



Safety and Fertility Potential of Kenyan *Grewia tenax* (*Mukawa Wa Guba*) Root Extract Secondary Metabolites

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Authors' contributions

This work was carried out in collaboration among all authors. Author PJK designed the study, wrote the protocol, performed laboratory work, did the statistical analysis and wrote the first draft of the manuscript. Author CKK worked on the acute oral toxicity studies and author COW developed extraction procedures and phytochemical screening and developed the manuscript. Author FOA managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To screen Kenyan *Grewia tenax* root extract phytochemicals and correlate the attribute fertility enhancing effects and safety in female albino rats.

Study Design: An experimental study design was used.

Place and Duration of Study: The phytochemical studies were done at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Botany Laboratory, while acute oral toxicity studies were done at the Department of Veterinary Anatomy and Physiology, University of Nairobi (UON) animal house. The study was done during the month of March to June 2019.

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Methodology: *Grewia tenax* was harvested with the help of a plant taxonomist. The roots were chopped into small pieces and dried under shade for three weeks. They were then ground into powder. Organic extracts were prepared by sequential extraction (petroleum ether, dichloromethane (DCM), ethyl acetate and methanol), by use of cold maceration. Aqueous extracts were obtained by hot maceration. Phytochemical screening of extracts was done by standard phytochemical procedures. A total of 12 female albino rats were used in acute oral toxicity studies as per OECD 423 guidelines.

Results: Methanol extract had the highest composition of phytochemicals, i.e. 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 are fertility enhancing compounds due to their antioxidant and radical scavenging properties. Flavonoids, alkaloids and glycosides improve fertility by inducing ovarian steroidogenesis and folliculogenesis. In the acute oral toxicity study, there were no adverse effects at 5000 mg/kg extract administration.

Conclusion: Kenyan *Grewia tenax* root extract has phytochemicals postulated to enhance fertility and is orally safe. These findings may require further verification using *in vivo* studies.

Keywords: *Grewia tenax*; phytochemicals; pro-fertility; toxicological profile.

1. INTRODUCTION

Medicinal plants have gained worldwide use in treatment, management and prevention of different diseases especially in Africa where up to 80% of the population use some form of herbal medicine [1]. These herbal products form a cheaper form of treatment and are available in the communities where they are used compared to the conventional medicine options. Herbal products play a major role in the management of basic health care needs in developing countries [2].

There are many plants traditionally used in the management of infertility including Kenyan *Grewia tenax* [3] *Abelmoschus esculentus* roots [4], *Cadaba fruticosa* [5] among others. *G. tenax*, a shrub in the Family of Teliaceae has been reported to be used as a pro-fertility plant in ethnobotanical studies in Kenya [3] and India [6]. *G. tenax* is also used to treat some intestinal and skin infections, cough, has antibiotic properties and possess free radical scavenging activities which aid in its therapeutic action against tissue damage [6]. It has iron and calcium which makes it useful in prevention of anaemia and strengthening of bones [6]. It's also a multipurpose plant species which is the source of food, fodder, fibre, fuel wood, timber. *Grewia tenax* is highly drought resistant and grows in areas with warm climate and average annual rainfall of between 200-1000 mm [6] and is native to sub tropics and tropics in Asia, Africa and Australia [7]. The reported fertility enhancing effects of Kenyan *Grewia tenax* was in an ethnobotanical study and is not supported by

any scientific research and data on its fertility effects is generally lacking.

There are several medical treatment options for infertility, but they are not available especially in rural areas and not affordable by many. Infertility medication cost is averaged at \$1,182 to as high as \$24,373 for *In vivo* fertilization (IVF) [8]. This cost is very high, especially in developing countries like Kenya, where per capita income is US\$ 1,361 [9]. Therefore, there is need to investigate traditional infertility treatment options and establish scientifically the potential to treat infertility. This would help people in rural areas and low-income communities who have difficulties in access or cannot afford other available treatment options.

This paper is focused on the use of *G. tenax* as a fertility enhancing plant by determining its phytochemical components and correlating them with their (phytochemical components) known pro-fertility effects. The paper also determines the safety (Acute toxicity) of Kenyan *G. tenax* root extract in female albino rats in order to ascertain if its secondary metabolites are toxic.

2. MATERIALS AND METHODS

2.1 Plant Material Collection, Preparation and Extraction

Grewia tenax roots of mature flowering plant were harvested from Kisamis, Kajiado West constituency, Kajiado County, Kenya in March 2019 with the help of a plant taxonomist. A Voucher specimen (Ref. no PJK 2019/001) was

deposited at the UON department of Botany herbarium. The roots were chopped and air dried under shade in ambient temperature for three weeks. The dried roots were ground into coarse powder using an electric grinder made at the Mechanical Engineering Department in JKUAT. Organic extraction was done using petroleum ether, dichloromethane (DCM), ethyl acetate and methanol by cold maceration, whereas aqueous extraction was done using hot maceration. Organic extraction was done by adding 500 mL of petroleum ether, methanol, DCM and ethyl acetate to each quantity of 50 g of the *G. tenax* root powder for 72 hours by cold maceration then the extract was concentrated by use of a rotary evaporator (BUCHI Vac® V-500) at 45°C and stored at 4°C [10]. 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 was freeze-dried using a freeze dryer (BUCHI Lyovapor™ L-300). The lyophilized sample was kept at 4°C [11].

2.2 Phytochemical Screening

Phytochemical screening was done by observing precipitate formation and colour change [12].

2.2.1 Saponins

A quantity of 20 mL of distilled water was added to 5 mL of the *G. tenax* extract in a graduated cylinder then shaken vigorously for 15 minutes. Formation of foam that persists for 15 minutes after shaking was indicative of presence of saponins [13].

2.2.2 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 solution was boiled and cooled. Concentrated sulfuric acid was added slowly along the sides of the test tube. Appearance of brown ring at the junction indicated the presence of terpenes [14].

2.2.3 Alkaloids

To 1 mL of extract in a test tube, 2 drops of Mayer's reagent was added. A white creamy precipitate indicated the presence of alkaloids [12].

2.2.4 Tannins

To 5 mL of distilled water, 50 mg of the extract was added and allowed to dissolve after which a

few drops of neutral 5% ferric chloride solution was added to the mixture. Presence of phenolic compound was indicated by the appearance of a dark green colour [12].

2.2.5 Sterols

Salkowski method was used. To 1 mL of extract in a test tube, 0.5 mL acetic anhydride and 0.5 mL chloroform were 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 [3].

2.2.6 Flavonoids

5 mL of 10% ammonium hydroxide solution was added to *Grewia tenax* extract. Appearance of a yellow fluorescence was indicative of the presence of flavonoids [12].

2.2.7 Glycosides-keller–killian test

To 1 mL of 3.5% ferric chloride in acetic acid was added to 1 mL of extract, followed by careful drop-wise addition of 1.5 mL concentrated sulfuric acid by the sides of the test tube to form a separate layer at the bottom. A brown ring at the interface indicated the presence of de-oxy sugar which was characteristic of cardenolides and pale green colour in the upper layer due to the steroid nucleus indicated cardiac glycosides presence [11].

2.2.8 Animal studies

Female Wistar albino rats weighing between 160–200 g were used. The animals were purchased from the Medical Physiology animal house, UON, and were housed in standard cages at the Veterinary Anatomy and Physiology animal laboratory; UON and provided with commercial rat pellets from Belmill feeds limited (Kenya) and water *ad libitum*. The temperature of the experimental room was maintained at 22°C±3 and relative humidity of 30–70%. The animals were exposed to 12 hours of light and 12 hours of darkness daily. Wood shavings were used for beddings and were changed on every other day. Acute oral toxicity studies were done to determine the plant extract safety using [15] Guidelines. Twelve nulliparous non pregnant rats weighing 160–200 g 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 CO₂, thereafter the carcasses were incinerated.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of *Grewia tenax* Root Extract

Plant phytochemical screening is important in determining different therapeutic agents present in the plant. *Grewia tenax* has been used traditionally as a fertility enhancing herb by traditional healers in treating women with fertility problems in the Kenyan coast, especially among the Pokomo community [3]. Its pro-fertility use has also been reported in India [6]. In this study, different extracts indicated the presence of flavonoids, alkaloids, terpenes, sterols, saponins and cardiac glycosides (Table 1).

Flavonoids, alkaloids and terpenes [16,17,18] 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 [19]. Elevated levels of reactive oxygen species (ROS) can damage the ovum after release and damage the sperm within the female reproductive tract since the sperm is sensitive to oxidative stress, hence affecting fertilization in females with no tubal problems [19]. 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 having a positive effect on steroidogenesis [20]. *Grewia tenax* methanol extract with antioxidant and radical scavenging activity has also been reported in a study "Evaluation of antioxidant activities by use of various extracts of *Abutilon pannosum* and *Grewia tenax* in the Kachchh region" of India [21]. *Abelmoschus esculentus* roots and *Cadaba fruticosa* were found to be rich in flavonoids and alkaloids and this was the attributable factor to their use as fertility enhancing agents because these active plant metabolites are antioxidants hence reduce stress within reproductive cells leading to enhanced fertility [4,5]. There exists a relationship between total flavonoid content and the antioxidant activity emphasizing the use of flavonoids as antioxidants [22]. Flavonoids, alkaloids and glycosides found in the leaves of

Justicia insularis improve fertility by inducing ovarian steroidogenesis and folliculogenesis in female rats [23].

Alkaloids and flavonoids reduce fertility [24] which contrasts this study in that they are reported to reduce luteinizing hormone (LH), estradiol and follicle stimulating hormone (FSH), levels therefore leading to alterations of circulating hormones seen on *Cnidioscolous aconitifolius*.

Saponins present in *Grewia tenax* have been reported to be responsible for regularization of estrus cycle in mice which had irregular cycle and were treated with a combined extract of *Turraeanthus africanus* and *Lepidium meyenii* [5]. Steroidal saponins, flavonoids and glycosides are phytoesteroidal compounds which have been reported to restore estrogen hormone balance, hence regularizing fertility [25]. *Ficus platyphylla* and *Anthocleista vogelii* which also have sterols and have been reported to promote fertility [26,18].

The phytochemicals especially flavonoids, saponins, glycosides and alkaloids present in *Grewia tenax* are probably responsible for the fertility enhancing effect of *Grewia tenax* reported by traditional medicine healers.

3.2 Acute Toxicity Studies of the Methanolic Kenyan *Grewia tenax* Root Extract on Female Albino Rats

The use of herbal medicine has gained popularity around the world, especially in developing countries, but this popularity has come with the assumption that plant products are safe. This assumption negates the health effects that the bioactive compounds in these plant products may have without proper toxicological data profile, given the fact that they are used as self-medication [27]. The lack of toxicological data necessitates the need for acute toxicity studies which would provide further ranges of doses in animal studies.

The starting dose was 300 mg/kg administered to 3 animals. All animals did not show any adverse effects such as lethargy, abnormal breathing, increased salivation, convulsions, coma or death within 24 hours observation during the entire 14 days period. No death occurred at this dose so the other 3 animals were administered with 2000 mg/kg of the extract. At this dose, the animals demonstrated normal breathing and no

Table 1. Phytochemical screening results

Solvents	Alkaloids	Flavonoids	Saponins	Sterols	Steroids	Tannins	Terpenes	Cardiac glycosides
Aqueous	+	-	+	-	-	-	-	+
MeOH	+	+	+	+	-	-	+	+
EtOAc	+	-	-	-	-	-	-	+
PE	+	-	-	-	-	-	-	+
DCM	+	-	+	-	-	-	-	+

*Key '+' Present, '-' absent; MeOH = Methanol; EtOAc = Ethyl acetate; PE = Petroleum ether; DCM = Dichloromethane

Table 2. Means body weight \pm SEM for *Grewia tenax* treated groups and the control group

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

adverse effects like changes in skin or fur, lethargy, increased salivation, convulsions, coma or death. Three more animals were administered with 5000 mg/kg body weight. All the animals showed fast breathing within the first thirty minutes but regrouped and resumed normal breathing and body grooming. The animals demonstrated normal breathing and no adverse effects like changes in skin or fur, lethargy, increased salivation, convulsions, coma or death at this dose. A control of three female albino rats was administered with 0.5 mL of normal saline and observed just like the rest. This group demonstrated normal breathing and normal activity throughout the fourteen days.

In the fourteen-day observation period, there was no mortality or morbidity observed. In this study, there was no adverse reaction noted even at 5000 mg/kg indicating the LD₅₀ was above 5000 mg/kg. There were no weight gain significant differences (Table 2) in treated animals and the negative control indicating that Kenyan *Grewia tenax* is non-toxic. This finding was in concurrence with a study by [25] on acute and sub-acute (28-Day) oral toxicity studies of ethanolic extract of *Celtis timorens* is leaves in rodents were they found no significant weight gain difference in both treated and negative control animal group and reported the extract as almost non-toxic. The safety of the plant was classified as Globally Harmonized System (GHS) as Category 5- > 2000.

3.3 Mean Body Weight \pm SEM

Mean body weight and \pm 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 that there was no significant difference between treatment groups and the control group ($P > 0.05$) as presented (Table 2).

4. CONCLUSION

In the current study, Kenyan *Grewia tenax* 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 oxidative 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. Kenyan *Grewia tenax* methanol root extract was found to be safe even at 5000 mg/kg dose so the LD₅₀ above 5000 mg/kg as per GHS Guidelines.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is

absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical review was sought from Biosafety, animal use and ethics committee of UON, Faculty of Veterinary Medicine (FVM/ BAUEC/2018/182).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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