500 and 889.56 mg/kg body weight. There was 20%, 60% and 100% mortality observed within 24 h for mice that received *P. africana* extracts at 1581.64, 2812.15 and 5000 mg/kg body weight respectively. Lethal dose (LD_{50}) for *P. africana* was 2201.207 mg/kg body weight. *W. ugandensis* extracts had no mortality recorded in all dose levels and the LD_{50} was >5000 mg/kg body weight. The weights of mice that survived the entire 14 days in all groups increased and were not significantly different from that of controls $p > 0.05$. From the *in vitro* and *in vivo* studies, the two extracts were safe to use. Though with their customary value among many Kenyan communities in management of asthma among other ailments there is a need for further validation of any anti-asthmatic properties and responsible chemical compounds to augment the findings.

1. Introduction

More than 60% of the world's population of which over 80% are in the developing countries depend upon traditional medicine for their primary health care (Bannerman et al., 1983). The knowledge of medicinal plants and their roles in treatment of diseases are individually held by the traditional health practitioners (THP). The information on the use of the herbal remedies was bequeathed to them by their fathers orally from generation to generation. Although herbal medication is thought to be more effective and having fewer side effects compared to synthetic medicines, self-prescription is a common practice in most rural communities. Adverse effects of these herbal therapies and sometimes life-threatening conditions have been noted among some ethnic communities (Chan, 2003; Elvin-Lewis, 2001). Scientific findings and the World Health Organization (WHO) specify that, many plants used as food and traditional medicine are potentially toxic, mutagenic and carcinogenic (Schimmer et al., 1994). Since few studies have been undertaken to ascertain the safety of traditional remedies, it is necessary to determine both efficacy and toxicity profile of medicinal plants prior to putting them to clinical use.

Prunus africana is commonly known as Pygeum or African cherry and it is in the Family Rosaceae (Kokwaro, 1976). Stem bark water extracts are traditionally used as an expectorant for disorders of the chest and lungs (Kokwaro, 1976). It is widely distributed in several provinces of Kenya though it is predominant in Mount Kenya forest and the environs. It is here where the herbal extract prepared from the bark of *P. africana*, is used as an alternative medicine for many ailments among them asthma and chest complications. These stem bark water extracts are used without scientific assessment of their safety and efficacy. Conversely, *W. ugandensis* is a species of evergreen tree native to Africa. The stem and roots of *W. ugandensis* are used as expectorant, for the common cold and against fever, malaria, stomachache, constipation and diarrhea (El Kamali and El kijalifa, 1997). Observational studies by clinicians indicate that people customarily use the plants-derived preparations for bronchial asthma management and that the

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http://dx.doi.org/10.1016/j.sajb.2013.07.007

patients showed great improvement (Aluoch et al., 1990a,b). From these observational findings it was apparent that these plant preparations are taken without any scientific screening for efficacy and safety. Due to the increased use of the stem bark extracts of the two plants traditionally in management of asthma, there was an ostensive need to carry out a comprehensive study on their toxicity to highlight any possible hidden toxic activity. This study was therefore designed to investigate the *in vitro* effect of the stem bark extracts to the Vero E6 cells and acute toxicity to BALB/c mice.

2. Materials and methods

2.1. Experimental animals

Eight week old healthy female BALB/c mice with a mean body weight of 20 ± 2 g bred at KEMRI, Nairobi, Kenya were used for the study. The animals were moved into the experimental room for acclimatization one week before onset of experiments. Five mice were housed in 15 cm × 21 cm × 29 cm transparent plastic cages bedded with wood shavings and equipped with continuous-flow nipple watering devices. They were feed with pellets (Mice pellets UNGA® feeds) and water *ad libitum*.

2.2. Experimental procedures

2.2.1. Collection of the medicinal plants

The medicinal plants were collected in August 2012 from their natural habitats in Meru forest Kenya. This was done with the assistance of a plant taxonomist/botanist. Five kilograms of fresh plant parts (stem barks) was collected and packaged in gunny bags. Acceptable bio-conservation/sustainable harvesting techniques such as peeling patches of barks from different sites on the tree/not cutting down the tree were applied (Harnischfeger, 2000). Voucher specimens were deposited at the East African Herbarium (voucher numbers, Table 1), National Museums of Kenya, Nairobi, Kenya. Plants of study are shown in Appendices A and B.

2.2.2. Preparation of plant extracts by water extraction

Clean stem barks were air-dried at room temperature under shade for 14 days and pulverized using a laboratory mill (Christy & Norris Ltd., Chelmsford, England). A hundred and fifty grams of the powdered plant material was extracted with 1500 ml of distilled water in a water bath at 60 °C for 1 h. The extracts were filtered and freeze dried using a Freeze Dryer (Edwards freeze dryer Modulyo). The freeze dried powder was weighed, labeled and stored in an airtight bijou bottle at 4 °C.

2.2.3. Preparation of plant extracts for in vitro bioassays

The water extracts of the plant samples prepared earlier, were retrieved from 4 °C and dissolved in distilled water so that the final highest concentration in the microtiter plates was 250 μg/ml. For these experiments 250 mg of the water extract was dissolved in 1 ml DMSO to form a stock solution of 10,000 μg/ml in 100% DMSO. This was diluted in media in a ratio of 1:100 that is 10 μl of extract in 990 of media to give a start concentration of 250 μg/ml in 1% DMSO. Each of the extracts was filter sterilized with syringe adaptable 0.22 μm filters into sterile Bijou bottles in the laminar flow hood (Bello Glasses Inc., USA) and stored at −20 °C).

Table 1
Plant species, voucher numbers and parts collected.

Botanical name	Family	Voucher number	Part used
Prunus africana	Rosaceae	TFm10	Stem bark
Warburgia ugandensis	Magnoliidae	TFm11	Stem bark

2.2.4. Cytotoxicity assay of the extracts in cell culture

In vitro cytotoxicity assay was carried out following a modified rapid calorimetric assay as described by Mosmann (1983) using actively dividing sub-confluent Vero E6 cells. Vero E6 cells were chosen in this study due to their sensitivity (quite fragile/delicate), subsequently if the drugs are not cytotoxic to them, the drugs are then safe in other cells which are more resistant (McGuinness and Mantis, 2006; Pauly et al., 2009, 2012). The Vero cells were maintained in Eagle's Minimum Essential Medium (MEM) containing 10% fetal bovine serum (FBS), in T-75 cell culture flasks incubated at 37 °C in 5% CO_2. Upon attainment of confluence, cells were detached by trypsinization and pooled in a 50 ml tube. The cells were then re-suspended in 40 ml fresh MEM. A 100 μl of cell suspension of 2×10^5 was seeded in 96-well microtiter plates in columns 1, 2, 4, 5, 7, 8, 10 and 11 in wells of rows A–H and incubated at 37 °C/5% CO_2 for 12 h. Cells without drugs (row A) served as negative controls while wells with no cells (columns 3, 6, 9 and 12) served as blanks. The cells were incubated overnight (24 h period) to allow cells to attach. The next day the media was removed from row H and care was taken not to leave cells without media for too long. Addition of 150 μl of 250 μg/1 ml DMSO of the highest drug concentration in triplicate (4 drugs per plate) was done in row H. Serial dilutions using a multi-channel pipette by removing 50 μl from wells of row H and adding to wells of row G were carried out. After mixing well, another 50 μl was transferred from row G to wells of row F and mixed well. This was continued up to row B discarding the last 50 μl of this row; leaving out row A (a threefold dilution is achieved). Row A wells were exempted since they served as controls (wells without drugs). Thus row H wells had a concentration of 250 μg/ml, G wells 83.3 μg/ml, F wells 27.77 μg/ml, E wells 9.26 μg/ml, D wells 3.08 μg/ml, C wells 1.03 μg/ml and B wells 0.342 μg/ml wells. Consequently row H had 100% drug concentration while those of B had only 0.137% of the test sample. The plates were incubated for 48 h at 37 °C, 5% CO_2 to allow drug to take effect on the growing cells. Addition of 10 μl of MTT dye to each well was done after ensuring normal growth of the cells in the plate under an inverted microscope. Plates were incubated for another 4 h at 37 °C. Media was removed from the wells and 100 μl of DMSO added to dissolve the formazin. The plates were read on a scanning multiwell spectrophotometer (Multiskan Ex labsystems) at 562 nm and 620 nm as reference. Chloroquine (100 μg/ml highest drug concentration) was used as a positive reference.

2.2.5. In vivo assay for the determination of safety (acute toxicity)

The mice were deprived of feed 12 h before and 3 h after administration of the plant extract. A total of 55 mice were used in the safety tests and these were divided into 11 groups of 5 mice each. One group received an oral gavage of water and therefore served as a control. Five groups for each extract received only one dose at concentrations of 500, 889.56, 1581.6, 2812.15 or 5000 mg/kg body weight once. The calculations of dose levels are shown in Appendix C. Animals were observed individually after dosing at least once within the first 30 min, then periodically during the first 24 h and twice daily thereafter for a total of 14 days. The parameters of interest were; changes in skin and fur, eyes and mucous membranes, respiratory, circulation, and autonomic and central nervous system for signs of toxicity that may include tremors, convulsions, salivation, diarrhea, lethargy, somnolence or coma (OECD, 2001). The mice were weighed on a daily basis and their weights recorded.

2.2.6. LD_{50} determination

This was carried out as described by Lorke (1983). At least five dose levels ranging 500–5000 mg/kg body weight of each compound was administered orally to the mice once. Five female mice were used per dose level and the diluent (distilled water) was administered as the control. Female mice were used in this study since literature surveys of convectional LD_{50} tests indicate that although there is little differences in sensitivity between sexes where differences are observed females are slightly more sensitive (Gad and Chengelis, 1988). Graphs of

Table 2
Plant species and percentage yields of water extracts.

Botanical name	Dry bark weight (g)	Freeze dried product (g)	(%) Yield of water extracts
Prunus africana	150	13.452	8.968
Warburgia ugandensis	150	19.481	12.987

Table 3
Cytotoxicity (CC_{50}) of plant extracts to Vero E6 cells.

Plant species	Initial concentration tested (μg/ml)	CC_{50} (μg/ml)
P. africana	250	104.08 ± 0.259
W. ugandensis	250	>250

probit against log dose of the aqueous extracts were drawn. The table for probit is shown in Appendix D. The log dose that responded to probit 5 (50% deaths) was calculated from the graph equation and its anti-log gave the LD_{50} (Finney, 1964).

2.3. Ethical clearance

Approval by KEMRI Scientific Steering Committee (SSC), KEMRI Ethical Review Committee (ERC) and Animal Care and Use Committee (ACUC) was sought before study implementation.

2.4. Data management and analysis

Probit–log analysis was used for LD_{50} calculations. Mean weights of mice in acute toxicity were obtained per group and means on the same scale were compared using student *T*-test. All values were expressed as mean ± standard error of mean and differences between the parameters of estimate were deemed statistically significant at $p < 0.05$.

3. Results

3.1. Yields of water extracts

The percentage yield of the water extract was calculated as follows:

$$\text{Percentage yield} = \frac{\text{Amount of freeze dried extract}}{\text{Amount of ground dry plant extract}} \times 100.$$

From 150 g of ground dry plant material, the percentage yield in *P. africana* was 8.968% while that of *W. ugandensis* was 12.9873%. Raw and percent yields are presented in Table 2.

3.2. Cytotoxicity assay

Growth of Vero E6 cells (%) incubated with high concentration of *P. africana* extracts is as shown in Fig. 1. *P. africana* extracts had CC_{50} of 104.08 μg/ml (Table 3). Growth of Vero E6 cells (%) when incubated with extracts of *W. ugandensis* is as shown in Fig. 2. Extracts of *W. ugandensis* at the highest concentration tested did not lyse/cause cytopathic effect to 50% of the cells and therefore the $CC_{50} > 250$ μg/ml (Table 3).

3.3. Acute toxicity

3.3.1. Acute toxicity in mice treated with water extracts of *P. africana*

Five groups of mice were administered each with one dose of *P. africana* water extract ranging from 500 to 5000 mg/kg body weight. Signs of toxicity observed in mice treated with *P. africana* at high dose levels (1581.64–5000 mg/kg body weight) include hypo-activity, pilo-erection, low appetite and hyperventilation. There was no mortality observed within 24 h for mice that received *P. africana* extracts at 500 and 889.56 mg/kg, while there was 20% mortality at 1581.64 mg/kg, 60% at 2812.15 mg/kg and 100% mortality at 5000 mg/kg body weight (Table 4). Oral LD_{50} of *P. africana* was calculated to be 2201.207 mg/kg body weight from probit analysis. The LD_{50} is the anti-log of 3.3426609 (Fig. 3) which is log dose that responded to probit 5 (dose that causes 50% deaths). Table for probit used to plot the graph of probit against log dose (Appendix D). The mice surviving after 24 h, survived for the entire 14 days of observation and their weights were noted. Their weights were plotted (Fig. 4). The weights of mice that received 500, 889.56 and 1581.64 mg/kg body weight continued to increase and had no significant difference with that of controls ($p > 0.05$). Mice that received 2812.15 mg/kg body weight had a decrease in weight the first day then continued increasing in weight in the consecutive days of the study but had no significant difference in weight from that of control mice ($p > 0.05$).

The equation of this graph was used to calculate the log dose for probit 5 (50%), from the graph the log dose that caused 50% animal deaths was 3.343 whose anti-log was 2201.202 mg/kg body weight which is the LD_{50} of *P. africana* stem bark extract.

3.3.2. Acute toxicity in mice treated with water extracts of *W. ugandensis*

Five groups of mice were administered each with one dose of *W. ugandensis* water extract ranging from 500 to 5000 mg/kg body weight. No signs of mortality were recorded at the doses tested. There was also no mortality observed within 48 h for mice that received *W. ugandensis* extracts in all dose levels (Table 5). *W. ugandensis* had $LD_{50} > 5000$ mg/kg body weight. There was an increase in body weight in all groups of mice treated with *W. ugandensis* in all dose levels (Fig. 5)

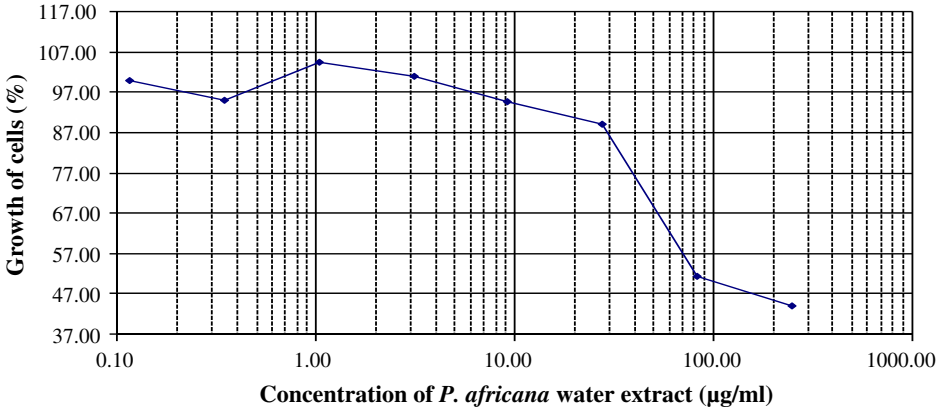


Fig. 1. Growth of Vero E6 cells (%) incubated with *P. africana* water extracts at different concentrations.

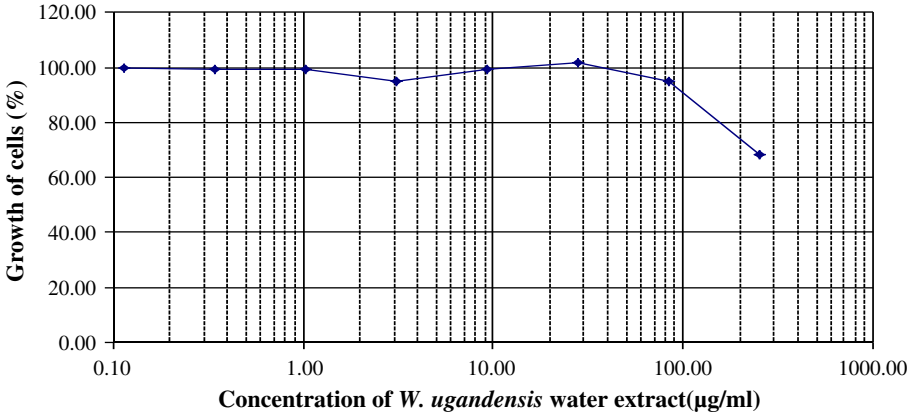


Fig. 2. Growth of Vero E6 cells (%) incubated with *W. ugandensis* water extracts at different concentrations.

and their weights were not significantly different from those of control mice ($p > 0.05$).

4. Discussion

4.1. Yields of water extracts

From 150 g of ground dried barks of *P. africana*, a yield of 13.452 g freeze dried water extract was obtained. The extraction was done using 1500 ml of water equivalent to six glasses of water (250 ml glass equivalent). Each glass of water extract therefore contains 2.242 g of freeze dried *P. africana* water extract (Appendix E). Ground dry stem barks yield in *W. ugandensis* was 19.481 g and using the same logic a glass of water extract contains 3.25 g of freeze dried extract. *W. ugandensis* extracts yielded 4.0193% more than those of *P. africana* in every 150 g of ground dried barks (Appendix E). Traditional health practitioners normally prescribe a glass of stem bark extract per day obtained by boiling a handful of dry stem barks in water. This is taken as a dose for an adult irrespective of age or body weight. This dose in the traditional setting is believed to be safe and effective against ailments according to the THP (Khalid and El Kamali, 1996; Kokwaro, 1976). Therefore if the same glass measure (250 ml) is used in the treatment of ailments, the patients receive more concentration of *W. ugandensis* than *P. africana* per glass in a boiled plant extract. However this does not imply that *W. ugandensis* is more efficacious than *P. africana.*

4.2. Cytotoxicity assay

Viable E6 cells take in MTT into their mitochondria; where mitochondrial succinate dehydrogenase reduced it to formazan crystals which are insoluble and on the addition of a detergent it solubilizes forming a purple color. The intensity of the color is an indication of cell activity/viability and this was read as absorbance from a spectrophotometer (Mosmann, 1983). Where the drug was cytotoxic the cells underwent apoptosis or had inhibited growth and as such had little absorbance. From the results of the MTT assay, CC_{50} of *P. africana* was 104.08 µg/ml. Albeit, CC_{50} is the concentration of the extract that lysis or causes cytopathic effect on 50% of the cells. *P. africana* extract was classified as not cytotoxic according to the criteria of Rukunga and Simons (2006). According to the classification, cytotoxicity was classified as: Cytotoxic at $CC_{50} < 2$ µg/ml, moderately cytotoxic at CC_{50} 2–89 µg/ml and not cytotoxic at $CC_{50} > 90$ µg/ml. Subsequently, other studies have demonstrated *P. africana* extracts as non-toxic, where cytotoxicity was >100 µg/ml (Tolo et al., 2006).

In accord to the Rukunga and Simons' (2006) classification, *W. ugandensis* was not cytotoxic at 250 µg/ml highest concentration tested and therefore classified as not cytotoxic at ≥250 µg/ml. Therefore CC_{50} for *W. ugandensis* was > 250 µg/ml. *W. ugandensis* stem bark extracts have been reported to have no significant cytotoxicity effect on infected BALB/c macrophages *in vitro* at 1000 µg/ml and were recommended as safe for use in further *in vivo* studies (Githinji et al., 2010). Moreover, *W. ugandensis* extracts were determined to be non-cytotoxic to Vero cells at 100 µg/ml (Ngure et al., 2009). However, a cytotoxic sesquiterpene, characterized as muzigadial was isolated from *W. ugandensis* (Olila et al., 2001) and it was highly toxic in the brine shrimp assay. It is critical to note, in all these studies, the stem bark extracts were not cytotoxic to Vero cells despite the fact that the stem barks were sourced from different localities in Kenya. The CC_{50} values of the two extract indicate that the extracts are not toxic to the Vero cells and thus are safe to use as herbal remedies.

Table 4
Acute toxicity in mice treated with *P. africana* water extracts.

Concentration of plant extract (mg/kg)	Number of mice that died	Total mice treated	% mortality	Log concentration	Probit
500	0	5	0	0	
889.56	0	5	0	2.949175	
1581.64	1	5	20	3.199108	4.16
2812.15	3	5	60	3.449038	5.25
5000	5	5	100	3.69897	5.25

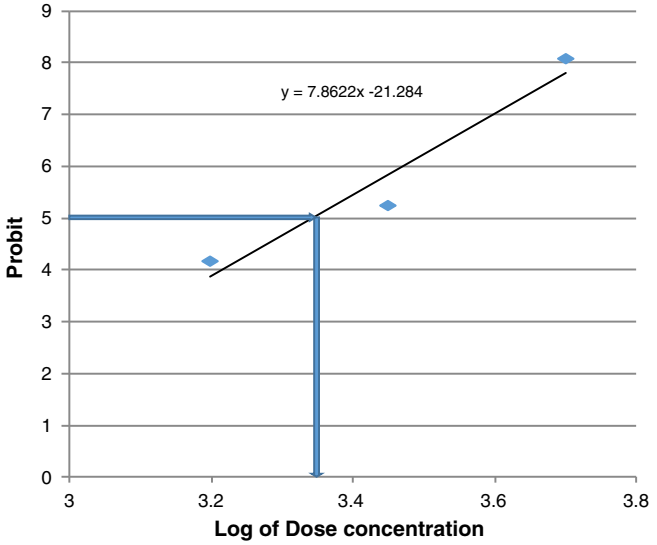


Fig. 3. Plot of probit against log dose of *P. africana* bark extracts.

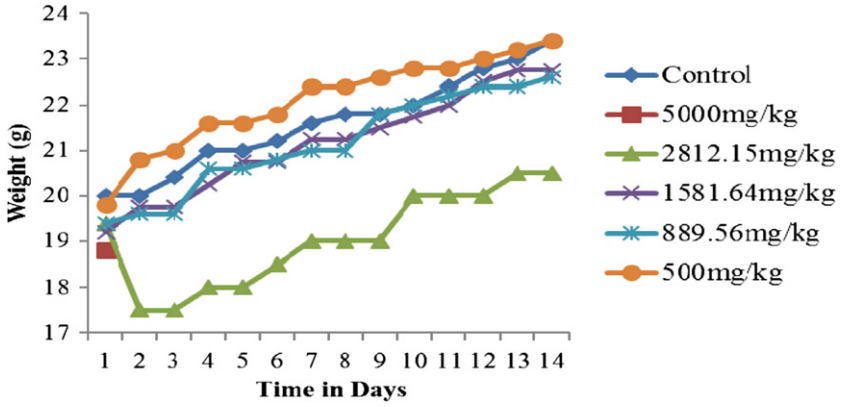


Fig. 4. Weight of groups of mice treated with different doses of *P. africana* water extracts.

4.3. Acute toxicity of *W. ugandensis* extracts

Scientific findings have pointed out that many plants used as food and traditional medicine are potentially toxic, mutagenic and carcinogenic (Schimmer et al., 1994) yet, very little evidence of systematic screening done on such plants. On administration of single doses of *W. ugandensis* to mice, no mortality was observed even at the highest concentration tested. Based on the scale of Loomis and Hayes (1996) classification of toxicity (Appendix F), *W. ugandensis* was classified as relatively harmless with LD_{50} > 5000 mg/kg body weight. Another pointer to the safety of *W. ugandensis* stem bark extracts was that, all the animals that received treatment with the extracts stayed alive for the entire period of study and the mean weights increased progressively with no significant difference from those of controls. The results showed that the stem bark water extracts of *W. ugandensis* were safe to use in mice as herbal medicine at the doses tested. Correspondingly, all parts of *W. ugandensis* have been shown to be edible and that leaves, barks, young shoots and fruits are used as food and medicine (Kokwaro, 1976; Mbuya et al., 1994). Suggesting that, the plant is safe for use in human. Besides, *W. ugandensis* bark extracts have been used for ages for treatment of stomach worms and malaria in Baringo Kenya with no adverse effects (Henke, 1994) while non-toxicity has been noted *in vitro* in BALB/c macrophages (Githinji et al., 2010). These augment *W. ugandensis* safety for use as a herbal extract.

4.4. Acute toxicity of *P. africana* extracts

Aqueous extracts of *P. africana* were mildly toxic to rats after repeated daily oral administration of 1000 mg/kg body mass (Gathumbi et al., 2000). There was no repeated therapy in the present study. Also, when *P. africana* extracts were administered at 500 and 889.56 mg/kg body weight dose levels, no mortality was noted in the BALB/c mice. Equally, *P. africana* stem bark extracts were determined to be non-toxic at therapeutic dose of 500 mg/kg body weight (Tolo et al., 2006). Lethal dose (LD_{50}) for *P. africana* was calculated by probit analysis to be 2201.207 mg/kg body weight. Based on the scale of Loomis and Hayes' (1996) classification of toxicity (Appendix F), *P. africana* was classified as slightly toxic with an LD_{50} of 2201.207 mg/kg body weight. Toxic signs were not noted in the present outcomes at 1000 mg/kg body weight. Therefore the toxic signs recorded in the repeated daily oral administration in the previous findings, would have been due to bio-accumulation of the extracts in the systems leading to high concentration of the medicine resulting to mild toxicity (Gathumbi et al., 2000). It can also be deduced that the differences in toxicity could be attributed to different factors such as; the stem bark extracts were from different plants of different ages, growing in different localities and storage periods owing to the fact that the drugs extracted are at times stored before use. The factors would be worthy to consider in profiling the toxicity of the stem bark extracts of *P. africana*.

Observational studies by clinicians have reported rare complains of diarrhea, constipation, dizziness and visual disturbances as adverse effects of *P. africana* among individuals under the medicinal therapy for varied condition (Andro and Riffaud, 1995; Murray, 1995). The current study had mortality in BALB/c mice on administration of higher doses (1581.6–5000 mg/kg body weight) of *P. africana* stem bark extracts. Signs of toxicity such as increased breathing rate and loss of body balance were also evident in the animals. This implies that doses above the LD_{50} were having lethal effects in the mice. Lower doses (<1581.6 mg/kg body weight) had no adverse effects in the mice. This corresponded to the studies that reported the absence of any significant deleterious effects of *P. africana* at concentrations of 100 mg/kg body weight (Andro and Riffaud, 1995; Murray, 1995), while pointing out on its constituents anti-carcinogenic and anti-mutagenic properties both *in vitro* and *in vivo* (Ishani et al., 2000). Mean weight loss for the first few days in the group of mice that received oral gavage of *P. africana* extract at 2812.15 mg/kg body weight accompanied the toxic signs noted. Nevertheless, weight gain or loss can be caused by a number of factors including motivation, eating behaviors, amount of activity, overall health metabolism and stress (Ekpo and Pretorius, 2008; Pretorius et al., 2007). Low appetite was noted in mice administered with the higher dose levels of the extract hence a potent factor to changes in animal physiology leading to abated body weight and survival (Marti et al., 1994; Ottenweller et al., 1992; Retana-Márquez et al., 2003). In one study using restraint stress, rats lost weight and remained hypophagic until a few days after the stress had ended (Harris et al., 1998). If stress would have caused weight loss in mice then, all the groups could have lost weight and would have had a significant difference with that of the control but this was not the case. Therefore, loss of weight in the first few days and less progressive weight gain in the animals treated with 2812.15 mg/kg body weight compared to controls and other treated groups would be attributed to; the drug exhibiting inhibitory effects in the organ systems or interference with cell functioning (Gathumbi et al., 2000).

All the mice that survived the entire 14 days of study in acute toxicity assay had their weight not different from those of control indicative that the lower doses were safe to use in mice. The survival of

Table 5
Acute toxicity in mice treated with *W. ugandensis* stem bark extracts.

Concentration of plant extract (mg/kg)	Number of mice that survived	Number of mice that died	Total
889.56	5	0	5
1581.64	5	0	5
2812.15	5	0	5
5000.00	5	0	5

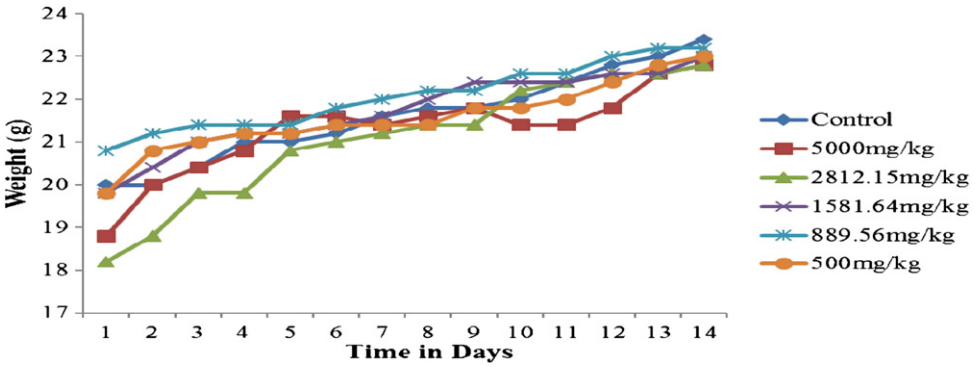


Fig. 5. Weight of groups of mice treated with different doses of *W. ugandensis* water extracts.

mice in the present study after treatment with *P. africana* extracts at doses less than the LD_{50} is in coherence with *in vivo* tests in rats. Concisely, *P. africana* stem bark water extracts did not cause clinical or pathological abnormalities in rats at daily doses of up to 1000 mg/kg for 8 weeks (Gathumbi et al., 2002). In view of the contention, *P. africana* extracts are evidently being used for treatment of prostrate hyperplasia as reported by Awang (1997). Despite the medicinal gains and advancement in utilization of *P. africana* extracts, the discourse points to the need for further validation of biochemical and hematological effects especially in cases of prolonged and repeated therapy.

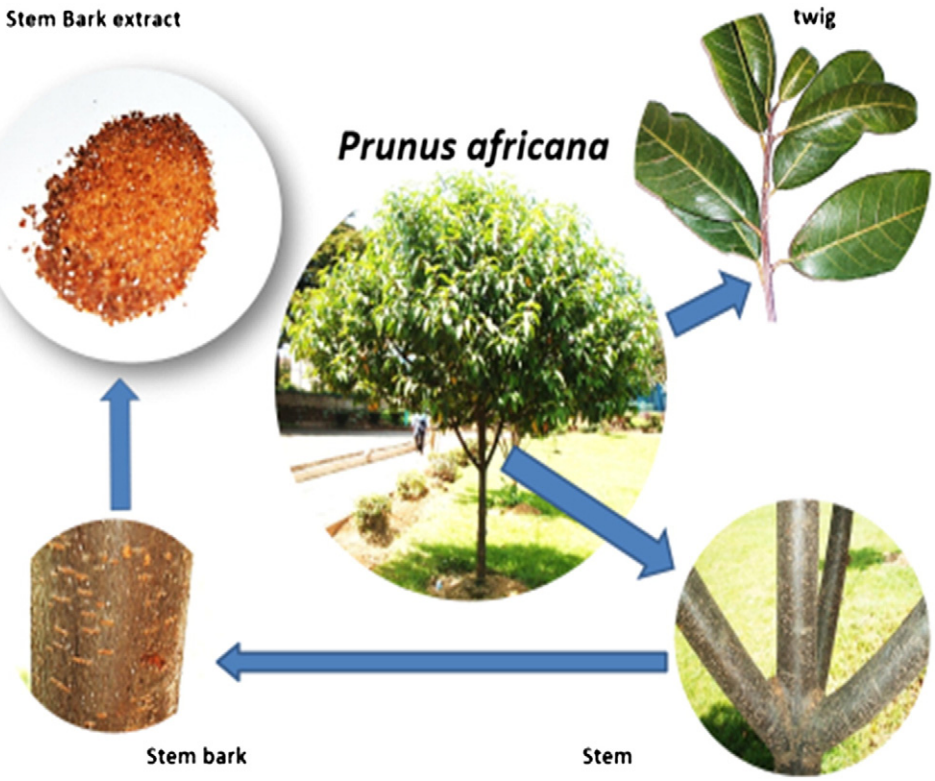
5. Conclusion and recommendations

In the present study, a preliminary determination of safety of the two selected medicinal plants commonly used by communities in Kenya for treatment of asthma was achieved in BALB/c mice. The findings revealed that the bark extract doses of *P. africana* and *W. ugandensis* administered had no obvious deleterious effect in the BALB/c mice. This information augments observational studies and forms a basis for further research for validation and development of the extracts as possible alternative therapies for management of asthma. To confirm on the non-toxic nature of *P. africana* and *W. ugandensis*, the effect that various factors such as the growth stage and maturity of the plant, the specific parts of the plant (leaves, roots, flowers and seeds), seasonal variations and storage conditions as well as where the plant is growing should be looked into. Further, investigations should be done using purified compounds and need to plan for future pre-clinical and clinical studies of the medicinal plants be considered.

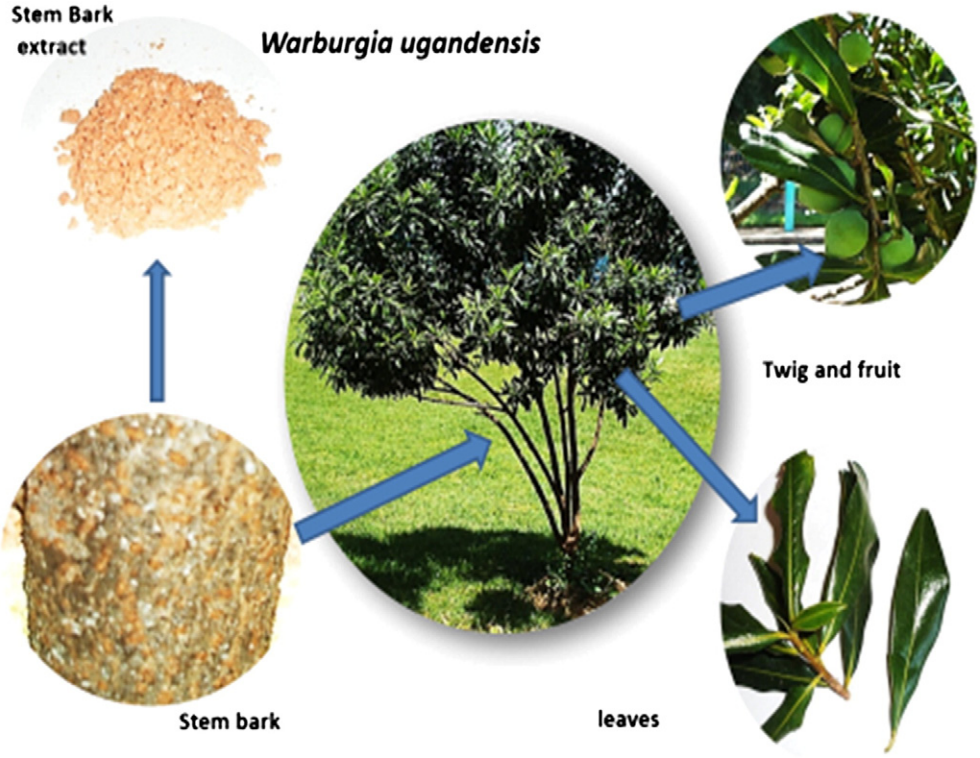
Acknowledgments

The authors wish to thank Mr. Lucas Ogutu in charge of KEMRI Animal house facility, Mr. Peter Mbugua and Mr. Enoch Moindi for technical assistance. We thank The KEMRI grantsmanship office for funding the work and the Director KEMRI for proving an enabling environment and permission to publish the work.

Appendix A. Photographs of *P. africana* and its stem bark water extract



Appendix B. Photographs of *W. ugandensis* and its stem bark water extract



Appendix C. Dose calculation in acute toxicity by Lorke's (1983) formula

$$r = \sqrt[n-1]{\frac{L}{l}}$$

Key:

r	ratio
n	number of doses (5)
L	largest dose level
l	smallest dose level

$$r = \sqrt[4]{\frac{5000\ \text{mg/kg/day}}{500\ \text{mg/kg/day}}} = \sqrt[4]{10} = 1.778$$

$$\text{Dose 1} = 5000\ \text{mg/kg/day},$$

$$\text{Dose 2} = \frac{5000\ \text{mg/kg/day}}{1.778} = 2812.148\ \text{mg/kg/day}$$

$$\text{Dose 3} = \frac{2812.14\ \text{mg/kg/day}}{1.778} = 1581.6358\ \text{mg/kg/day}$$

$$\text{Dose 4} = \frac{1581.6358\ \text{mg/kg/day}}{1.778} = 889.559\ \text{mg/kg/day}$$

$$\text{Dose 5} = 500\ \text{mg/kg/day}.$$

Appendix D. Transformation of percentages to probits

%	0	1	2	3	4	5	6	7	8	9
0	–	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
–	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.40	7.51	7.58	7.65	7.75	7.88	8.09

Appendix E. Calculation of amount of freeze dried extract per glass (250 ml glass)

P. africana: 150 g dry barks extracted with 1500 ml/6 glasses of water yields 13.452 g,

$$1\ \text{glass contains}\quad \frac{13.452\ \text{g}}{6} = 2.242\ \text{g of freeze dried stem bark extract.}$$

W. ugandensis: 150 g dry stem barks extracted with 1500 ml/6 glasses of water yields 19.481 g

$$1\ \text{glass contains}\quad \frac{19.481\ \text{g}}{6} = 3.247\ \text{g of freeze dried stem bark extract.}$$

Percentage quantity difference between *P. africana* and *W. ugandensis* freeze dried stem bark water extracts from 150 g of dry ground stem barks:

$$\frac{19.481-13.452}{150}\times 100 = 4.019\%.$$

Appendix F. Loomis and Hayes' (1996) classification of toxicity

1 or less	Extremely toxic
1–50	Highly toxic
50–500	Moderately toxic
500–5000	Slightly toxic
5000–15,000	Practically non-toxic
More than 15,000	Relatively harmless

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