Macrothink Institute™

Journal of Biology and Life Science
ISSN 2157-6076
2023, Vol. 14, No. 2

Phytochemical Composition of Extracts from *Acacia nilotica* Pods and *Albizia lebbeck* Stem Bark and Their Effect on Morphologies of Rat Spermatozoa

Lusekelo Msomba Mwangengwa

(Corresponding Author)

Department of Veterinary Physiology, Biochemistry and Pharmacology. Sokoine University

of Agriculture (SUA). P. O. Box 3017, Morogoro, Tanzania.

Email: lusamwange@yahoo.com

Noel Lemree Kanuya

Department of Veterinary Surgery and Theriogenology. Sokoine University of Agriculture

(SUA). P.O. Box 3021, Morogoro,

Email: kanuya@sua.ac.tz

Robert Arsen Max.

Department of Veterinary Physiology, Biochemistry and Pharmacology. Sokoine University

of Agriculture (SUA). P. O. Box 3017, Morogoro, Tanzania.

Email: robertmax@sua.ac.tz

Received: September 14, 2022 Accepted: January 17, 2022 Published: January 30, 2023

doi:10.5296/jbls.v14i2.20271 URL: https://doi.org/10.5296/jbls.v14i2.20271

Abstract

The study evaluated the phytochemical constituents of crude extracts from *Acacia nilotica* pods and *Albizia lebbeck* stem bark and tested the potential effects of their extracts on *M.*

natalensis spermatozoa morphologies. A portion of the grounded materials from each plant was extracted in distilled water and 70% methanol. The aqueous and methanolic plant extracts were assessed for the phytochemicals with antifertility potentials. Also, a total of 90 male rats were randomized into 9 groups (n = 10) and treated with either *A. lebbeck* or *A. nilotica* methanolic extracts for 15, 30, or 60 days. Results indicated that in both plants flavonoids, tannins, saponins, steroids, terpenoids and phenols were present in the aqueous and methanolic extracts. Judged by the intensity of colour change after a chemical reaction, saponin was more intensely present in both the aqueous and methanolic extracts of the *A. lebbeck* stem barks while flavonoids and tannins were relatively more present in the *A. nilotica* sourced corresponding extracts. Spermatozoa with normal head-abnormal tail, normal head-tailless, abnormal head-normal tail, and both head and tail distortion were more revealed in rats treated with either *A. nilotica* or *A. lebbeck* extracts relative to the control. In conclusion, the studied *A. nilotica* and *A. lebbeck* extracts are rich in flavonoids, tannins, saponins, steroids, terpenoids and phenols that possess some antifertility properties as reflected on the observed adverse effects on rat spermatozoa morphologies.

Keywords: phytochemical analysis, antifertility, A. nilotica, A.lebbeck, spermatozoa, morphologies

1. Introduction

In our previous studies (Mwangengwa *et al*., 2022 a and b) we reported the effect of crude extracts from *Acacia nilotica* and *Albizia lebbek* on the reproductive systems of male and female *Mastomys natalensis*. The studies demonstrated clearly that male rats had significantly reduced fertility success rates as a result of testicular structure distortion and disruption of the spermatogenic process. Female rats treated with the same crude extracts were equally affected by several lesions in their reproductive system such as decreased number of follicles suppressed ovulatory activities and induction of follicular atresia. All these antifertility effects were probably associated with secondary metabolites (phytochemicals) contained in the tested crude extracts.

Secondary plant metabolites are compounds synthesized from the primary plant metabolites such as carbohydrates, proteins and lipids (Cheynier, 2012). Their role in plants varies from structural, growth and protection against insects and herbivores (Dai and Mumper, 2010). The most common phytochemicals include phenolics, terpenoids, and alkaloids (Cheynier, 2012) and are at the centre stage of the discipline of pharmacognosy. Phytochemicals have been shown to cause a range of beneficial and non-beneficial biological activities when consumed by animals (Dai and Mumper, 2010; Cheynier, 2012). Biological effects such as antimicrobial, anticancer, toxic actions, anti-nutritional and antifertility have been observed in many studies (Samtiya *et al*., 2020; Dai and Mumper, 2010; Cheynier, 2012). For instance, treatments with isoflavonoids inflicted some antispermatogenic and anti-implantation activities in rats (Nayakam *et al*., 2014). Also, tannins have been associated with some potent spermicidal effects when treated in rats (Benhong, 2012). Terpenoids such as triterpenes, saponins and b-sitosterol were found to cause some antifertility actions when fed in rats (Chaudhary *et al*., 2007; Shu *et al*., 2015; Singh and Gupta, 2016). Another compound includes quinolizidine

which showed adverse effects on sperm cell counts, motility, viability, and morphology in treated rats (Olayemi and Raji, 2020). Also, quinolizidine affected significantly the plasma levels of testosterone, luteinizing hormone and follicle-stimulating hormone in comparison to the control when treated orally in rats (Olayemi and Raji, 2020).

Following our successful demonstration of the antifertility effects of the crude extracts from *A. lebbeck* stem bark and *A. nilotica* pods the current study evaluated the phytochemical constituents of powdered raw materials and corresponding extracts from the two plants. Since the effects of the plant extracts on spermatozoa morphologies were not ascertained in the previous studies, the current work also evaluated the potential effects of *A. lebbeck and A. nilotica* extracts on the morphology of the *M. natalensis* spermatozoa.

2. Methodology

2.1 Study Area

Collection of *Acacia nilotica* pods took place in Nangurukuru in Kilwa, Lindi Region in southeastern Tanzania (8° 56′0″S, 39°30′45″E), while stem barks of *Albizia lebbeck* were collected in Morogoro Urban, Tanzania (6° 50' 42.66" S, 37° 39' 29.14"E). Authentication of the two trees was carried out by a botanist from the Department of Ecosystems and Conservation, at Sokoine University of Agriculture (SUA).

2.2 Processing of the Plants

Processing of the plants for phytochemical analysis was carried out at the Toxicology laboratory in the College of Veterinary Medicine and Biomedical Sciences (CVMBS) at SUA. A total of 10 kg each of *A. lebbeck* stem barks and fresh matured pods of *A. nilotica*, were collected in August and transported in bags to the laboratory where they were dried under the shed until they were breakable.

The dried plant materials were then chopped into smaller pieces using a knife before being ground into fine particles (1 mm) using an electric grinder. Extraction involved the soaking of 500 g of powdered materials of each plant in 1.5 L of 70% methanol (Gupta *et al.*, 2004) for 72 hours. The crude extracts from *A. lebbeck* and *A. nilotica* were obtained by filtering the methanolic extract through gauze and then cotton wool. Methanol was removed from the filtrates using a vacuumed rotary evaporator at 80^0C. The resultant filtrates were further concentrated using a water bath at 40^0C until they were a solid mass.

2.3 Phytochemical Analysis

Evaluation of phytochemical constituents was carried out as per Edeoga *et al.* (2005) methodology with some minor modifications. Briefly, a total of 5 g of the dried powdered samples of *A. lebbeck* stem bark and *A. nilotica* pods were each boiled in 200 ml of distilled water in beakers and then filtered to prepare the stock solution of aqueous extracts. Also, 5 g of the methanolic extracts from *A. lebbeck* stem bark and *A. nilotica* pods were separately dissolved into 200 ml of distilled water and then filtered to prepare stock solutions.

2.3.1Test for Flavonoids (Alkaline Reagent Test)

A total of 5 ml of dilute NH_3 solution was added to 3 ml of the stock solution of *A. lebbeck* stem bark or *A. nilotica* pods extracts followed by the addition of 2 ml of concentrated H_2SO_4. The formation of yellow colouration that disappeared on standing was an indication of the presence of flavonoids.

2.3.2 Test for Saponins (Frothing Tests)

About 1 mil of stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added separately in test tubes followed by the addition of 5 mils of distilled water. The mixture was then shaken vigorously for 5 minutes and then allowed to settle for 30 minutes. The appearance of honeycomb froth was an indication of the presence of saponin.

2.3.3 Test for Steroids (Libermann Burchard's Test)

A total of 2 ml of stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added with 2.5 ml of chloroform followed by slow addition of 2.5 ml of concentrated H_2SO_4. A colour change from violet to blue or green indicated the presence of steroids.

2.3.4 Test for Triterpenoids (Salkowski's Test)

About 5 ml of the stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were mixed with 2 ml of chloroform. Then 3 ml of concentrated H_2SO_4 was added slowly and carefully. The formation of a reddish-brown coloration indicated the presence of triterpenoids.

2.3.5 Test for Tannins (Ferric Chloride Test)

A total of 2 ml of stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added to the test tube followed by the addition of 3 ml of 10% ferric chloride solution. A brownish-green or blue-black coloration indicated the presence of tannins.

2.3.6 Test for Phenol (Gelatin Test)

About 2 ml of the stock solution of *A. lebbeck* stem bark or *A. nilotica* pod extracts were added to the test tube followed by the addition of 2 ml of gelatin solution. The formation of white precipitate indicated the presence of phenolic compounds.

2.3.7 Preparation of the Plain and Extract Containing Test Feed

The extracts of *A. lebbeck* and *A. nilotica* were treated in the *M. natalensis* rats through feed baits. Details describing the preparation of the plain and extracts containing taste feed have been presented in our previous publication (Mwangengwa *et al*. (2021). Just briefly a stiff porridge was prepared by boiling and stirring a mixture of 10 kg of maize flour, 6 kg of roughly crushed maize, 250 g of fish meal, and 1 kg of cane sugar for 20 min. A standard pelletizer machine (KENWOOD, type MG51, designed at Hampshire, PO 9NH in the UK, made in China) was employed to process a portion of the stiff porridge mixture to prepare the basal feed pellets. The remaining stiff porridge mixture was spiked with the extract of either *A. nilotica* pods or *A. lebbeck* stem barks before pelleting. Spiking was done in such a way

that the treated feed contained 2% (w/w) of either of the two crude extracts.

The 2%w/w was equivalent to 0.2g (200mg) of the extract added to 9.8g (9 800 mg) of feed making 10g (10 000mg) of the feed extract mixture. The assumption was that rats may consume up to 10 g/100g body weight per meal per day (Krishnakumari *et al*., 1979). A dosage of 200mg of the extract used in the current study considered the dosage range of 100 to 200 mg/ kg body weight of rats which have been used in other antifertility studies involving *A. lebbeck* (Gupta *et al*., 2004 and 2006) and *A. nilotica* (Lampiao 2013).

2.3.8 Experiment Setup and Treatments

The detail of the experimental setup has been explained elsewhere (Mwangengwa *et al*., 2001). Briefly, a total of 90 male rats were stratified on a bodyweight basis and randomized into 9 groups (Table 3.1). Treatment was carried out in parallel for 15 (to assess immediate effects), 30 (intermediate effects), and 60 days (to evaluate for potential chronic effects) (Table 3.1). The amount of test feed supplied daily for each rat was 10 g, assuming that rats consume up to 10g/100g bodyweight meals per day (Krishnakumari *et al*., 1979).

The *M. natalensis* were collected from the University farms by using Sherman LFA aluminium traps. Out of the 220 collected male rats, 90 of them were selected for this study. The selected rats were housed singly in plastic cages and left to adapt to the standard conditions of a 12/12 light-dark cycle, 25±5^{o}C, and 35%−60% relative humidity for two weeks while feeding on broiler finisher and *adlib* water.

The criterion for inclusion and exclusion of experimental animals was the bodyweight category of 25-50 g for sexually mature rats (Lalis *et al*., 2006) and a healthy body condition. The Tanzanian Commission for Science and Technology (COSTECH) granted the research permit (Permit No 2019-225-NA-2019-47) before the study's commencement. Guidelines for caring and using laboratory animals were properly followed during the handling and restraints of the experimental rats.

Table 1. Experimental design. To evaluate the contraceptive potential of *A. nilotica* pods and *A. lebbeck* stem bark methanolic extracts in the male *M. natalensis*

Group (n=10)	**Treatment allocation**	**Trial duration**
Control (C)	10 g of plain pellets per rat/day	
A. lebbeck (AL)	10 g of pellets with 2 % of *A. lebbeck* stem bark extract per rat/day	15 (days)
A. nilotica (AN)	10 g of pellets with 2 % of *A. nilotica* pods extract per rat/day	
Control (C)	10 g of plain pellets per rat/day	
A. lebbeck (AL)	10 g of pellets with 2 % of *A. lebbeck* stem bark extract per rat/day	30 (days)
A. nilotica (AN)	10 g of pellets with 2 % of *A. nilotica* pods extract per rat/day	

Control (C)	10 g of plain pellets per rat/day	
A. lebbeck (AL)	10 g of pellets with 2 % of *A. lebbeck* stem bark extract per rat/day	60 (days)
A. nilotica (AN)	10 g of pellets with 2 % of *A. nilotica* pods extract per rat/day	

2.4 Determination of Sperm Cell Morphologies

The effects of *A. lebbeck* and *A. nilotica* extracts on sperm cell counts, motility and vitality have been presented in our previous publication (Mwangengwa *et al*., 2021b). The current study presents the effects of plant extracts on sperm cell morphologies. The morphology of spermatozoa was determined as per Lucio *et al*. (2013) methodologies.

The rat testes' caudal epididymides were dissected out and homogenized into 1 mL of warm (37 ^{0}C) normal saline in a small beaker using a dissection scissor to make a sperm cell suspension. One drop of the sperm cell suspension was added on a microscopic slide followed by one drop of nigrosin-eosin and mixed with a toothpick. Then several slides of thin smears were prepared and air-dried before being examined by a bright-field microscope at x 200 magnification.

The percentages of normal-shaped sperm cells with sickle-shaped heads and large straight elongated tails versus the abnormal sperm cells with weird heads, tails or tailless were determined from the total counts of 200 sperm cells from five different fields of the nigrosin and eosin-stained microscopic slides (Lucio *et al*., 2013).

Sperm cell morphological classification was done according to the shape of the head and flagellum as described by Kempinas and Lamano-Carvalho (1988) and Ahmed *et al*. (2013). Normal-shaped spermatozoa had an intact head and flagellum. Abnormal-shaped sperm cells were classified as follows (a) normal head separated from flagellum, (b) abnormal head separated from flagellum, (c) abnormal head with normal flagellum, (d) defects on both head and flagellum, (e) degenerative flagella with normal head and (f) other flagella defects with the normal head.

2.5 Data Analysis

IBM SPSS statistics 20 was used for data analysis to analyze the proportion of sperm cells with normal and abnormal morphologies. Statistical association related to treatment, treatment duration and sperm cell morphologies were assessed by Chi-squared test using the multinomial logistic regression model. Statistical significant differences and associations were considered at $p< 0.05$).

3. Results

Phytochemical analysis revealed the presence of significant amounts of flavonoids, tannins, saponins, steroids, terpenoids and plant phenols in both the aqueous and methanolic extracts from both *A. nilotica* pods and *A. lebbeck* stem bark (Table 2 and 3). While the presence of saponin was more intense in both the aqueous and methanolic extracts from *A. lebbeck* stem barks compared to related extracts from *A. nilotica* pods, the reverse was true for Tannins

(Tables 2 and 3). Moreover, the presence of flavonoids was less intense in the aqueous than in the methanolic extracts from both *A. lebbeck* and *A. nilotica*. The presence of other plant compounds including plant phenolics, terpenoids and plant steroids was equally highly intense in both the aqueous and methanolic extracts from both the *A. nilotica* pods and *A. lebbeck* stem barks.

Table 2. Phytochemical constituents of aqueous extracts and methanolic extracts from *A. lebbeck* stem bark powder using color intensity methods

Compound	***Test method***	***Color intensity***	
		Aqueous extracts	Methanolic extract
Flavonoids	Alkaline reagent test	++	+++
Saponins	Frothing tests	+++	+++
Steroids	Libermann Burchard's test	+++	+++
Tannins	Ferric chloride test	++	++
Terpenoids	Salkowski's test	+++	+++
Phenols	Gelatin test	+++	+++

+++: highly present, ++: moderately present, +: Low/absent.

Table 3. Phytochemical constituents of aqueous extracts and methanolic extracts from *A. nilotica* powder using color intensity methods

Compound	***Test method***	***Color intensity***	
		Aqueous extracts	Methanolic extract
Flavonoids	Alkaline reagent test	+++	+++
Saponins	Frothing tests	+	+
Steroids	Libermann Burchard's test	+++	+++
Tannins	Ferric chloride test	+++	+++
Terpenoids	Salkowski's test	+++	+++
Phenols	Gelatin test	+++	+++

+++: highly present, ++: moderately present, +: Low, -: absent.

Morphological analysis of the rat spermatozoa indicated a significant reduction of the normal-shaped sperm cells in the *A. nilotica* pods and *A. lebbeck* stem barks extract-treated rats relative to the control rats. Spermatozoa reductions in extracts treated rats were vivid at all the treatment duration of 15, 30 and 60 days (Figure. 1). However, in the 15 days treated rats the *A. lebbeck* stem barks extract caused more reduction of normal shaped spermatozoa than the *A. nilotica* pods extract. Nevertheless, after 30 and 60 treatment duration, the extracts from *A. nilotica* pod caused more reduction of normal-shaped spermatozoa in treated rats than the *A. lebbeck* stem bark extracts (Figure. 1). Spermatozoa with normal head and abnormal tail were significantly ($p<0.05$) more revealed in the *A. lebbeck* and *A. nilotica* extracts treated than in the control rats across all the treatment durations of 15, 30, and 60 days (Figure 2). However, the treatment duration of 15 days indicated a large proportion of sperm cells with normal-shaped heads and abnormal tails in the *A. lebbeck* stem bark than in the *A. nilotica* extracts treated rats (Figure 2). Also, the 30 days and 60 days treated groups of rats revealed a large proportion of spermatozoa with normal heads and abnormal tails in the *A. nilotica* extracts treated relative to the *A. lebbeck* extracts treated rats (Figure 2).

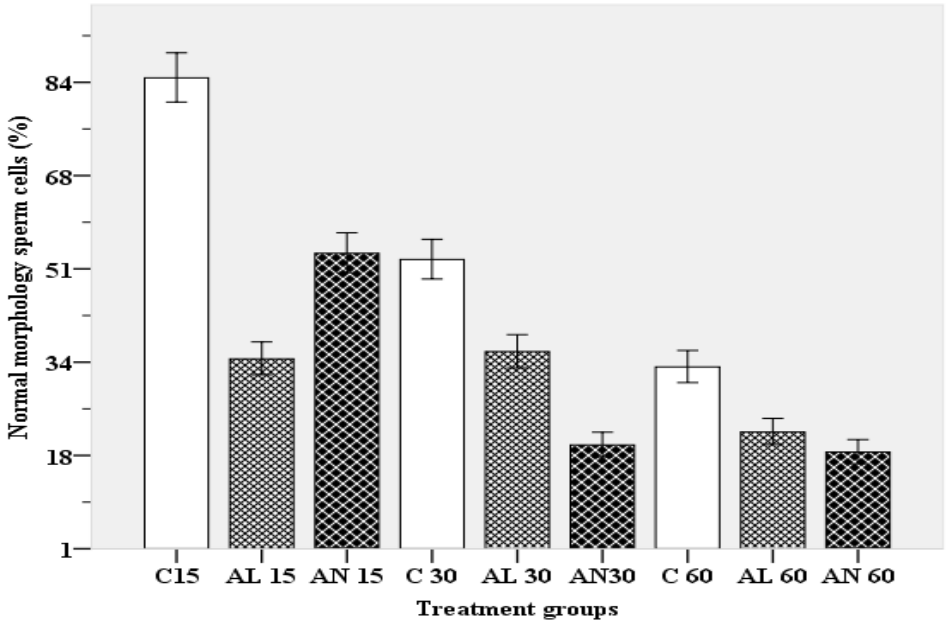

Figure 1. Effect of crude plant extract in feed on the percentage of spermatozoa with normal morphology following its treatment for 15, 30, or 60 days in the *M. natalensis* rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract

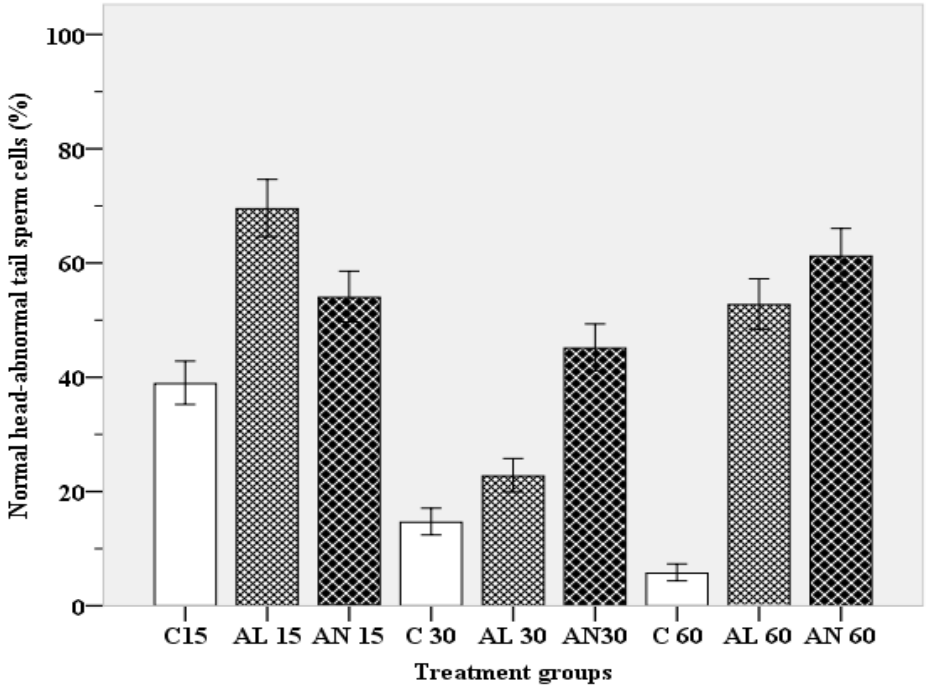

Figure 2. Effect of crude plant extract in feed on the percentage of spermatozoa with the normal head-abnormal tail following its treatment for 15, 30, or 60 days in the *M. natalensis* rats**.** C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract

Sperm cells with a normal head but tailless (Figure 3) and those with an abnormal head but normal tail (Figure 4) were significantly more displayed in the *A. lebbeck* and *A. nilotica* extracts treated rats relative to the control. No significant difference in the proportion of sperm cells with a normal head but tailless and those with an abnormal head but a normal tail was observed between the *A. nilotica* and *A. lebbeck* ($p > 0.05$) treated rats after a treatment duration of 15 or 30 days (Figure 3 and 4). However, after 60 days of treatment length, sperm cells with normal head-tailless and those with an abnormal head-normal tail were significantly ($p < 0.05$) more revealed in the *A. nilotica* extract-treated relative to the *A. lebbeck* treated rats (Figures 3 and 4).

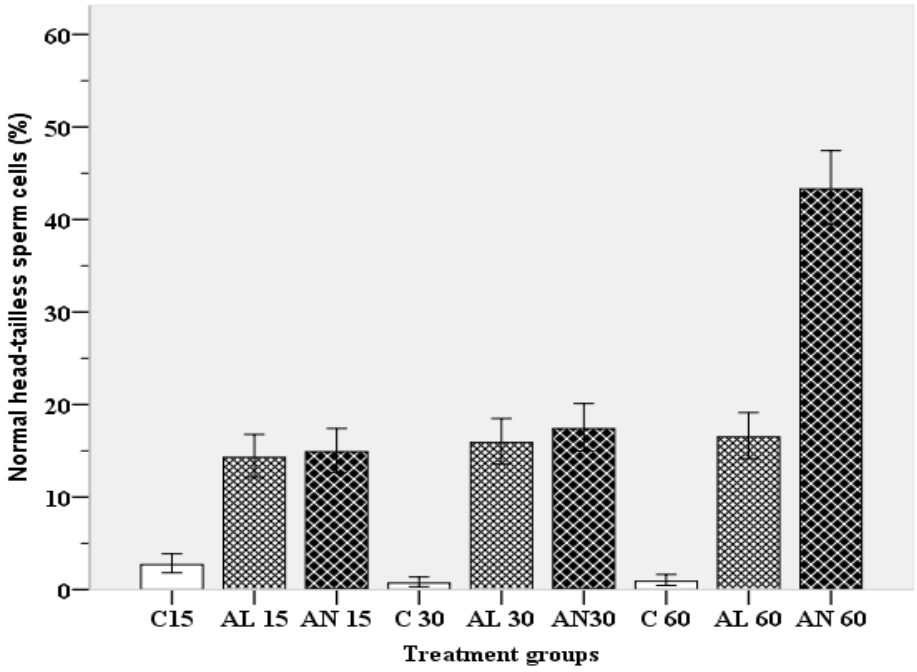

Figure 3. Effect of crude plant extract in feed on the percentage of spermatozoa with normal head-tailless following its treatment for 15, 30, or 60 days in the *M. natalensis* rats.
C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract

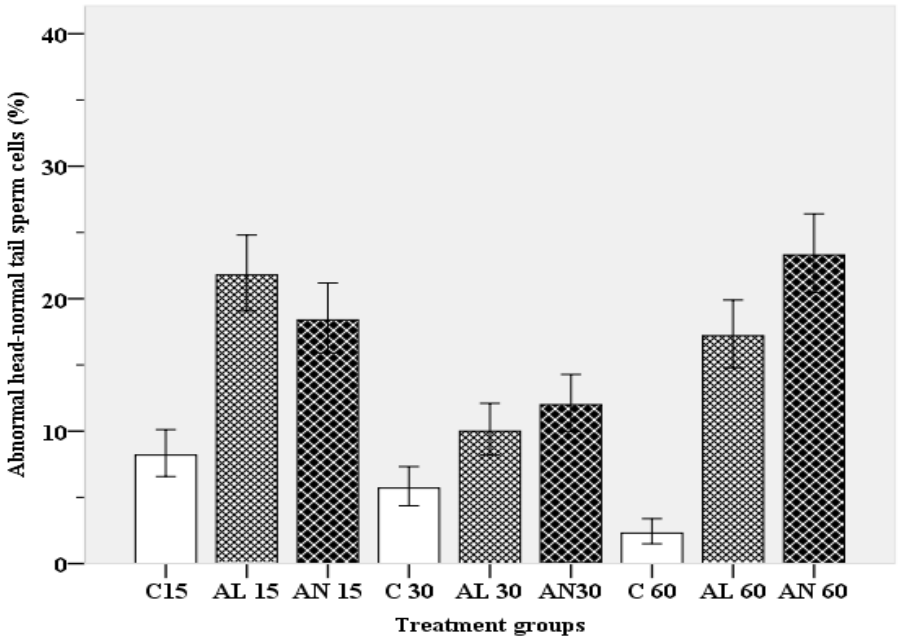

Figure 4. Effect of crude plant extract in feed on the percentage of spermatozoa with abnormal head- normal tail following its treatment for 15, 30, or 60 days in the *M. natalensis* rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract

Spermatozoa with distortion on both the head and tail were significantly ($p < 0.05$) more present in the *A. lebbeck* and *A. nilotica* extracts treated rats relative to the control across all the treatment duration of 15, 30, and 60 days (Figure 5). The treatment made for 15 days indicated the proportion of sperm cells with both head and tail defects not differing significantly ($P > 0.05$) between the *A. lebbeck* and *A. nilotica* extracts treated rats (Figure 5). However, the 30 days treated rats revealed a significantly ($p < 0.05$) larger number of sperm cells with both head and tail abnormalities in the *A. nilotica* extract treated compared to the *A. lebbeck* extracts treated rats (Figure 5). Nevertheless, in the 60 days treated rats, sperm cells with head and tail defects were significantly ($p < 0.05$) more in numbers in the *A. lebbeck* extract treated relative to the *A. nilotica* extract treated rats (Figure 5). Sperm cell morphological defects revealed by counts were further revealed on the histo-morphological pictures presented in Figure 3.6. The spermatozoa micrographic pictures from the 15, 30 and 60 days treated rats indicated sperm cells in the control groups of rats to be dominated by those with normal morphologies. However, the pictures from the *A. lebbeck* and *A. nilotica* treated rats revealed a dominance of spermatozoa with deformities on the head or tail or both tail and head (Figure 6).

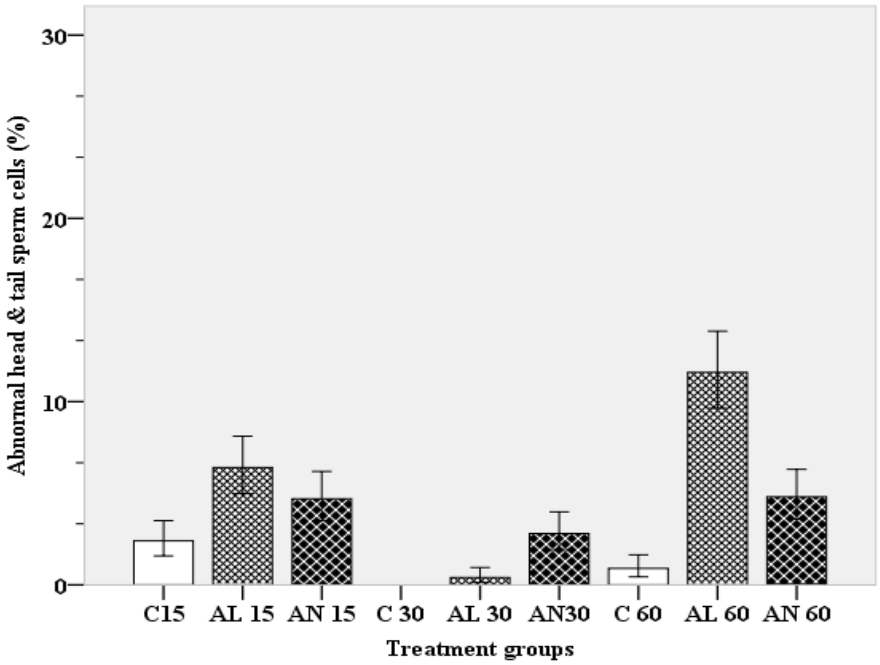

Figure 5. Effect of crude plant extract in feed on the percentage of spermatozoa with defects on both head and tail following its treatment for 15, 30, or 60 days in the *M. natalensis* rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract

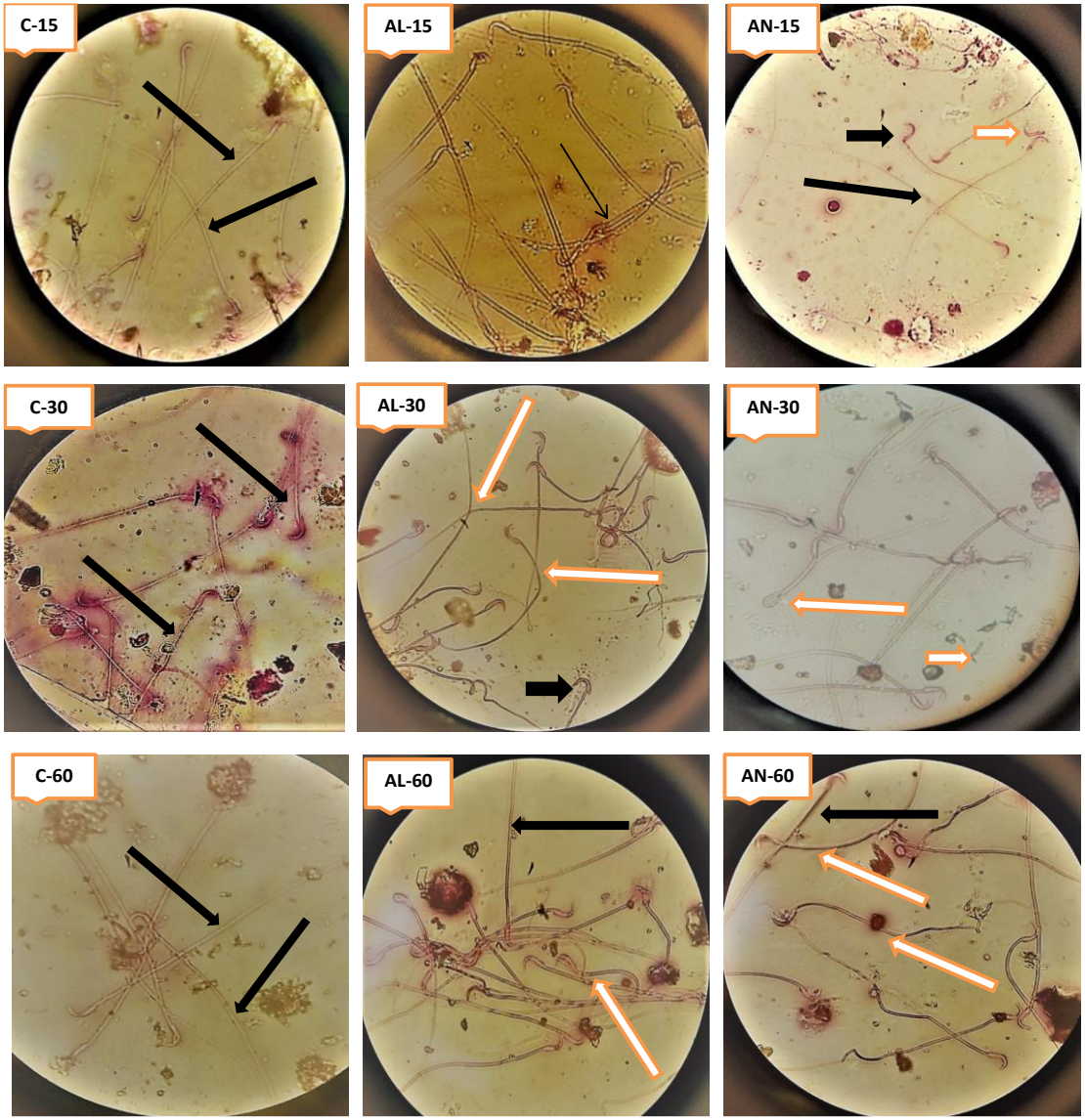

Figure 6. Micro-graphic pictures of sperm cells at x200. Shows the effect of crude plant extract in feed on the morphology of the head and tail of spermatozoa following its treatment for 15, 30, or 60 days in the *M. natalensis* rats. C = control groups, AL= *A. lebbeck* stem bark methanolic extract, AN = *A. nilotica* pods methanolic extract. A long black arrow shows normal spermatozoa, a long whitish arrow shows sperm cells with a bent tail, a short white arrow shows tailless sperm cells, short black arrow shows sperm cells with a bent head

4. Discussion

The phytochemical analysis of crude extracts from the *A. nilotica* pods and *A. lebbeck* stem bark in the current study revealed the presence of several compounds with antifertility properties in both plants. The phytochemical profiles shown presently agreed well with the results of Auwal *et al.* (2014) who analyzed pods, leaves, stem bark and root extracts from *A. nilotica* Okoro *et al*. (2014). The revealed phytochemical results were also agreeable with the results of Elshiekh *et al*. (2020) who assessed the phyto-compounds present in *A. lebbeck* seeds, Lawan *et al*. (2018) who reported phytochemical constituents in *A. lebbeck* leaf

extracts.

The various phytochemicals detected in *A. nilotica* and *A. lebbeck* in the current study are therefore discussed in the context of their effects on the *M. natalensis* reproductive system. Flavonoids were shown to be more predominant in both plant extracts. While in plants, flavonoids offer some key biological functions in pigmentation and protection, in animals, these compounds work as antioxidants, anti-inflammatory and anti-microbial agents (Cushnie and Lamb, 2005; Panche *et al*., 2016). However, some types of flavonoids such as iso-flavanoids are phytoestrogen. In animals, iso-flavanoids can mimic the natural estrogens hence triggering multiple oestrogenic effects including disruption of reproductive activities (Wocławek-Potocka *et al*., 2013). Tannins were also shown be moderately present in the aqueous and methanolic extracts from *A. lebbeck* while being abundant in *A. nilotica*. Tannins are water-soluble polyphenols largely found in the bark, leaves, buds, stems, fruits, seeds and roots of several plant species (Chung *et al*., 1998). In animals, tannins are known to have no useful role besides their anti-nutritional properties once they are consumed in food (Chung *et al*., 1998). However, the antifertility properties of tannins have also been reported in some studies whereby the plant compound caused some spermicidal effects in-vitro and in-vivo when tested in rabbits (Benhong *et al*., 2012). Another phytochemical detected in the current study is saponins; these were especially present in *A. lebbeck* extracts. Structurally, Saponins are triterpenoid or steroidal aglycones linked to oligosaccharide moieties (Mugford and Osbourn, 2013; Faizal and Geelen, 2013). The toxicological importance of saponins is shown by their various pharmacological properties including their significant antifertility effects in rats (Gupta *et al*., 2005; Shu *et al*., 2015). Furthermore, the current study revealed the presence of steroids in both the aqueous and methanolic extracts from both plants. Apart from their multiple medicinal benefits Phyto-steroids are also associated with infertility effects when treated in animals (Gebrie *et al*., 2005; Taur and Patil, 2011; Qasimi *et al*., 2017).

There was an abundance of sperm cells with morphological defects in the plant extract-treated rats in the current study most likely reflecting the *A. lebbeck* and *A. nilotica* extracts spermicidal potential. The dominant spermatozoa defects in the current study were the secondary abnormalities mostly dominated by the sperm cells with normal head-abnormal tails followed by those with normal head-tailless and those with abnormal head-normal tails. Also, there were few spermatozoa with deformities on both head and tail in rats treated with the extracts of *A. lebbeck* stem bark or *A. nilotica* pods. Results on sperm cell morphologies obtained in this study were related to the results of Saba *et al*. (2009) who revealed a significantly large proportion of sperm cells with secondary deformities in the *Lagenaria breviflora* fruit extract-treated Wister rats. Also, Oridupa *et al*. (2018) reported related results to the current study where a high percentage of spermatozoa with secondary morphological defects were revealed in male Wister rats exposed to the acetone extracts of *Combretum sordidum.*

In conclusion, the current study has demonstrated the presence of several phytochemicals namely: flavonoids, tannins, saponins, steroids, terpenoids and phenolics in the pods of *A. nilotica* and stem bark of *A. lebbeck*. Most of these phytochemicals are associated with reduced fertility in the tested rat species. This assertion corroborates well with the adverse effects on the rat sperm cell morphologies observed in this study.

Conflict of Interests

The authors have not declared any conflict of interest.

Acknowledgments

Much acknowledgment from the Authors goes to the ACEII-IRPM and BTD project for funding this study. More thanks go to G. Nicera and J. Nalengwe for their outstanding contribution to field works during plant collection. We also offer our sincere thanks to Z. Mbilu for his generous assistance in the laboratory work.

References

Ahmed, M., Al-Daghri, N., Alokail, M. S., & Hussain, T. (2013). Potential changes in rat spermatogenesis and sperm parameters after inhalation of *Boswellia papyrifera* and *Boswellia carterii* incense. *International Journal of Environmental Research and Public Health, 10*(3), 830-844. https://doi.org/10.3390/ijerph10030830

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Veterinary Research Forum : *An International Quarterly Journal, 5*(2), 95-100.

Benhong, Z. (2012). Spermicidal and antigonococcal effects of tannins from pomegranate rind. *Journal of Medicinal Plants Research, 6*(7), 1334-1339. https://doi.org/10.5897/JMPR11.1488

Chaudhary, R., Gupta, R. S., Kachhaw, J. B. S., Singh, D., & Verma, S. K. (2007). Inhibition of spermatogenesis by Triterpenes of *Albizzia lebbeck* (L.) Benth pods in male albino rats. *Journal of Natural Remedies, 7*(1), 86-93.

Cheynier, V. (2012). Phenolic compounds: From plants to foods. *Phytochemistry Reviews, 11*(2), 153-177. https://doi.org/10.1007/s11101-012-9242-8

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A Review. *Critical Reviews in Food Science and Nutrition, 38*(6), 421-464. https://doi.org/10.1080/10408699891274273

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343-356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dai, J., & Mumper, R. J. (2010a). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*(10), 7313-7352. https://doi.org/10.3390/molecules15107313

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology, 4*(7), 685-688. https://doi.org/10.5897/AJB2005.000-3127

Elshiekh Y. H, Alagbash R. E, Ali R. A, Saad F. O., & Musharaf M. (2020). Phytochemical constituents, antibacterial screening and antioxidant activity of *Albizia lebbeck* (L.) Benth (Seed). *World Journal of Advanced Research and Reviews, 07*(01), 035-040. https://doi.org/10.30574/wjarr.2020.7.1.0225

Faizal, A., & Geelen, D. (2013). Saponins and their role in biological processes in plants. *Phytochemistry Reviews, 12*(4), 877-893. https://doi.org/10.1007/s11101-013-9322-4

Gebrie, E., Makonnen, E., Debella, A., & Zerihun, L. (2005). Phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extract of *Rumex steudelii*. *Journal of Ethnopharmacology, 96*(1), 139-143. https://doi.org/10.1016/j.jep.2004.08.026

Gupta, R. S., Kachhawa, J. B. S., & Chaudhary, R. (2004). Antifertility effects of methanolic pod extract of *Albizzia lebbeck* (L.) Benth in male rats. *Asian Journal of Andrology, 6*(2), 155-159.

Gupta, R. S., Kachhawa, J. B. S., & Chaudhary, R. (2006). Antispermatogenic, antiandrogenic activities of *Albizia lebbeck* (L.) Benth bark extract in male albino rats. Phytomedicine. *Phytomedicine*, *13*(4), 277-83. https://doi.org/10.1016/j.phymed.2004.11.008

Kempinas, W. G., & Lamano-Carvalho, T. L. (1988). A method for estimating the concentration of spermatozoa in the rat cauda epididymidis. *Laboratory Animals, 22*(2), 154-156. https://doi.org/10.1258/002367788780864547

Krishnakumari, K. M., Rajalakshmi, D., Sreenivasan, V., & Ramasundaram, C. P. (1979). Feeding responses of young and adult albino rats (*Rattus norvegicus*) to a mixed basal diet. *Proceedings of the National Academy of Sciences, India, 88*(5), 367-375. https://doi.org/10.1007/BF03179116

Lalis, A., Lecompte, E., Cornette, R., Moulin, S., Machangu, R. S., Makundi, R., & Denys, C. (2006). Polymorphism of the age population structure of two wild *Mastomys natalensis* (Rodentia: Muridae) Tanzanian habitat samples: A multicriteria comparison. *Mammalia, 70*(3-4), 293-299. https://doi.org/10.1515/MAMM.2006.052

Lampiao, F. (2013). The anti-fertility effects of *Acacia nilotica* in male wistar rats. *Journal of Reproduction and Infertility, 14*(1), 39-42.

Lawan, S. A., Saleh, A., Sani, B. G., Fa'iza, A. M., & Sadiya, A. Z. (2018). Preliminary phytochemical constituents and phytotoxic effect of *Albizia lebbeck* (L.) Benth on Sorghum bicolor. *Bayero Journal of Pure and Applied Sciences, 10*(1), 405. https://doi.org/10.4314/bajopas.v10i1.79S

Lucio, R. A., Tlachi-López, J. L., Eguibar, J. R., & Ågmo, A. (2013). Sperm count and sperm motility decrease in old rats. *Physiology and Behavior, 110*, 73-79. https://doi.org/10.1016/j.physbeh.2012.12.015

Mugford, S. T., & Osbourn, A. (2013). Saponin Synthesis and Function. In: *Isoprenoid Synthesis in Plants and Microorganisms: New Concepts and Experimental Approaches.*

(Edited by Bach, T. J. and Rohmer, M.), pp. 405-424. https://doi.org/10.1007/978-1-4614-4063-5_28

Mwangengwa, L. M., Bakari, G. G., Kanuya, L. L., & Max, A. R. (2021a). Antifertility effects of crude extracts from *Acacia nilotica* pods and *Albizzia lebbeck* stem bark in female multimammate rats, *Mastomys natalensis*. *Journal of Physiology and Pathophysiology, 12*(1), 1 – 1. https://doi.org/10.5897/JPAP2021.0137

Mwangengwa, L. M., Bakari, G. G., Kanuya, L. L., & Max, A. R. (2021b). Effect of A. nilotica pods and A. lebbeck stem bark extracts on the reproductive system of male Mastomys natalensis: An Anti-fertility studies. *African Journal of Biological Sciences, 4*(2), 106-119. https://doi.org/10.33472/AFJBS.4.2.2022.106-119

Nayakam B. H., Londonkar, L. R., & Umesh, M. K. (2014). Evaluation of Potential Antifertility activity of Total Flavonoids, Isolated from *Portulaca oleracea* L on female albino rats. *International Journal Pharmatical Technical Research, 6*(2), 783-793. https://doi.org/10.1155/2014/175851

Okoro, S., Kawo, A., & Arzai, A. (2014). Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts. *Bayero Journal of Pure and Applied Sciences, 7*(1), 105. https://doi.org/10.4314/bajopas.v7i1.20

Olayemi, F. O., &Raji, Y. (2011). Quinolizidine alkaloids: the bioactive principles in *Cnestis ferruginea* (de Candolle) with male antifertility activities. *African Journal of Medicine Science, 40*, 253-263.

Oridupa, O. A., Samuel, B. B., & Amolegbe, A. A. (2018). Sperm Morphology and Characteristics in Male Rats Treated with Leaf Extract of *Combretum sordidum*. *Africa Journal of Biomedical Research, 21*, 51-55.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5*, e47. https://doi.org/10.1017/jns.2016.41

Qasimi, M. I., Nagaoka, K., & Watanabe, G. (2017). The effects of phytosterols on the sexual behavior and reproductive function in the Japanese quail (*Coturnix coturnix* japonica). *Poultry Science, 96*(9), 3436–3444. https://doi.org/10.3382/ps/pex161

Saba, B. A., Oridupa A, O., Oyeyemi O. M., & Osanyigbe, D. O. (2009). Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of *Lagenaria breviflora* Roberts. *African Journal of Biotechnology, 8*(7), 1170-1175.

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, *2*(1), 1-14. https://doi.org/10.1186/s43014-020-0020-5

Shu, Y., Cao, M., Yin, Z. Q., Li, P., Li, T. Q., Long, X. F., & Zhao, J. (2015). The reproductive toxicity of saponins isolated from *Cortex Albiziae* in female mice. *Chinese Journal of Natural Medicines, 13*(2), 119-126. https://doi.org/10.1016/S1875-5364(15)60015-2

Singh, K., & Gupta, R. S. (2016). Antifertility activity of β-sitosterol isolated from *Barleria prionitis* (L.) roots in male albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(5), 88-96.

Taur, D. J., & Patil, R. Y. (2011). Some medicinal plants with antiasthmatic potential: A current status. *Asian Pacific Journal of Tropical Biomedicine, 1*(5), 413-418. https://doi.org/10.1016/S2221-1691(11)60091-9

Wocławek-Potocka, I., Mannelli, C., Boruszewska, D., Kowalczyk-Zieba, I., Waśniewski, T., & Skarzyński, D. J. (2013). Diverse effects of phytoestrogens on the reproductive performance: Cow as a model. *Internation.* https://doi.org/10.1155/2013/650984