African Journal of Pharmacology and Therapeutics Vol. 3 No. 3 Pages 85-94, 2014
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Research Article

In vivo antifertility activity and phytochemical screening of selected Kenyan medicinal plants

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Background: Medicinal plants are reported in folklore to play a role as fertility control agents. Very few studies have been carried out to confirm the safety and efficacy of medicinal plants used as anti-fertility agents.

Objective: To establish anti-fertility activity, safety, effect on genital organs and estrous cycle, and phytochemical profile of total extracts from *Terminalia brownii*, *Ximenia americana*, *Bridelia micrantha, Rhoicissus revoilii,* and *Ocimum masaiense*.

Methodology: Extracts of water and organic solvents were administered to female mice at a dose of 800 mg/kg orally for antifertility tests and at a dose of 0 to 5000mg/kg orally for acute toxicity test. Phytochemical screening was done using thin layer chromatography.

Results: The leaf water extracts of the *Bridelia micrantha*, *Ximenia americana* showed a reversible anti-fertility effect while ethyl acetate extracts of the stem bark of *Terminalia brownii* had an irreversible anti-fertility effect. The bioactive extracts had an effect on the estrus cycle and had different phytochemical compounds with no signs of toxicity.

Discussion: The plant extracts tested exhibited antifertility activity, suggesting potential alternative to the current birth control methods. Compounds such as steroids, terpenoids, alkaloids, saponins and flavonoids present in the bioactive extracts may have contributed to the anti-fertility activity.

Key words: Anti-fertility; *Bridelia micrantha*; *Terminalia brownii*; *Ximenia Americana;*

Received: July, 2014
Published: September, 2014

1. Introduction

In a report released by the United Nations (United Nations, 2011), 62.9% of the women in the reproductive stage worldwide use either a modern or a traditional method of birth control. While the greater number of them used modern contraceptives, 6.7% of these women used traditional methods. The number of women, married or in union, using birth control in Kenya is still low. Recent studies have indicated that more than 40% of births recorded in Kenya are unplanned on average, and this figure rises to 47% among teenagers. In Africa 22.2% of the population, still have unmet needs for family planning (African Population and Health Research Center, 2001; United Nations, 2011). High rates of discontinuation of these family planning methods and complaints about their discomforting side effects are indications that contraceptive methods need to be made more acceptable (World Health Organization, 1997). Some of the side effects that have been reported include stroke, risk of pelvic inflammatory disease (Jain, 2006), myocardial infarction, irreversibility (Rutagwera, 1990), high failure rate, and discomfort among others (Schwartz and Gabelnick, 2002).

Family planning in the rural parts of Kenya is usually based on the use of either medicinal plants or rhythm

 ISSN 2303-9841

method (African Population and Health Research Center, 2001). Rhythm method also known as Knaus-Ogino method relies on estimating a woman's likelihood of fertility, based on a record of the length of previous menstrual cycles. Pregnancy is achieved by timing unprotected intercourse for days identified as fertile, or avoided by restricting unprotected intercourse to days identified as infertile. Success of the rhythm method, however, is subjective and requires careful record keeping and diligence (Kimball, 2012). The use of medicinal plants as contraceptives has been reported (Ravichandran et al, 2007) but their safety and efficacy have not been carried out exhaustively.

Various plants have been studied for their anti-fertility effects. A strong anti-implantation and abortifacient activity have been reported in the hydroalcoholic extract of stem bark of *Ailanthus excelsa* (Roxb.) (Ravichandran et al, 2007). The effect of the extract of *Martynia annua* L. root on reproduction was studied on male rats where dose related reduction in the testicular sperm count, epididymal sperm count and motility, number of fertile males, ratio between delivered and inseminated females and number of pups were reported (Mali et al, 2002).

Ethanol extract from the seeds of *Jatropha curcas* L. have been reported to have antifertility activity when tested in adult female rats. The ethanol extracts halted the normal estrus cycle at diestrus phase as well as significantly reducing the weight of ovaries (Ahirwar et al, 2010). Goonasekera et al (1995) reported pregnancy-terminating effect of *Jatropha curcas* seeds in rats. In India, *Mimosa pudica* Linn (Fabaceae) is used traditionally as a birth control agent among rural people (Tiwari et al, 1982). The root extracts prolonged the length of the estrous cycle when administered orally at a dose of 300 mg/kg and reduced the number of litters in albino mice. Principal hormones analysis showed that the root extracts altered estradiol secretion and gonadotropin release (Ganguly et al, 2007). These root extracts have also been reported to significantly reduce the number of normal ova in rats (Valsala and Karpagaganapathy, 2002). A study conducted by Johri et al, (2009) reported a low anti-implantation and anti-fertility activity of between 19% and 38% when extracts from *Moringa oleifera*, *Momordica tuberosa* (Roxb.) and *Jasminum arborescens* (Roxb.) were administered to rats.

A number of plants were considered for antifertility activity testing in the current study. These plants include *Terminalia brownii* Fries (Combretaceae), *Ximenia americana* L. (Olacaceae), *Bridelia micrantha* (Hochst.) Baill. (Phyllanthaceae), *Ocimum masaiense Ayob.ex* A.J. Paton (Lamiaceae) and *Rhoicissus revoilii* Planch (Vitaceae).

*Terminalia brownii*is a leafy tree and has an attractive layered appearance. The tree has different traditional medical uses in the different areas where it is found (Fyhrquist et al, 2002). The leaves are used by traditional healers in Tanzania to treat diarrhea and stomachache, gastric ulcers, colic, and heartburn (Mbuya et al, 1994). In Kenya, extracts obtained from the leaves are reportedly used for birth control among the Mbeere ethnic community (Kareru et al., 2007). Parts of this plant are also used in the treatment of malaria by Kenyan traditional healers (Heine and Heine, 1988).

Ximenia americana is a semi-scandent shrub that is bush-forming or a small tree of not more than 7 m in height (Booth and Wickens, 1988). In Kenya, the leaves and twigs are traditionally used to treat colds, fevers, toothache, eye infection and as a laxative (Beentje, 1994). The leaves are also used for angina, headache and as poison antidote (Beentje, 1994). A paste prepared using the stem-barks is applied to skin ulcers and on the forehead to treat headaches (Kokwaro, 2009; Orwa et al, 2009).

Bridelia micrantha is a shrub or tree that grows to a height of 2-8 m. Extracts obtained from parts of this plant are used for headaches, eye infections, abdominal pains, constipation, common cold, and scabies, as an antidote, an antibortifacient and as a laxative (Duke, 2012).

Ocimum masaiense is a shrub with a height of up to 1.5 m (Paton et al, 2009). Extracts of *Ocimum masaiense* roots exhibited antinociceptive properties in the formalin test (Mwangi et al, 2011).

Rhoicissus revoilii is a woody climber with tendrils about 1-8 m in height (Verdcourt, 1993). The aerial parts are used as an antiseptic (Beentje, 1994). The roots are used in the treatment of wounds while the sap from the stem is applied to cuts, burns and sores. The root decoction is taken as a remedy for venereal diseases and bloody constipation (Shivalingappa et al, 2001).

Medicinal plants are reported in folklore to play a role as contraceptives. These claims and/or the mechanisms of action have not been demonstrated scientifically. Very few studies have been carried out to confirm the safety and efficacy of medicinal plants used as anti-fertility agents. Selection of the plants studied was was based on ethno-botanical information (herbalists who visited the Centre for Traditional Medicine and Drug Research (CTMDR), at the Kenya Medical Research Institute (KEMRI) Headquarters, Nairobi, Kenya) and literature (Kokwaro, 2009).

The study thus aimed to establish anti-fertility activity of total extracts from *Terminalia brownii* (stem bark), *Ximenia americana* (leaves), *Bridelia micrantha* (aerial parts), *Rhoicissus revoilii* (roots), and *Ocimum masaiense* (aerial parts), test for the safety of the bioactive extracts, their effect on the weight of the ovaries and uterus, and on the estrous cycle. The study also aimed at conducting phytochemical screening of the bioactive extracts..

2. Materials and Methods

2.1 Sample Collection and Preparation

Plant materials were collected from different areas of Kenya in Kajiado and Makueni counties, Kenya. A plant taxonomist was engaged during the field study to identify all the plant materials collected. Voucher specimens of each were then deposited at the East African Herbarium in the National Museum of Kenya. All the plant parts collected were washed, dried at room

temperature for two weeks, and weighed. The plant materials were then ground into powder using an electric mill and the powder stored in labeled airtight bags for storage ready for extraction. The voucher assigned to each of the specimens deposited at the East African Herbarium in the National Museum of Kenya were 711-*Ximenia americana* (Leaves), 712-*Bridelia micrantha* (Leaves), 743-*Ocimum masaiense* (Aerial Parts), 744-*Rhoicissus revoilii* (Roots), and 745-*Terminalia brownii* (Stem bark).

2.2 Extraction procedure

The ground materials were extracted using water and different organic solvents. The organic solvents included petroleum ether, dichloromethane, ethyl acetate and methanol. The sample (50-100g) was soaked in the methanol, petroleum ether, dichloromethane and ethyl acetate (approx. 200 mL) and left to stand for 48 hours. Samples were then filtered using Whatman® (No.1) filter paper. The filtrate was kept in a conical flask while the residues were re-soaked for another 48 hours. The samples were then filtered again, and the filtrate added to the previous filtrate. The filtrates were then concentrated under reduced pressure using a rotatory evaporator at controlled temperature of between 40- 60^{o}C and then transferred into weighed vials. The samples were then left to dry after which their weight was obtained. The percentage yield was calculated and recorded. Water extraction was done using water and the extracts were dried using a freeze-drying machine (Mallikharjuna et al, 2007). Fifty grams of the samples was weighed and transferred into a conical flask. The materials were then covered with distilled water (100 mL) and placed in the water bath at 60^{o}C for 2 hours. The samples were filtered and divided into two 50 ml portions and transferred into round bottomed flasks. The filtrate was frozen in an acetone-carbon-ice bath and freeze-dried using a freeze drying machine. The percentage yield of the extract obtained was calculated and recorded.

2.3 Animal Handling

Healthy Swiss mice of both sexes, weighing 21-25 g from KEMRI animal house were used in the study. The animals were housed spaciously in standard polypropylene cages clearly labeled with experimental details. The mice were maintained in a room temperature of about 22^{o}C and 60-70% relative humidity range appropriate for their species. This enabled them to acclimatize with minimal stress and physiologic alteration. The mice were fed on commercial rodent food and water *ad libitum* (Adeneye et al, 2006). A cannula was used for oral administration. The size of the needle that was used to draw blood was 25 gauge. After the experiment, the mice were sacrificed by euthanizing in diethyl ether. The euthanized mice were then placed in biohazard disposable bags for incineration. The study was done after obtaining clearance from KEMRI ethical review committee.

2.4 Extract Constitution

On the day of drug administration, each of the organic extract was freshly prepared by dissolving in a solution consisting of 70% Tween 80, 30% ethanol and diluted 10 folds with double distilled water. The aqueous extracts were prepared using distilled water (Houghton and Raman, 1998). Both the organic and water extracts were prepared to make a dosage of 800mg/kg (Mishra et al, 2009). A conventional drug (Femiplan®) was used as the positive control at a concentration of 90 mg/kg while the solvents used to dissolve the organic and water extracts were included as negative controls.

2.5 Fertility Test

The female mice were divided into 5 mice per cage. The male were introduced to virgin 8 weeks old female mice and were allowed to mate. After two weeks, the male mice were withdrawn after pregnancy was noted in the female mice. The expectant females were allowed to go to term and deliver and the number of pups was recorded (Mali et al, 2002).

2.6 Effects of extracts on fertility

Screening for Anti-fertility Effect in Mice

This study was done according to the method described by Ganguly et al (2007). Thirteen groups (each group containing 3 mature female mice) were selected for the study. Ten groups received the test drug at a concentration of 800mg/kg while three groups served as controls, the two different vehicles (double distilled water as negative controls) and a positive control (Femiplan® at a concentration of 90 mg/kg dissolved in double distilled water). Administration of the test drug and controls was first carried out continuously for 8 days before the introduction of males. All the experimental mice were then allowed to mate with mature fertile male mice, and the drug administration continued daily until the end of 21 days. The number of litters was determined after the completion of one gestation period of 21 days in all experimental groups.

Reversibility Test

The reversibility of the anti-fertility effect of the extract was also studied in the treated groups according to the method described by Salhad et al (1997). Briefly, the extract was administered continuously for 21 days, and then withdrawn. After 21 days of extract withdrawal, animals were allowed to mate with male mice. The number of litters was determined after the completion of one gestation period.

Effect of the Extract on the Estrous Cycle

Five mature female mice were employed for the study. Vaginal smears from each animal were examined under a microscope every morning for 21 days. This accounted for about 4 - 5 cycles. Each mouse was held in a supine position and the vaginal secretion was collected after cleansing with 0.2 ml of normal saline (NaCl 0.9%) contained in a smooth plastic pipette (Marcondes et al, 2002). Vaginal smears were assessed under a light microscope at x10 and x40 magnification once each day between 9.00 and 10.00 a.m. The smears were evaluated to determine the phases of estrous cycle

 ISSN 2303-9841

using the proportion of characteristic cell types such as the leucocytes, cornified and epithelial cells (Malaivijitnond et al, 2006; Abu and Uchendu, 2011). The duration of the estrous cycle together with that of the various phases was determined as described by Makonnen et al (1997) for 21 days. Drug administration was done at 800mg/kg orally for another 21 days and the same parameters were determined.

2.7 Effect of the Extract on the Weight of Genital Organ and Body Weight

The experiment was done according to Makonnen et al (1997) with some few modifications. Five groups of mature female mice were employed. The experimental mice received the extract for 10 days through the oral route. The control group received the vehicle for the same number of days by the same route. On the 11th day, all the animals in all the groups were weighed and sacrificed using diethyl ether anesthesia. The ovaries and uteri were dissected out, separated from the surrounding tissues, and then blotted on aluminum foils (Gebrie et al, 2005). The organs were weighted and a ratio calculated by dividing the weight of the ovary, as well as, that of the uterine in milligrams by body weight in grams. The rise in the uterine ratio was an indication of the estrogenic effect of the extract as described by Vogel (1997).

2.8 Acute Toxicity Assay

The acute toxicity study of the plant extracts was done according to the method described by Mukinda and Syce (2007). The aqueous extracts were aseptically reconstituted in water while the organic extracts were dissolved in 10% Tween 80 and administered in single doses orally of 0, 1000, 2000, 3000, 4000 and 5000 mg/kg. The general behavior of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h with especially the first 4 hours (Hilaly et al, 2004), and then daily thereafter, for a total of 14 days. Tween 80 solution was used as the negative control. Changes in the normal activity of mice were monitored and the time at which signs of toxicity or death appeared recorded.

2.9 Phytochemical Screening of the Extract

Phytochemical screening of the bioactive extracts was done by use of simple qualitative and quantitative methods. The methods as described by Harborne (1998) with slight modifications were used to test for the presence secondary metabolites such as steroids, terpenoids, tannins, flavonoids, alkaloids, saponins, glycosides and reducing sugars in the test sample. The extracts were reconstituted using their respective extracting solvent and using a micro-capillary, and the sample was plotted on the thin layer chromatography (TLC) plate. The TLC plate was then placed in a tank with a suitable solvent system to enable separation of compounds in the extracts (Mallikharjuna et al, 2007). Dichloromethane (DCM) extracts used a solvent system of petroleum ether: DCM: Methanol in the ratio of 6:3:1. Petroleum ether extracts used a solvent system of petroleum ether: DCM in a ration of 7:3. Ethyl acetate extract used a solvent system of petroleum ether:DCM: methanol in a ratio of 5:3:2 while methanol extracts used butanol: acetic acid: water in the ratio of 4:1:1.

2.10 Statistical Analysis

The litter number and weight of the uterus and body weight ratio were expressed as mean ± standard deviation (S.D). A Significant difference between control and experimental groups was assessed by the use of Student's t-test while the analysis of variance (ANOVA) was used to assess any significant difference among the groups. The level of significance was set at ρ values less than 0.05.

3. Results

Percentage Yield

The weight of the extracts was determined in grams and the percentage yield from the plant parts calculated (Anokwuru et al, 2011). The percentage yields of extracts are shown in **Table 1**. In general extraction with water gave the highest percentage yield followed by methanol, dichloromethane, ethyl acetate and finally petroleum ether. The highest yield was the methanol extracts of the aerial parts of *Ximenia americana* (22.9%). *The* lowest percentage yield was recorded in the dichloromethane extract of the aerial parts of *Lippia kituensis* (0.1%). ND = Not Done.

Fertility Test

Determination of the fertile female mice gave a high rate of fertility in the female mice. Out of the 65 mice that were selected, 40 (61.5%) of them were confirmed fertile. The results of fertility test (**Table 2**) suggested that some of the mice were not receptive for the 21 days that the male mice were in the cage, and thus did not give birth during the first gestation period. The litter size per mouse recorded an average of 6 litters. The mice that gave birth were considered as fertile and were used in the proceeding anti-fertility screening of the candidate plants.

Screening for Anti-fertility Effect

At a dose of 800mg/kg, the organic extracts of the roots extracts of *Terminalia brownii*, aqueous extracts from the leaves of *Ximenia americana*, and aerial parts of *Bridelia micrantha* showed anti-fertility activity. However, extracts from *Ocimum masaiense* and *Rhoicissus revoilii* did not show anti-fertility activity at 800mg/kg. Femiplan™, used as a positive control also proved to reduce the fertility of mice at a concentration of 180mg/kg when dissolved in distilled water. The water extracts of stem bark of *Terminalia brownii* were toxic to the mice at 800 mg/kg. Consequently the dose for this extract was lowered to 600 and then 400 mg/kg at which the extract was still toxic. All the mice died within the first week of drug administration in all cases. The ethyl acetate extract of the stem bark of *Terminalia brownii,* and aqueous extracts from the leaves of *Bridelia micrantha* and *Ximenia americana* reduced the fertility of the female mice to zero indicating that they have promising anti-fertility activity.

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Fertile mice gave birth at an average of 7 litters per mouse. However, there was no significant difference observed in the litter size in any group. All delivered pups were normal and healthy. The results for the screening are as shown in **Table 3**.

Table 1: Percentage yield of the medicinal plant extracts used in the study

	Percentage Yield (%)				
Sample	**Methanol**	**DCM**	**Pet. Ether**	**Ethyl Acetate**	**Water**
Bridelia micrantha (Leaves)	16.4	4.6	3.0	3.9	10.5
Ocimum masaiense (Aerial Parts)	1.9	0.7	0.5	0.9	6.0
Rhoicissus revoilii (Roots)	4.0	7.5	1.1	1.0	8.3
Terminalia brownii (Stem bark)	14.3	1.769	0.3	4.4	16.6
Ximenia americana (Leaves)	22.9	ND	ND	ND	18

Table 2: Fertility of female mice after one gestation period (Male: Female ratio, 1:5)

Group	No. of mice per groups	No. of fertile mice	Total Litter	Litter size ±S.D
1	5	3	16	5.33±0.58
2	5	4	24	6.00±0.82
3	5	3	18	6.00±1.0
4	5	3	18	6.00±1.0
5	5	3	11	3.67±0.58
6	5	4	31	7.75±1.26
7	5	4	23	5.75±0.96
8	5	4	19	4.75±0.96
9	5	3	24	8±1.00
10	5	2	16	8±0.71
11	5	3	30	10±1.00
12	5	2	10	5±1.41
13	5	2	9	4.5±0.71

Reversibility Test

The bioactive extracts in the screening process were subjected to a reversibility test to check for the reversibility of their anti-fertility effect.

From the experiment, water extracts from the leaves of *Bridelia micrantha* and *Ximenia americana* had reversible anti-fertility effect (**Table 4**).

However, ethyl acetate extract of the stem bark of *Terminalia brownii* had a permanent anti-fertility effect and mice that were treated with the extract did not give birth after withdrawal. The group that was administered with Femiplan™ also showed reversibility after the withdrawal of the drug. The effect of *Bridelia micrantha* and *Ximenia americana* was reversible in that the mice gave birth after the test extracts were withdrawn.

Effect of the Extracts on the Estrous Cycle

Administering the bioactive extracts of the water extracts of the leaves of *Bridelia micrantha* and *Ximenia americana* arrested the cycle of the mice at the diestrus phase and at the proestrous phase. Mice in these groups were in the proestrus phase for 5 and 8.33 days and diestrus phase for 16 and 12.67 days in *Bridelia micrantha* and *Ximenia americana* respectively. The mice did not show either the estrous or the metestrus phase during the 21 days of test extract administration (**Table 5**). Most of the days during the administration of ethyl acetate extracts of *Terminalia brownii,* the mice exhibited diestrus phase followed by proestrus.

Most mice exhibited either estrus or metestrus phases during the first days of test extracts adnimistration probably before the drug could take effect. The extracts thus exhibited strong anti-estrogenic property.

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Table 3: Fertility of female mice after 21 days of treatment with 800mg/kg of the test drug extract (Male: Female ratio, 1:3)

Drug	Solvent	Drug Dose mg/kg	No. of fertile/ Total	Total no. of pups	Litter size ±S.D
Terminalia brownii (stem bark)	Ethyl acetate	800	0/3	0	0
Terminalia brownii (stem bark)	Water	800	0/0	0	0
Ximenia americana (leaves)	Methanol	800	3/3	21	7±1.00
	Water	800	0/3	0	0
Rhoicissus revoilii (Root)	Methanol	800	2/3	14	7.00±0.50
	Water	800	2/3	16	8.00±1.00
Bridelia micrantha (Aerial)	Methanol	800	2/3	10	5.00±1.00
	Water	800	0/3	0	0
Ocimum masaiense (Aerial)	Methanol	800	2/3	18	9.00±1.00
	Water	800	2/3	12	6.00±1.00
Negative control	Water	N/A	3/3	24	8.00±0.58
	Tween 80	10 %	3/3	21	7±1.53
Positive control (FemiplanTM)		90	0/3	0	0

Table 4: Fertility of female mice after 21 days of treatment and then withdrawal of the treatment

Drug	Solvent	Drug Dose mg/kg	No. of fertile/ treated	Total no. of pups	Mean Litter size ± S.D
Terminalia brownii (stem bark)	Ethyl Acetate	800	0/3	0	0
Bridelia micrantha (Leaves)	Water	800	3/3	16	5.3±1.15
Ximenia americana (leaves)	Water	400	3/3	17	5.6±0.58
Femiplan	Water	90	3/3	22	7.3±0.58

Table 5: Effect of the active extract on estrus cycle of female mice (n = 3)

Phases (days)	Control	*Bridelia micrantha* (Leaves)	*Ximenia americana* (leaves)	*Terminalia brownii* (stem bark)
Estrous cycle	4.33	0	0	0
Proestrus	1.15	5	8.33	7
Estrus	1.46	0	0	0.33
Metestrus	1.23	0	0	0.33
Diestrus	1.08	16	12.67	13.33

The data is represented in days and shows days that each phase took in one cycle

 ISSN 2303-9841

Table 6: The effect of the extract in comparison with that of control on fresh weight of mice uterus and ovaries

Treatment	Ovary/Body Weight± S.D (mg/g)	Uterus/Body Weight ± S.D (mg/g)
Control	0.474±0.086	1.900±0.369
Bridelia micrantha (Leaves)	0.378±0.090	1.464±0.349
Ximenia americana (Leaves)	0.578±0.084	2.003±0.569
Terminalia brownii (stem bark)	0.376±0.140	1.547±0.594

Data are expressed as uterine or ovarian ratio ± S.E.M, with n=5

Effect of the Extracts on the Weight of Genital Organ and Body Weight

Oral administration of the water extracts of the leaves of *Bridelia micrantha*, ethyl acetate extracts of the stem bark of *Terminalia brownii,* and the water extracts of the leaves of *Ximenia americana* did not have a suppressive effect on the ovary as well as the uterus. The data was expressed in mg/1000 gm of body weight (**Table 6**). The administration of water extracts of *Bridelia micrantha* (leaves), ethyl acetate extracts of *Terminalia brownii* (stem bark), and water extracts of *Ximenia americana* (leaves), at a concentration of 800mg/kg for 10 days did not affect the uterine wet weight ($\rho > 0.05$), as well as, the fresh weight of the ovaries ($\rho > 0.0.5$). There was also no significant difference among the different treatments at $\rho=0.05$ significant level on the size of the ovary with a ρ value of 0.0611. In the same way, there was no significant difference among the different treatments at ($\rho > 0.05$) significant level on the size of the uterus with a ($\rho > 0.05$).

Acute Toxicity Assay

In this study, there were no deaths or any signs of toxicity observed after the oral administration of the water extracts of the leaves of *Bridelia micrantha* at any dose level up to the highest dose tested (5000 mg/kg). There were no deaths or other toxicity signs after the oral administration of the ethyl acetate extract of the stem bark of *Terminalia brownii* were administered at any dose level up to the highest dose tested (5000 mg/kg). The water extracts of the leaves of *Ximenia americana* had a mortality rate of 0% up to a dose of 4000 mg/kg and of 20% at 5000 mg/kg. The mortality at 5000 mg/kg was noted after 48 hours after drug administration.

Phytochemical Studies of the Extract

The TLC and qualitative studies of the extract revealed the presence various compounds. Phytochemical analysis revealed the presence of terpenoids and steroids in the leaves of *Bridelia micrantha*. The stem bark of *Terminalia brownii* contains saponins, flavanoids, triterpenoids, terpenoids and steroids. *Ximenia americana* leaves contained terpenoids, glycosides, steroids, phenols and triterpenoids. However, anthraquinones, cardiac glycosides and alkaloids were not present in any of the extracts

4 Discussion

The methanol extracts generally gave the highest yield for most of the plant parts as compared to the other organic solvents, while ethyl acetate gave the least. For example, *Bridelia micrantha* yielded 16.384% using methanol and 3.948% using ethyl acetate. Water extraction gave higher yields than the organic solvents, underscoring its efficiency as a universal solvent (Kolb et al, 2001).

The result of the percentage yield provides a suggestion that methanol is a better organic solvent for the extraction of aerial parts of *Bridelia micrantha*, stem barks of both *Terminalia brownii* and *Ximenia Americana,* the leaves and the root bark of *Ximenia americana.* The results of higher yield using methanol have also been reported (Singh et al, 2002; Anokwuru et al, 2011) and this is usually due to the high polarity of methanol as compared to other solvents. The use of methanol alone as an extraction solvent resulted in greater yield when compared to a mixture of different solvents (Jayaprakasha et al, 2001).

Oral administration of 800mg/kg water extracts of *Bridelia micrantha* and *Ximenia americana,* as well as ethyl acetate extracts of *Terminalia brownii,* demonstrated promising anti-fertility activities. Administering these extracts to the mice did not show a significant difference in the uterine or even ovarian ratio from the control group. This indicates that the extracts have no estrogenic effect. The extracts also arrested the cycle of the mice at the diestrus and proestrus phases. This suggests that the extracts prevent the ovaries as well as the uterus from undergoing the normal preparations during the estrous cycle. This could be due to the presence of high level of compounds such as saponins (Oluyemi et al, 2007). The results of this study confirm the reports of the ability of some plant extracts to prolong the estrus cycle, as well as the diestrus phase of the cycle (Shibeshi et al, 2006). However, Shukla et al (1987) reported complete abolition of the proestrus phase, shortened diestrus and prolonged estrus phases of the cycle after butanolic extract of *Pueraria tuberosa* was administered in rats. Shivalingappa et al (2001) also reported similar observations on the prolongation of the estrous cycle when ethanolic extract of *Rivea hypocrateriformis* was administered in rats. Similarly, extract of *Anethum graveolens* prolonged the estrus cycle in rat (Monsefi et al, 2006). Prolonging the cycle reduces fertilization in the affected experimental animals (Uchendu et al, 2000).

 ISSN 2303-9841

The phytochemical screening indicated the presence of compounds such as steroids, terpenoids, and alkaloids in the extracts. Some of these compounds exhibit anti-fertility activity (Hiremath and Rao, 1990) and the effect of these extracts may be due to the presence of one or more of these compounds. Omer and Elnima (1999) have also reported the presence of abundant amount of saponins and flavonoids in stems of *Terminalia brownii.* These compounds in *Terminalia brownii* could be responsible for the 100% fertility reduction in the experimental mice.

The acute toxicity study shows that an aqueous extract of the *Bridelia micrantha* and the ethyl acetate extract of *Terminalia brownii* stem bark are non-toxic via the oral route in mice at least up to the maximum doses. However, water extracts of the leaves of *Ximenia americana* showed a low level of toxicity at the highest dose tested. The no-observed-adverse-effect level (NOAEL) (Alexeeff et al, 2002) for *Bridelia micrantha,* and *Terminalia brownii* may thus be said to be 5000mg/kg. Unlike the methanolic extract of the bark of the *Terminalia brownii* that has been reported, to have mortality of 66% at a dose of 1000mg/kg in rat orally (Thoria et al, 2012); the ethyl acetate extract of the same was safe at the highest concentration of 5000mg/kg. Differences in toxicity could have been as a result of the differences in the compounds extracted by the different solvents. Compounds extracted by ethyl acetate may be safer than those extracted using methanol. In a previous toxicity study, methanol extracts of the leaves of *Ximenia americana* showed that the extract is not toxic where it caused no death up to a concentration of 5000 mg/kg orally (Siddaiah et al, 2011).

5. Conclusion

From the results of the study, it can be concluded that the extracts of the leaves of *Bridelia micrantha* and the stem bark of *Terminalia brownii* have promising anti-fertility effect. The study of the effect of the active extracts of the estrus cycle confirmed that the extracts could control fertility by having the anti-estrogenous activity in mice. The presence of one or more compounds detected such as steroids, terpenoids, alkaloids, saponins, flavonoids in the bioactive extracts may be contributing to the anti-fertility activity, and hence, further phytochemical investigations should be carried out to establish the compounds present in the bioactive extracts. The weight of the uterus, as well as that of the ovaries, did not change significantly; hence the extracts may be said to be safe to the reproductive organs of the mice. These active extracts did not show severe signs of toxicity at the highest concentration tested, (5000mg/kg) and their LD_{50} is thus considered higher than 5000mg/kg. These results indicate potential of the extracts as alternative fertility control agents and investigations are warranted with the aim of developing affordable and alternative herbal contraceptive.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

We would like to thank the National Commission for Science Technology and Innovation for funding the project. In addition, we thank all the staff members at the Center for Traditional Medicine and Drugs Research in Kenya Medical Research Institute and the University of Eldoret staff for their much valued support.

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