Contraceptive and Phytochemical Profile of Lime-Yellow Pea (*Macrotyloma axillare*, E. Mey) Verdc: A Tropical Climber

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RSO and DWO designed the experiments. Author RSO carried out the experiments and wrote the manuscript. Authors PGK, EKM, and DWO supervised the work and provided the facilities for the study. The editing and approval of the final manuscript was done by all the authors.

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ABSTRACT

Background: Rising human population has detrimental effects on quality of life and lead to increased cases of pollution and poverty. Modern forms of contraceptives also have severe side effects and result in high cases of unwanted pregnancies. As a result, several cases of illegal abortion, leading to serious health complications have been reported, and more than 30 million US dollars are used to treat post-abortion complications in Kenya annually. Herbal contraceptives offer

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alternatives to health problems, and this study was novel with significant findings, since little information exists about the pharmacological properties of *M. axillare*, unlike its closely related species, *M. uniflorum*, which has been extensively studied and established to have great pharmacological potential.

Aims: To establish and validate the use of *M. axillare* extracts as traditional contraceptives and also to profile the phytochemicals responsible for its contraceptive activity.

Place and Duration of Study: Department of Veterinary Anatomy and Physiology, University of Nairobi, Kenya.

Methodology: The contraceptive effects were investigated by *in vivo* studies using Virgin Wistar female rats (*Rattus norvegicus*) with regular oestrus cycle. Qualitative and quantitative phytochemical profiling was carried out using standard procedures while GC-MS analysis was employed in the identification of compounds present in the methanolic extracts.

Results: Macroscopic and microscopic examination of female reproductive tract revealed reduced ovary size, involuted uterus and persistent corpus luteum in the ovary and no births were recorded in groups treated with higher doses of the plant extracts. The presence of saponins, alkaloids, steroids and terpenoids, as well as phenolic compounds like tannins and flavonoids, which could be responsible for the contraceptive activity observed, either acting independently or synergistically, was confirmed through qualitative phytochemical analysis. GC-MS profile also led to the identification of compounds that included sitosterol, stigmasterol, stigmasta-3,5-dien-7-one, stigmastan-3,5-diene and campesterol that have structural similarities to known reproductive hormones and conventional contraceptives and could, therefore, be responsible for the observed contraceptive activity of the plant extract.

Conclusion: The tested methanolic extracts have a potential contraceptive activity that warrants further investigations towards the development of novel plant-based contraceptives.

Keywords: Contraceptive; population; phytochemicals; estrous; antifertility.

1. INTRODUCTION

Rising human population has serious repercussions on life supporting systems leading to increased pollution and excruciating poverty [1]. Consequently, control of population growth is an important facet of global health concern and use of contraceptives forms a vital component of reproductive health [2]. Several approaches for induction of infertility, including hormonal, chemical, surgical and immunological have been evaluated [1]. Unfortunately, these methods of contraception lead to side effects that include hormonal imbalance, hypertension, increased risk of cancer, weight gain and stroke. Other side effects include pelvic inflammatory disease, myocardial infarction, irreversibility, discomfort and even lead to unacceptable rates of unwanted pregnancies [3,4,5,6]. Moreover, these conventional contraceptives are not readily affordable or available to most women, especially those in developing nations and are also not culturally acceptable in some communities. Therefore, this calls for research for alternative substitutes that are more potent, safer, affordable and require less administration frequency but with long lasting and reversible antifertility effects. Natural products pose as potential candidate substitute. Therefore, screening of

plants with antifertility activity and the subsequent identification and characterization of active components may provide leads for the formulation of cheaper and affordable contraceptive with fewer side effects.

A report released by the United Nations [7] revealed that 37.1% of women in the reproductive stage worldwide do not use either modern or traditional methods of birth control. The number of women using birth control in Kenya is still low and studies have shown that more than 40% of births recorded in the country are unplanned and, for teenagers, this rises to 47%. 22.2% of the population in Africa still have unmet needs for family planning [8] and cases of illegally procured abortions are also high, especially among teenagers. More than 450,000 abortions are procured annually in Kenya, with about 120,000 cases of post-abortion complications reported. The government of Kenya spends over 30 million US dollars in treating post-abortion complications; an amount that could be channeled towards solving other pressing health needs of the country, such as treatment of cancer. Therefore, promotion of contraceptive use is vital, both for maternal health and for the economic development of the country. Documentation on the use of medicinal

plants as contraceptives exists [9], but exhaustive evaluation of their safety and efficacy has not been done. Medicinal plants contain secondary metabolites such as tannins, alkaloids, terpenoids, steroids and flavonoids among others which have definite physiological action on humans [10]. Plant phytochemicals are derived from barks, leaves, flowers, roots, fruits as well as seeds [11]. As a result of better cultural acceptability, better compatibility with the human body, lesser side effects, and greater effectiveness, traditional medicine is an accepted fact, and more than 35,000 plant species are in use around the world for medicinal purposes [12].

Macrotyloma axillare (E. Mey.) Verdc., also known as lime-yellow pea, perennial horse gram, *Dolichos axillaris* or *Archer axillaris* belongs to the genus *Macrotyloma* and family Fabaceae whose members comprise of edible and non-edible legumes. It is a perennial trailing and twining plant native to Africa, parts of Asia, Middle East and some islands in the Indian Ocean but naturalized in Australia and Papua New Guinea [13]. *M. axillare* is widely used as fodder [14] and, in some instances; it has found use in the treatment of malaria [15]. There is, however, a dearth of information regarding its medicinal value even though closely related plants have been shown to have profound and diverse medicinal effects. In a study investigating the abortifacients of plant origin in Tanzania, extracts of *M. axillare* did not show any appreciable *in vitro* effects on either the magnitude or force of uterine contraction in rats [16]. Nevertheless, *M. uniflorum*, a plant in the same genus, and closely related to *M. axillare*, has been reported to cause reduction in sperm count, motility, fertility and viability as well as increase in the number of abnormal sperms in male rats [17,18]. In addition, its extracts have been shown to deplete androgens in cauda epididymis, hence, interfering with sperm maturation [19]. In India, women always avoided contact with *M. uniflorum* during pregnancy because it was thought to instigate abortion [20]. On the other hand, extracts of this plant found use in treating menstrual disorders, bleeding during pregnancy, leucorrhoea and excessive postpartum discharges [21]. Furthermore, *in vivo* studies have shown that methanolic seed extracts of *M. uniflorum* lowers lipid levels of rats fed high-fat diet [22]. Seed decoction of this plant also possess anti-arthritis [23], anti-urolithic [24] and hypoglycaemic [25] properties. Additionally, it has shown antihelminthic, antidiabetic,

antioxidant, antibacterial and antifungal activities [26]. These properties are mainly attributed to the phytochemicals found in the plant including alkaloids, saponins, flavanoids, phytosterols, tannins, fatty acids and terpenoids [27]. In addition, dolichin A and B, pyroglutaminylglutamine, kaempferol-3-O- β -D-glucoside, stigmasterol, β -sitosterol, and other phenolic compounds have also been isolated [27]. These compounds possess diverse pharmacological effects, including estrogenic activity [27], hence, their potential to be used as contraceptives.

In vitro and *in vivo* contraceptive activity of other plant species in the family Fabaceae have also been evaluated and found to be highly effective. For example, *Dolichos lablab* and four other plants in a mixed form has been shown to modulate morphological changes of the endometrial surface in adult rats when treated orally [28]. *Abrus precatorius* exhibit both anti-implantation and antifertility effects and abridine, isolated from the plant, showed 100% contraceptive activity in female rats [29,30]. Butin, isolated from seeds of *Butea monosperma*, possessed anti-implantation activity in female rats leading to a reduction in the number of implantation sites, and since it is a weak estrogen, it also had a significant uterotrophic effect [31]. *Acacia arabica* and *A. catechu*, have been reported to be effective oral contraceptives in rats through inhibition of implantation while *A. concinna* has spermicidal activity [32]. Other plants in the family Fabaceae whose contraceptive activity have been evaluated include *Cassia fistula* (anti-estrogenic), *Derris brevipes* (anti-implantation and abortifacient), *Sesbania sesban* (anti-implantation), *Trigonella foenum-graecum* (anti-estrogenic), *Afromosia laxiflora* (antigonadotrophic, disrupts estrous cycle), and *Crotalaria juncea* (anti-implantation) among others [33].

Based on the foregoing, *M. axillare* extracts were also expected to exhibit contraceptive activity since, in most cases, presence of certain secondary metabolites is usually genus or family-specific. Moreover, phytochemicals such as alkaloids, saponins, flavonoids, tannins, terpenoids and phytosterols have been confirmed to be present in *M. axillare*. So far, no scientific study has been done to validate the use of *M. axillare* as a herbal contraceptive. The purpose of this study was, therefore, to establish the potential of this plant as a contraceptive

agent and identify the secondary metabolites likely to be responsible for the observed activity.

2. METHODOLOGY

2.1 Sample Collection, Preparation, and Extraction

Leaves and stem samples of *M. axillare* obtained from Ugunja sub-county of Siaya County, Kenya were botanically identified and authenticated at the University of Nairobi herbarium, where voucher specimens were also deposited (RSO/2016/001 and RSO/2016/002). The plant samples were washed thoroughly in water, chopped, air dried for two weeks, pulverized in an electric grinder and exhaustively extracted using analytical grade methanol (Sigma-Aldrich). The extracts were concentrated using a rotary evaporator, dried and stored at 4°C until required for bioassay.

2.2 Evaluation of Contraceptive Activity

2.2.1 Animal care

The Biosafety, Animal Care and Use Committee (BACUC) of the Faculty of Veterinary Medicine (FVM), University of Nairobi approved this study, which was conducted based on The Basel Declaration on animal use. Thirty five (35) virgin female albino rats (*Rattus norvegicus*), colony bred and approximately twelve weeks old were employed for this study. The rats weighed between 190 and 200 g and exhibited regular estrous cycles. Seven (7) male rats of proven fertility were also used in the study, and all the animals were housed in cages at controlled room temperature (24±2°C) and 12 h light and 12 h darkness. They were fed pelleted standard rat feed (Unga Feeds Ltd, Nairobi) and provided with water *ad libitum*.

2.2.2 Experimental design

Thirty five (35) adult female rats with regular estrous cycle confirmed by daily vaginal smear analysis were used for this assay. Animals were identified based on the presence of two consecutive 4 - day estrous cycle. The animals were randomly divided into seven (7) treatment groups each comprising five animals while the extracts were reconstituted in distilled water to various concentrations before oral administration. Group, I animals were orally administered distilled water (2 mL) only and served as control. Groups II, III and IV orally

received 2 mL of 50, 100 and 200 mg/kg bwt of leaves methanolic extracts respectively while groups V, VI, and VII animals were orally given 2 mL of stem methanolic extracts at the same respective dosage rates.

The animals were treated three times a week for two weeks, at the end of which fertile males were introduced; one male per group and allowed to cohabit with the females for the entire duration of the study (3 months) which was equivalent to four gestation periods. The administration of the extracts (3 times a week) continued even after the introduction of the males for the entire duration of the experiment. Mating was confirmed by the presence of dead sperm cells in the vaginal smear [34]. The animals were regularly observed for pregnancy signs and litter size in cases where births were observed. The number of pregnant animals per group was noted and presented as percentages while for each group, the average litter size was calculated.

2.2.3 Histological evaluations

During the termination of the experiment, the animals were anesthetized using diethyl ether, abdomen opened through the ventral midline and the ovaries randomly selected. The tissues were first examined for pathological lesions before being fixed in 10% buffered formaldehyde solution for 24 hours. Subsequently, the tissues were dehydrated in graded concentrations of alcohol, cleared in xylene and embedded in paraffin wax. The paraffin blocks were sectioned using a rotary microtome (LEICA RM 2255) at 7 µm thickness. Haematoxylin and eosin were used for staining of the tissue sections followed by examination and photography under Leica microscope.

2.3 Phytochemical Profiling

2.3.1 Qualitative analysis

Screening for the presence of saponins, tannins, flavonoids, alkaloids, triterpenes and sterols was done using standard phytochemical procedures [35,36].

2.3.2 Quantitative analysis

2.3.2.1 Saponins

20 g of ground plant sample was put into a conical flask, and 100 mL of 20% C₂H₅OH added to the plant sample. The mixture was heated at

55°C for four hours in a water bath with continuous stirring then filtered. The residue was re-extracted twice using 100 mL of 20% C₂H₅OH then filtered. The filtrates were combined then reduced to 40 mL over a water bath at 90°C. The reduced, concentrated extract was then transferred into a 250 mL separating funnel and 20 mL of diethyl ether added to the extract and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process repeated thrice. 60 mL portions of butanol were then added thrice, and the combined butanol extracts washed twice with 10 mL portions of 5% sodium chloride. The extract was subsequently heated in a water bath and after evaporation; the samples were dried in the oven to a constant weight. The weight was expressed as mg/g of the sample weight analyzed. The experiment carried out in triplicate and the findings recorded as the average of the three replicates [37].

2.3.2.2 Alkaloids

The alkaloid content was determined gravimetrically [35]. 5 g of the sample was weighed into a beaker and 200 mL of 10% acetic acid in ethanol added to the sample. The mixture was shaken well, covered and allowed to stand for four hours. After that, it was filtered and the extract concentrated in a water bath till it reached ¼ of the original volume. The alkaloids were precipitated using concentrated ammonium hydroxide that was added dropwise. A pre-weighed filter paper was used to filter off the precipitate, which was then washed using 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60°C for 30 min to a constant weight before being transferred into a desiccator to cool. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as mg/g of the sample weight analyzed. The experiment was performed in triplicate, and the result recorded as the average of the three replicates.

2.3.2.3 Total phenols

Total phenol content was determined using Folin-Ciocalteu Reagent (FCR) as described by Makkar [38]. 200 mg sample weighed into a glass beaker of approximately 25 mL capacity. 10 mL of aqueous acetone (70%) was then added, the beaker suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20 min at room temperature. The content of

the beaker was then transferred to centrifuge tubes, cooled by keeping the centrifuge tubes on ice centrifuged for 10 min at approximately 3000 g (Centurion 6000 Series) and the supernatant collected and kept on ice. 0.1 mL portions of the supernatant were aliquoted into test tubes, and the volumes made up to 0.5 mL using distilled water. 0.25 mL of 1N FCR was added followed by 1.25 mL of Na₂CO₃ solution. The tubes were then vortexed and absorbance recorded at 725 nm after 40 min. The concentration of total phenols in mg/g tannic acid equivalents (TAE) was calculated from the calibration curve established using tannic acid as standard. The samples were prepared in triplicate for each analysis and the mean value of absorbance obtained.

2.3.2.4 Total flavonoid

The total flavonoid content of each sample was estimated by the method described by Zhishen et al. [39]. 1.0 mL of each sample was mixed with 4 mL distilled water and subsequently with 0.30 mL of 10% NaNO₂ solution. After 5 min, 0.30 mL of 10% AlCl₃ solution followed by 2.0 mL of 1% NaOH solution were added to the mixture. Immediately, the mixture was thoroughly mixed and absorbance determined at 510 nm versus the blank. A standard curve of catechin was prepared (0-1.25 mg/mL) and the flavonoid concentration expressed as catechin equivalents (mg catechin/g dried sample).

2.4 GC-MS Analysis of the Extract

2.4.1 Sample preparation and analysis using GC-MS

One milligram of each of the sample was weighed (in triplicates) and dissolved in 1 mL dichloromethane. The samples were vortexed for 10s, ultra-sonicated for 1 h, centrifuged at 14,000 rpm and the supernatant dried through anhydrous Na₂SO₄. Centrifugation was further done at 14,000 rpm before analyzing with GC-MS.

2.4.2 Instrumental conditions

The samples were analyzed by GC-MS on a 7890A gas chromatograph linked to a 5975C mass selective detector. The inlet temperature was 270°C while the transfer line temperature was 280°C. The column oven temperature was programmed between 35 and 285°C and the initial temperature was maintained for 5 minutes

then maintained at to 10°C/ min to 280°C where it was held for 30 mins. Low bleed capillary column (HP-5 MS, 30 m × 0.25 mm i.d., 0.25 µm) was used while helium was used as carrier gas at a flow rate of 1.25 mL/min. The mass selective detector, with an ion source and quadrupole temperatures, maintained at 230°C and 180°C, respectively, was employed while electron impact (EI) mass spectra were obtained at the acceleration energy of 70 eV. An aliquot of the extract (1.0 µl) was injected in the split/splitless mode and fragment ions analyzed, in full scan mode, over 40 – 550 m/z mass range. The filament delay time was set at 3.3 min for the samples. Identification of the structures of the compounds was based on a comparison of their fragmentation patterns with reference spectra from Library–MS databases: National Institute of Standards and Technology (NIST) 11, 08 and 05, Adams and Chemical mass spectral databases.

2.5 Statistical Analysis

Data on litter size, quantitative analysis of phytochemicals and concentration of plant constituents as established by GC-MS are expressed as a mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Contraceptive effects

This is the first research that has attempted to scientifically and experimentally validate the use of *M. axillare* as a herbal contraceptive. During the experiment, the study animals exhibited good health, fed normally and no adverse effects were noted in any of the animals. The numbers of pregnant rats for each group, together with the

average litter sizes for the various treatment doses are as shown in Table 1.

The rats in the control group had two sets of litters during the whole duration of the study with an average litter size of 8.7±0.82. However, rats treated with the highest dose of both leaves and stem methanolic extracts (200 mg/kg bwt) did not litter for the entire duration of the study. For the medium dose (100 mg/kg bwt), an average litter size of 1.0±0.0 and 2.0±0.0 was recorded for leaves and stem methanolic extracts, respectively. These animals only littered once during the study period, unlike the control animals that had two sets of litters. Moreover, no implantation sites were observed at the time of termination of the study. For rats treated with the lower dose of 50 mg/kg bwt, the percentage pregnancy was 40% and 75% for leaves and stem methanolic extracts, respectively. However, even in these groups, the litter sizes were lower compared to rats in the control group. For both medium and lower doses, conception was fundamentally delayed and, for the entire duration of the study, only one full gestation period was observed.

3.1.2 Histological observations

Histologically, control ovary displayed normal and consistent progression of follicular development from primary follicles through secondary to mature Graafian follicle (Fig. 1a). Corpora lutea were also evident in normal proportions. The ovaries of animals treated with 50 mg/kg bwt of both leaves (Fig. 1b) and stem (Fig. 1e) methanolic extracts did not show any appreciable difference from the controls. All stages of follicular development were recognized. At the medium dose of 100 mg/kg bwt of leaves (Fig. 1c) and stem (Fig. 1f)

Table 1. Fertility and average litter size of female rats (Male: Female ratio, 1:5)

Plant extract	Dosages (mg/kg bwt)	No. of rats per group	No. of pregnant rats	% pregnancy	Total litters in 3 months	Average litter size ±SD
Leaves	50	5	2	40	3	1.5±0.71
methanolic extract	100	5	1	20	1	1.0±0.00
	200	5	0	0	0	0.00
Stem	50	5	3	60	5	1.67±0.58
methanolic extract	100	5	2	40	4	2.0±0.00
	200	5	0	0	0	0.00
Control	Control	5	5	100	87	8.7±0.82

The average litter size for the control is computed for two parturition cycles unlike the treatment groups that only littered once during the entire experiment period

methanolic extracts, there was evidence of slowed progression of follicular development with fewer or no secondary follicles transforming into mature Graafian follicles. However, animals treated with 200 mg/kg bwt of both leaves (Fig. 1d) and stem (Fig 1g) methanolic extracts showed predominance of corpora lutea and few or no developing follicles. Where there was follicular development as in stem methanolic extract treated animals (Fig. 1g), the development seemed to have been arrested at the secondary stage.

3.2 Phytochemical Profile of the Extracts

Qualitative phytochemical studies indicated the presence of flavonoids, tannins, alkaloids, saponins sterols and triterpenes in both leaves and stem methanolic extracts. The quantities of saponins, alkaloids, flavonoids and total phenols were established, and the results are as shown in Table 2. These phytochemicals have pharmacological properties that may be associated with the observed contraceptive activity of the plant extracts in the study.

Fig. 1. Photomicrographs of the ovaries of control (a) and female rats treated with 50 (b), 100 (c) and 200 (d) mg/kg bwt of leaves methanolic extracts and rats treated with 50 (e), 100 (f) and 200 (g) mg/kg bwt of stem methanolic extract of *M. axillare*

C: Corpus luteum; P: Primary follicle; S: Secondary follicle; G: Graafian follicle; IT: Interstitial tissue; A: Atretic follicle. All magnifications are at X40

Table 2. Quantities of phytochemicals with known contraceptive activity

Phytochemicals	Quantity of phytochemicals			
	Saponins (mg/g)	Alkaloids (mg/g)	Flavonoids (mg/g catechin equivalents)	Total phenols (mg/g tannic acid equivalents)
<i>M. axillare</i> leaves	35.44±4.99	13.74±2.34	2.48±0.05	0.23±0.023
<i>M. axillare</i> stem	17.92±1.45	10.00±1.64	2.21±0.04	0.12±0.067

3.3 GC-MS Profile of the Extracts

The GC-MS TIC profiles for the leaves and stem methanolic extracts of *M. axillare* are shown in Figs. 2 and 3 respectively. The identities of compounds present in these extracts, based on comparison with reference spectra are as shown in Table 3.

3.4 Discussion

In general, this study has shown that at higher dose rates, there was a reduction in the number of developing and mature Graafian follicles, the persistence of corpora lutea accompanied by increased number of atretic follicles in histological sections of the ovary. Phytochemical

Fig. 2. Total Ion Chromatogram (TIC) of leaves methanolic extract of *M. axillare* Fig. 3. Total Ion Chromatogram (TIC) of stem methanolic extract of *M. axillare*

Table 3. Identity and quantities of compounds present in leaves methanolic extract

S/N	Constituent	Leaves methanolic extract		Stem methanolic extract	
		RT	Average concentration (µg/mg)±SDEV	RT	Average concentration (µg/mg)±SDEV
1	Limonene	11.98	0.962±0.00114	11.975	0.44±0.00025
2	3-acetamido-s-Triazole,	16.275	2.932±0.00114	16.243	2.54±0.00025
3	DL-Proline, 5-oxo-, methyl ester	17.478	1.083±0.00114	17.441	1.54±0.00025
4	<i>trans</i> -Pinane	22.664	7.947±0.00114	22.663	1.19±0.00025
5	Methyl linoleate	25.182	8.807±0.00114	25.179	2.67±0.00025
6	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	25.245	13.363±0.00114	25.234	1.68±0.00025
7	Linoleic acid ethyl ester	-	-	25.782	3.71±0.00025
8	Eicosanoic acid, methyl ester	27.201	7.857±0.00114	-	-
9	1,2,5,8-tetrahydroxy-9,10-Anthracenedione	-	-	29.99	2.24±0.00025
10	Farnesoic acid<2E,6E->	31.355	6.225±0.00114	31.349	4.83±0.00025
11	Stigmastan-3,5-diene	35.01	3.023±0.00114	35.0	5.75±0.00025
12	Campesterol	37.625	3.418±0.00114	37.554	12.30±0.00025
13	Stigmasterol	38.298	5.403±0.00114	38.227	12.22±0.00025
14	Sitosterol	39.611	16.371±0.00114	39.52	9.94±0.00025
15	β-Amyrin	40.383	10.964±0.00114	-	-
16	α-amyrenone	-	-	40.733	10.33±0.00025
17	α-Amyrin	41.436	8.203±0.00114	41.417	10.52±0.00025
18	Stigmasta-3,5-dien-7-one	41.649	8.969±0.00114	41.659	10.70±0.00025

RT: Retention time

Most of the compounds identified were terpenoids, alkaloids, steroids and carboxylic acids

profiling of the plant has revealed that it contains phytoestrogenic substances in reasonable quantities that are likely to have influenced the fertility patterns in the animals under study. These phytoestrogenic substances include stigmastan-3,5-diene, stigmastan-3,5-dien-7-one, campesterol, stigmasterol, sitosterol, α-amyrenone, α-amyrin, and β-amyrin. Many compounds isolated from plants have been associated with contraceptive activity. The compounds often belong to classes of secondary metabolites that include flavonoids, terpenoids, saponins, steroids, and alkaloids. β-sitosterol and ergosterol peroxide isolated from *Ananas comosus* have abortifacient activity [40]. Other isolated compounds with contraceptive potential include; campesterol, chalinasterol, stigmasterol, oleanolic acid-3β-glucoside, 5,7,3'-trihydroxy-6,8,4'-trimethoxyflavones, n-hexacosanol, butin, and *p*-coumaric acid. Other compounds are aristolochic acid, 5α-stigmastane-3β,5,6β-triol-3-monobenzoate, ferujol, triterpene glycoside and cirantine [40]. Estrogenic substances cause infertility by shortening the time of transport of egg from the ovary, disrupting estrous cycle, expelling ova from the oviduct or blocking ovulation, inhibiting uterine development, lowering the plasma progesterone thereby

decreasing pregnanediol leading to termination of endometrium development [41,42]. Phytoestrogens bind or inactivate some enzymes causing modulation of endogenous estrogens. Hence, influence the bioavailability of sex hormones through binding or stimulation of the synthesis of sex hormone binding globulin [43]. Studies on alterations in normal cyclicity, reduction in the number of litters and implantation sites have been attributed to high saponin concentrations and other phytoestrogens in plants with known antifertility effects [44,45]. The steroidal structure of saponins are vital and are precursors for the hemisynthesis of birth control pills containing progesterone and estrogens as well as similar hormones and corticosteroids [46].

Phytochemicals such as saponins, tannins, steroids, alkaloids, glycosides, and terpenes have also been established to possess antiovarulatory and anti-fertility properties [47] while flavones and isoflavones on the other hand, exhibit estrogenic [48,49,50] and androgenic properties [51,52]. Alkaloids and flavonoids have also been shown to reduce the concentrations of reproductive hormones such as follicle stimulating hormone (FSH), luteinizing

hormone (LH), estrogen and progesterone [53-56]. Also, decreased aromatase activity due to alkaloids may lead to lower serum concentration of estradiol [57,58] consequently inhibiting ovulation, preparation of the reproductive tract for zygote implantation, and subsequent maintenance of the pregnancy state [59]. Probable reduction in the levels of FSH by the extracts may have hampered folliculogenesis and delayed maturation of the follicles in the pre-ovulatory phase [60]. It is also possible that the extracts may have exerted their effects on the anterior pituitary gland or the hypothalamus since the secretion of FSH is regulated by the gonadotropin-releasing hormone (GnRH) [61]. Other studies have also demonstrated that LH surges at the proestrus stage are responsible for ovulation [62,63] and any substance that inhibits this release could disrupt ovulation by reducing the number of mature follicles or inducing disruption of estrous cycle at rest [64]. Therefore, the reduction in the number of mature follicles may be explained by an inhibitory effect of the extracts on the release of LH which may have triggered disruption of ovulation, leading to impairment of estrous cycle; thereby inhibiting conception and normal reproduction in the females. The possible reduction in the levels of progesterone by *M. axillare* leaves and stem methanolic extracts may also have reduced conception in females by impeding ovulation resulting in anovulation sequelae. Therefore, the effect of methanolic extracts of *M. axillare* on these hormones may be responsible for the contraceptive activity observed in the ovaries of the treated animals and based on the present results; we presume that the phytochemicals present in appreciable quantities in the extracts may individually or synergistically be responsible for the observed contraceptive properties. The findings of this study are in agreement with other previous studies that have established the existence of contraceptive action of extracts of some medicinal plants [65,66,67]. These findings have important implications for female contraceptive development.

4. CONCLUSION

M. axillare exhibit excellent contraceptive activity and the findings of this research validates its ethnomedicinal use. The majority of secondary metabolites with known contraceptive activity and with structural similarities to conventional contraceptives are also present in appreciable quantities and could be acting either independently or synergistically to bring about

the observed bioactivity. These secondary metabolites could be working through their influence on the levels of reproductive hormones. However, further research is recommended to ascertain the particular class of compounds that are majorly responsible for the observed activity. Evaluation of contraceptive activity, through the administration of the extracts through other routes of drug administration and use of other species of animals such as nonhuman primates, are also highly recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by Biosafety, Animal Care and Use Committee (BACUC) of the Faculty of Veterinary Medicine (FVM), University of Nairobi. The establishment of contraceptive activity was done in accordance with the guidelines by BACUC and according to the internationally accepted guidelines (OECD) on animal use. The herbal plant materials collected were also authenticated by taxonomist before use for the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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