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Phytochemical and toxicological profile of *Markhamia zanzibarica* root extract using female Wistar rats

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Abstract

The study evaluates *Markhamia zanzibarica* root extract phytochemicals, toxicological profile and fertility enhancing potential using female rat model. The plant was harvested, chopped, dried, ground into powder. Organic extracts were prepared by sequential extraction (petroleum ether, dichloromethane (DCM), ethyl acetate and methanol), by use of cold maceration. Aqueous extract was obtained by hot maceration. Phytochemical screening of extracts was done by standard phytochemical procedures. Twelve female rats were used for the acute oral toxicity study as per OECD guideline 423. Methanol extract had the highest composition of phytochemicals such as alkaloids, flavonoids, saponins, sterols, terpenes and cardiac glycosides. Aqueous and DCM extracts showed presence of alkaloids, saponins and cardiac glycosides while petroleum ether and ethyl acetate extracts showed presence of alkaloids and cardiac glycosides. Steroids and tannins were absent in all extracts. Flavonoids, alkaloids and terpenes enhanced fertility probably due to their antioxidant and radical scavenging abilities. Flavonoids, alkaloids and glycosides probably improves fertility by inducing ovarian steroidogenesis and folliculogenesis. The plant extract had no adverse effects at over 2000 mg/kg through intra-abdominal gavage. *Markhamia zanzibarica* root extract has phytochemicals that enhance fertility and potentially safe when given orally. These findings however require further verification using *in vivo* studies.

Key words: *Markhamia zanzibarica, Toxicology profile, Phytochemicals, Pro-fertility*

1. Introduction

Infertility is the inability to conceive after 12 month of unprotected intercourse (Ruder *et al.*, 2008). Global infertility is approximated at 8-12% in couples within the reproductive age, affecting around 50 to 80 million people (WHO, 2018). In as much as infertility does not confer direct threat to life, it confers mental distress to

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couples interested in having children (Amudha and Rani, 2016). Many plants have been used traditionally to manage infertility including *Markhamia zanzibarica* (Kaingu *et al.*, 2014), *Abelmoschus esculentus* roots (Ashidi *et al.*, 2013), *Cadaba fruticosa* (Amudha and Rani, 2016) among others. The reported fertility enhancing effects of *Markhamia zanzibarica* as reported by (Kaingu *et al.*, 2014) is not backed by any scientific research and data of its toxicology profile unknown, *Markhamia zanzibarica* grows in areas with hot temperatures and minimal rainfall (Sharma and Patni, 2012). Markhamia zanzibarica traditionally referred to Mubwoka (Pokomo) belongs to the family Bignoniaceae. It is used to treat infertility in both females and males, manage post partum hemorrhage, menorrhagia, dysmenorrhea, threatened abortion, retained afterbirth in humans and livestock and induce milk letdown in livestock. There are several conventional treatment options for infertility, but majority are unavailable in rural areas and to a large extent expensive. Infertility medication cost is averaged at $1,182 to as high as $24,373 for *In Vivo* Fertilization (IVF) (Katz *et al.*, 2012). This cost is too high especially in developing countries like Kenya, where per capita income is US$ 1,361 (Kenya Economic Report, 2017). Therefore, there is need to increase infertility treatment options by validating traditional infertility treatment options. This increases infertility treatment options in rural areas low-income communities who have difficulties in affording conventional treatment options.

2. Materials and methods

2.1. Plant material collection, preparation and extraction

Markhamia zanzibarica roots were harvested from Garsen and Itsowe village, in Tana River County Kenya in March 2025. A voucher specimen (BAUCUC/UON/FVM/2026/650) was deposited at the UON department of Biology in the botany herbarium. The roots were chopped and air dried under shade in ambient temperature for three weeks. They were ground into course powder using an electric grinder from the Department of Veterinary Anatomy and Physiology, University of Nairobi. Organic extraction was done using petroleum ether, dichloromethane (DCM), ethyl acetate and methanol by cold maceration, whereas aqueous extraction was done using hot maceration. In aqueous extraction, 50 g of the powder was added to 500 mL of distilled water in a 1 L flask then boiled for 15 minutes. The boiled mixture was then filtered using Whatman No. 1 filter paper and the extract evaporated to a powder by use of a freeze dryer (BUCHI Lyovapor™ L-300) then the extract was kept at 4 °C (Wangia *et al.*, 2016). In organic extraction, 500 mL of petroleum ether, methanol, DCM and ethyl acetate were added to 50 mg of *Markhamia zanzibarica* root powder for 72 hours then the extract was concentrated by use of rotary evaporator (BUCHI Vac® V-500) at 45 °C then stored at 4 °C (Wangia, 2017).

2.2. Phytochemical screening

Phytochemical screening was done by observing precipitate formation and color change (Banu and Cathrine, 2015). Saponins; 20 mL of distilled water was added to 5 mL of *Markhamia zanzibarica* extract in a graduated cylinder then shaken vigorously for 15 minutes. Formation of foam that persisted for 15 minutes after shaking, was indicative of the presence of saponins (Savithramma *et al.*, 2011).

Terpenes–liebermann-burchard test: Chloroform was added to the extract then the solution was filtered. A few drops of acetic anhydride was added to the filtrate. The mixture was boiled and cooled. Concentrated sulphuric acid was added slowly along the sides of the test tube. Appearance of brown ring at the junction indicated the presence of terpenes (Tiwari, 2011).

Alkaloids: 2 drops of Mayer's reagent was added to 1 mL of *Markhamia zanzibarica* extract in a test tube. White creamy precipitate appearance indicated the presence of alkaloids (Banu and Cathrine, 2015).

Tannins: Five (5 mL) of distilled water was added to 50 mg of the extract and allowed to dissolve then a few drops of neutral 5% ferric chloride solution added to the mixture. Presence of phenolic compound was indicated by appearance of a dark green colour (Banu and Cathrine, 2015).

Sterols: Salkowaski method was used. 1 mL of *Markhamia zanzibarica* extract in a test tube was added 0.5 mL, acetic anhydride and chloroform each added. Concentrated sulfuric acid was slowly added along the sides of the test tube. A red coloration was an indication of the presence of sterols (Kaingu *et al.*, 2014).

Flavonoids: 10% ammonium hydroxide solution was added to *Markhamia zanzibarica* extract. Yellow fluorescence appearence was indicative of the presence of flavonoids (Banu and Cathrine, 2015).

Glycosides-keller–killian test: 1 mL of 3.5% ferric chloride in acetic acid was added to 1mL of *Markhamia zanzibarica* extract followed by careful drop- wise addition of 1.5 mL concentrated sulphuric acid to form a separate layer at the bottom. A brown ring at the interface because of the presence of de-oxy sugar was characteristic of cardenolides and pale green color in upper layer due to the steroid nucleus indicated cardiac glycosides presence (Wangia, 2017).

2.3. Animal studies

Female Wistar rats weighing between 150-200 g were used. They were housed in standard cages and provided with commercial rat pellets from @Bellmill feeds limited (Kenya) and water provided *ad libitum*. The rats were purchased from Biochemistry department and housed in Veterinary Anatomy and Physiology animal house; University of Nairobi. The experimental room temprature was maintained at 22 °C ± 3 and relative humidity of 30-70%. The rats were exposed to approximately 12 hours of light and 12 hours of darkness daily. Wood shavings were used for beddings and changed every other day.

2.4. Acute oral toxicity study

Acute oral toxicity studies was undertaken to determine the plant extract safety as per OECD guideline number 423. Twelve nulliparous non pregnant rats weighing 160-200 grams divided into three groups of three were used. All animals were weighed before fasting, at day zero, day one, day two then day seven and day fourteen. After the study, all the animals were humanely sacrificed by inducing hypoxia using CO_2, the carcasses were incinerated.

3. Results and discussion

3.1. Phytochemical screening of Markhamia zanzibarica root extract

Medicinal plant phytochemical screening is important in determining presence of various therapeutic agents responsible for the treatment of various diseases. *Markhamia zanzibarica* has traditionally been used as a fertility enhancing plant by traditional medicinal practitioners in treating infertility in women in Tana River County among the Pokomo community (Kaingu *et al.*, 2014). Its pro-fertility use has also been reported in India (Sharma and Patni, 2012). In the current study, phytochemical studies indicated presence of flavonoids, alkaloids, terpenes, sterols, saponins and cardiac glycosides in different extracts as shown in Table 1. Flavonoids, alkaloids and terpenes (Oke and Hamburger, 2002; Oladimeji *et al.*, 2014a and 2014b) are phenolic compounds which have been reported as fertility enhancing compounds in several studies due to their reported antioxidant and radical scavenging abilities. Oxidative stress has been reported to affect ovulation, fertilization, embryo development and implantation hence negatively impacting female fertility (Tvrda *et al.*, 2011). Elevated Reactive Oxygen Specials (ROS) can damage the ovum after ovulation and importantly it can damage the spermatozoa within the female reproductive tract since the spermatozoa are very sensitive to oxidative stress resulting in failed fertilization in females with no tubal problems (Tvrda *et al.*, 2011). Antioxidants have been shown to improve fertility by improving blood supply to the endometrium, decreasing insulin resistance within reproductive system cells, improving cervical mucus to ease fertilization, and causing a positive influence on steroidogenesis (Smits *et al.*, 2018). *Markhamia zanzibarica* methanolic extract strong antioxidant and radical scavenging activity have also been reported in a study (Dave, 2017) *Cadaba fruticosa* roots were found to be very rich in flavonoids and alkaloids and this was the attributable factor to their use as fertility enhancing plants. It was reported that anti-oxidants properties *in Cadaba fruticosa* reduced stress in reproductive cells leading to enhanced fertility (Amudha and Rani, 2016; Ashidi *et al.*, 2013). There exists a relationship between total flavonoid content with the antioxidant activity emphasing the use of flavonoids as antioxidants (Pourmorad *et al.*, 2006). Flavonoids, alkaloids and glycosides found on the leaves of *Justicia insularis* have been proven to improve fertility by inducing ovarian steroidogenesis and folliculogenesis in female rats (Telefo *et al.*, 2012). Alkaloids and flavanoids reduce fertlity (Yakubu, 2008) which is in contrast to this study in that they have been reported to reduce levels of reproductive hormones (Luteinizing Hormone (LH), estrodial and Follicle Stimulating Hormone (FSH)) therefore leading to compromised reproductive parameters. Saponins present too in *Markhamia zanzibarica* have been reported to be responsible for regularization of estrus cycle in mice who had irregular cycles. The mice were treated with a concortion of *Turraeanthus africanus* and *Lepidium meyenii* (Amudha and Rani, 2016). Steroidal saponins, flavonoids and glycosides are phytoesteroidal compounds

which have been reported to increase estrogen hormone levels thereby improving fertility (Kaaria *et al.*, 2019). *Ficus platyphylla* and *Anthocleista vogelii* promotion of fertilty was associated with the presence of phytosteroids (Ugwah-Oguejiofor *et al.*, 2011; Oladimeji *et al.*, 2014b). Flavonoids, saponins, glycosides and alkaloids present in *Markhamia zanzibarica could* probably be responsible for the fertility enhancing effect reported by traditional medicinal healers.

Table 1: Phytochemical screening results

Solvents	Alkaloids	Flavonoids	Saponins	Sterols	Steroids	Tannins	Terpenes	Cardiac Glycosides
Aqueous	+	-	+	-	-	-	-	+
Methanol	+	+	+	+	-	-	+	+
Ethyl acetate	+	-	-	-	-	-	-	+
Petroleum ether	+	-	-	-	-	-	-	+
Dichloromethane (DCM)	+	-	+	-	-	-	-	+

Note: '+' Present; '-' Absent.

3.2. Acute oral toxicity study of Markhamia zanzibarica root methanol extract on female Wistar rats

The use of herbal medicine has gained popularity globally especially in developing countries. The toxicological profile of most medicinal plants is yet to be studied. Without proper toxicological profile of such medicinal plants, the assumption that plant extracts are safe, is a myth (Prasanth *et al.*, 2014). The lack of toxicological profile necessitates the need for acute oral studies and provides further range of doses in animal studies. This study undertook to establish the acute oral toxicity of *Markhamia zanzibarica* methanol root extract in female albino rats.

The starting dose was 300 mg/kg administered to three animals. All animals had normal breathing and were active following the extract administration up to 24 hours and in the entire 14 days. No death occurred at this dose so other three animals were administered with 2000 mg/kg of the extract. At this dose the animals demonstrated fast breathing in the first 30 minutes but returned to normal breathing and activity within first one hour of administration. Lack of mortality at 2000 mg/kg dose necessitated three more animals to be administered with 5000 mg/kg body weight. All the rats exhibited fast breathing within the first thirty minutes but regrouped and resumed normal breathing and body grooming after one hour. The control (three female albino rats) were administered with 0.5 mL of physiological saline and observed intensively during the first 30 minutes. Hourly up to 4 hours then once every twenty four hours for 14 days as per OECD guideline 423. The control rats demonstrated normal activity and breathing throughout the fourteen days. On the fourteenth day observation period, there was no mortality or morbidity observed in the rats. In this study there was no adverse reaction noted even at 5000 mg/kg indicating the LD_{50} was probably above 5000 mg/kg. There was no significant

Table 2: Means body weights ± SEM for *Markhamia zanzibarica* treated groups and the control group

Markhamia zanzibarica (mgs/kg)	Fasting Day	Day 0	Day 1	Day 7	Day 14
Control	177.80 ± 2.747	171.90 ± 1.751	178.67 ± 0.998	184.78 ± 1.358	188.95 ± 2.193
300	183.74 ± 1.529	168.21 ± 3.292	182.51 ± 1.150	188.63 ± 3.549	194.97 ± 3.703
2000	176.95 ± 4.084	165.02 ± 5.269	173.63 ± 4.713	180.27 ± 4.159	183.34 ± 4.709
5000	177.06 ± 0.890	166.22 ± 4.462	178.69 ± 4.737	187.40 ± 3.640	189.31 ± 4.066
P-value	0.275	0.639	0.393	0.365	0.270

weight gain differences (Table 2) in treated rats compared to the negative control indicating *Markhamia zanzibarica* is non-toxic. This finding corroborates a study by Prasanth *et al.* (2014) on acute and sub-acute (28-Day) oral toxicity studies of ethanolic extract of *Celtis timorensis* leaves in rodents which caused a non significant weight gain difference in treated and negative control rats. *Markhamia zanzibarica* is therefore classified as Globally Harmonized System (GHS) as Category 5- > 2000.

3.3. Mean body weight ± SEM

Mean body weights and ± SEM and *P*-value ($P = 0.05$) during the fasting day, days 0, 1, 7 and 14 in treated animals at doses 300, 2000, and 5000 mg/kg and the control group in the current study showed there was no significant difference between treatment groups and the control group ($P > 0.05$) as presented in Table 2.

4. Conclusion

In the current study, *Markhamia zanzibarica* root extract was found to have flavonoids, alkaloids, terpenes, sterols, saponins and cardiac glycosides in different solvent extracts. These phytochemical compounds have antioxidant and radical scavenging abilities hence postulated to reduce stress within reproductive cells leading to enhanced fertility. Flavonoids, alkaloids and glycosides have also been postulated to improve fertility by inducing ovarian steroidogenesis and folliculogenesis. *Markhamia zanzibarica* methanol root extract was found to be safe even at 5000 mg/kg. Therefore, *Markhamia zanzibarica* LD_{50} is above 5000 mg/kg as per GHS Guidelines.

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Author contribution

This study was conducted in collaboration between all authors. Author 1* designed the study, wrote the protocol, performed laboratory work, did the statistical analysis and wrote the first draft of the manuscript, Author 2 worked on the acute oral toxicity studies, developed extraction procedures and phytochemical screening and developed the manuscript, managed literature searches. All authors approved the final manuscript.

Ethical approval

Ethical review was sought from Biosafety, animal use and Ethics committee of UON, Faculty of Veterinary Medicine (BAUCUC/UON/FVM/2026/650).

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