Journal of Medicinal Herbs

journal homepage:**www.jhd.iaushk.ac.ir**

Effects of methanol root extract and fractions of *Waltheria Indica* (linn) on sexual orientation and sexual behavioral parameters in male wistar rats

Sunday Yakubu*, Sunday Otimenyin, Samuel Otokpa

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria;

Email:** ***sabos@unijos.edu.ng

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *May 26th2023*
Accepted *September 08th2023*

Key words:
- ✓ *aphrodisiac*
- ✓ *medicinal plants*
- ✓ *mount*
- ✓ *intromission*
- ✓ *ejaculation*
- ✓ *fractions*

ABSTRACT

Background & Aim: *Waltheria indica* is a shrub whose roots are used in many communities of Northern Nigeria to manage several sexual behavioral disorders; however, there is no scientific study to verify this claim. The aim of the study is to determine the effect of methanol root extract of *Waltheria indica* and its fractions (ethyl acetate, n-butanol, and residual ethanol) on sexual orientation and sexual behavioral parameters in male albino rats.

Experimental: Male rats were divided into five groups each containing 6 rats. Group 1 rats were treated with normal saline. Rats in Groups 2, 3 and 4 were treated with 50, 100 and 200 mg/kg crude extracts while rats in Group 5 were treated with Sildenafil 5 mg/kg. Treatment was administered once daily for 7 days. Female rats were artificially brought to oestrus by administering oestradiol 25 µg/kg and progesterone 500 µg/kg 48 and 8 h respectively prior to mating The mating was done in a 1:1 ratio and mating behaviour was observed on days 1 and 7. Similar procedure was repeated, normal saline, 12.5 and 25 mg/kg of the ethyl acetate, n-butanol and mother liquor fractions, respectively and sexual behavioral parameters were observed.

Results: Crude extract and fractions of *Waltheria indica* significantly increased sexual behavioral parameters, with the n-butanol and mother liquor fractions being most active.

Recommended applications/industries: The study showed that methanol root extract of *Waltheria indica* and its fractions have aphrodisiac activity with the n-butanol and residual methanol fractions being more active. These revelations further substantiates the traditional use of *Waltheria indica* for the management of infertility in male, thus such discovery can serve as a lead for the development of drugs for the management of sexual dysfunctions.

1. Introduction

It is generally believed that the consumption of medicinal plant by humans have significantly led to reduction in burden of various diseases through their preventive and curative mechanisms (Oigbochie *et al.*, 2019; Njoga *et al.*, 2022). This can be attributed to the numerous active phytochemicals found in the plants that have demonstrated pharmacological actions against a variety of diseases (Ezebuiro *et al.*, 2022).

Over the years, medicinal plants have played vital roles as agents for the management of sexual disorders such as erectile dysfunction, premature ejaculation, lack of sexual desire, and infertility (Msiska *et al.*, 2020). An aphrodisiac can be defined as substances that improve sexual behaviour and satisfaction in humans and other animals (Enema *et al.*, 2018). Several herbal plants used in African folkloric medicine have

demonstrated male fertility enhancement or regulatory Potentials (Ajuogu *et al.*, 2020), however very few medicinal plants have been scientifically certified to enhance sexual behavior, increase testosterone levels, improve erectile function and spermatogenesis among others in animal models and randomized clinical trials (Msiska *et al.*, 2020; Kotta *et al.*, 2013).

Some of these plants include *Blepharis edulis, Tribulus terrestris,* Anacyclus *pyrethrum and Allium tuberosum* (Singh *et al.*, 2013; Tang *et al.*, 2017). *Waltheria indica linn* also known as sleepy morning belongs to the family Sterculiaceae; it is wide spread in West Africa. The Hausas call it Hankufa while the Yorubas call it korikodi. In traditional medicine *Waltheria indica* is used as an aspirin-like anti-inflammatory drug (Basiru *et al.*, 2014; Sanders *et al.*, 2001). *Waltheria indica* whole plant is use to treat malaria, peptic ulcer and dysentery (Jansen *et al.*, 2010; Oluranti *et al.*, 2012). The plant is also used for the management of chronic diseases such as cancers, leprosy and epilepsy (Graham *et al.*, 2000; Olajuyigbe *et al.*, 2011). Traditional medicine practitioners have also used the plant for the management of infertility, bladder ailments, erectile dysfunction and impotence (Zongo *et al.*, 2013).

Even though the ability of *Waltheria indica* to treat infertility, erectile dysfunction and impotency in male has been reported in ethno-medicine, there is no scientific study to verify this claim. This study seeks to evaluate the effect of methanolic root extract of *Waltheria indica* and its fractions on sexual orientation and sexual behavioral parameters in male albino rats.

2. Materials and Methods

2.1. Plant collection, authentication and preparation

Waltheria indica roots were collected from Geji village in Bauchi State, Nigeria in November 2022. The plant was identified and authenticated at the Department of Horticulture and Landscape Technology by Mr. Christopher Abok a taxonomist with Federal college of Forestry, Jos, Nigeria with assigned voucher numbers of FHJ 273. The plant was deposited in the Federal College of Forestry herbarium. The roots were cleaned of debris and dried under shade in the department of Pharmacology and Toxicology university of Jos. The dried roots were pulverized pestle and mortar into coarse powder. Five hundred (500) grams of the pulverized plant material was poured into a 6-L round bottom flask and 3 L of 70% methanol was added onto the plant powder to be extracted by maceration. The mixture was allowed to stand for 72 h with constant shaking at intervals of 12 h. After 72 h, the mixture was successively passed through sieves of varying pore sizes (800, 500 and 150 µm); followed by filtration using cotton wool plug and finally Whatmann No. 1 filter paper to obtain a clear filtrate.

The filtrate was dried by passing a steady stream of air using an exhaust fan to reduce the volume to about one third of the original volume. The remainder was transferred into an oven to dry at a temperature of 40°C after which the dried extract was scrapped and stored in air tight containers at 25 ± 2°C pending use.

2.2. Fractionation of crude extract

Fractions of the crude methanol root extract of *Waltheria indica* were obtained using the method described by Hossain *et al.* (2014). 50 g of the crude extract was suspended in 200 ml of distilled water in a 500-ml separating funnel. The suspension was successively extracted using solvents of increasing polarity (n-hexane, ethyl acetate and n-butanol). First, 100 mL of n-hexane was added to the suspension and gently mixed. The mixture was allowed to stand and separate into the aqueous and hexane layers. The hexane layer was collected and the process was repeated 3 times to obtain the hexane fraction. The same process was carried out using ethyl acetate and butanol to obtain the n-hexane, ethyl acetate (EA), butanol (NB) and mother liquor (ML) fractions.

2.3. Preliminary phytochemical screening

The crude extract was tested for the presence of secondary metabolites using the methods described by Evans (2009). The phytochemicals tested for were saponins, flavanoids, carbohydrates, tanins, anthraquinones, cardiac glycosides and steroids.

2.4. Determination of acute toxicity (LD_{50})

The LD_{50} of the crude extract was determined using the method described by Lorke (1983). Male rats weighing between 150 to 200 g were used. The determination was carried out in two phases. In Phase 1, nine rats were divided into 3 groups of 3 rats each and labeled as Groups 1, 2 and 3. They were administered 10, 100 and 1000 mg/kg body weight

dose of *Waltheria indica* extract orally and were observed for mortality or any signs of toxicity. After 24 h, the phase II treatment was initiated with administration of 1600, 2900 and 5000 mg/kg body weight dosage of the extract each to 1 rat. The rats were observed for mortality or signs of toxicity hourly for the first 6 h, then after 24 h observation continued for 14 days to assess for signs of delayed toxicity.

2.5. Evaluation of mating performance of male rats

Healthy sexually experienced male wistar rats weighing between 200-300 g with age ranging from 20-20.5 weeks and female wistar rats weighing between 150-200 g with age ranging from 12.5- 16 weeks were obtained from the Animal House Unit of the Department of Pharmacology; University of Jos, Nigeria and used for the studies on the aphrodisiac potential of the plants. The male and female rats were kept in separate cages (Temperature: 22-28^{0}C; 12 h natural light and 12 h dark; humidity 50-55 %) with free access to rat pellets. Ethical clearance was obtained from the animal care and use committee of the Department of Pharmacology and toxicology, Faculty of Pharmaceutical Sciences University of Jos.

2.6. Preparation of female wistar rats

The method modified by Gotep *et al.* (2021) was employed. Female rats were artificially brought to oestrus by administering oestradiol 25 µg/kg and progesterone 500 µg/kg 48 and 8 h respectively prior to mating via subcutaneous injection under the skin of the neck. The receptivity of the female rats was tested by mating with male rats other than the ones used for the experiment

2.7. Experimental design

For evaluation of the crude extract, male rats were divided into five groups each containing 6 rats. Group 1 rats served as the control and were treated with normal saline at equivalent volume with the rats taking the extract. Rats in Groups 2, 3 and 4 were treated with 50, 100 and 200 mg/kg body weight of the crude extracts while rats in Group 5 were treated with Sildenafil 5 mg/kg. Treatment was administered once daily for 7 days. The male rats were paired with the females and mating behaviour observed on days 1 and 7.

For evaluation of the fractions (ethyl acetate, butanol and residual Methanol fractions), male rats weighing 250 to 300 g were divided into 7 groups each containing 6 rats. Two doses per fraction (12.5 and 25 mg/kg) and a control group that was administered normal saline with volume equivalent to that of fractions administered to constituted groups. The sexual behavioral activity was observed on the first day of treatment.

2.8. Determination of mating parameters

Individual male rats were placed in separate observation glass chamber measuring 60 cm × 30 cm × 30 cm (L × B × H) and allowed to acclimatize for 10 min. Thereafter, a female that had been experimentally brought to oestrous was introduced into the cage and allowed to cohabit for 30 min. The mating was done in a 1:1 ratio. The sequence of events was captured using a document camera. The recorded video was then played back to evaluate mating behavioral parameters. The experiment was conducted between the hours of 18:00 h and 22:00 h in the same laboratory and under the light of same intensity.

2.9. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Dunnett's test to determine the level of statistical significance. Significance level was set at $P<0.05$ and confidence level at 95%. Statistical analysis was carried out using Graph pad Prism 7.0. Results were presented as Mean ± Standard Error of Mean.

3. Results and discussion

Plant-based in-vivo research has made significant rewarding progress in many important areas such as research on sexual dysfunction in males worldwide. The phytochemical screening of the root extract of *Waltheria indica* revealed the presence of the saponins, flavanoids, carbohydrates, tanins, anthraquinones, cardiac glycosides and steroids; These results also confirms the phytochemical studies carried out by Musa *et al.* (2016) . Saponins and alkaloid have been shown to be responsible for aphrodisiac activity especially penile erection (Kim *et al.*, 1998, Yakubu and Afolayan 2009). Similarly, prosexual stimulatory has also been attributed to its Steroid and triterpene contents (Drewes *et al.*, 2003). Saponin also enhances androgen production (Gauthaman *et al.*, 2002) and this action can be attributed to steroidal nature of saponins which plays an intermediary role in the steroidal

pathway of androgen production (Gauthaman *et al.*, 2008). Research evidence has shown the potentials of saponins to facilitate penile erection by directly inducing the vasodilation and relaxation of the penile corpora cavernosa via a nitric oxide dependent mechanism (Chen and Lee, 1995), including arginase inhibition (Corine *et al.*, 2015). Flavonoids are said to have inhibitory effects on phosphodiesterase (PDE) enzymes that breaks down cyclic AMP (cAMP), which activates synthesis of nitric oxide leading to vasodilatation thus increasing penile blood flow and sustaining penile erection (Gakunga *et al.*, 2014). Base on the studies flavanoids, saponins and steroid might be responsible for the observed aphrodisiac activity.

Acute toxicity study defines the intrinsic toxicity of the chemical (Padhy *et al.*, 2017). It also provides initial information about the mode of toxic action of a substance by which the dose of a new compound can be fixed. LD_{50} helps in dose determination in animal studies (Ghosh *et al.*, 2019). In the study, the LD_{50} for *Waltheria indica* is said to be within the save limits using Lorke's method after single administration of extract with increasing doses.

Sexual orientational activity such as genital sniffing is a parameter used to measure pre-copulatory sexual behavior, the purpose being to stimulate sexual excitement. An increase in the number of times the male seeks out the female to sniff her odors is an indication of pre-copulatory sexual stimulation in the male (Padashetty and Mishra, 2007). Equally, increase in anogenital grooming is indicative of increased sexual stimulation in male rats. It plays a major role in the readiness of the adult male rat for reproduction (Ofeimun and Ayinde, 2017). It is also an important measure of the erectile status of the penis (Hernandez-Gonzalez, 2000). In the present study the methanolic root extract of *Waltheria indica* showed an increase in genital sniffing, licking, non-genital grooming and genital grooming. The 200 mg/kg dose of the extract exhibited significant increase in all the parameters except genital sniffing. *Waltheria indica* is said to have more potential aphrodisiac activity.

Table1. Effects of methanolic root extract of *Waltheria indica* on male sexual orientation in male albino rats parameters on day 1 of administration.

	Mean±SEM				
Parameters	**Normal saline 10ml/kg**	***W. indica* 50mg/kg**	***W. indica* 100mg/kg**	***W. indica* 200mg/kg**	**Sildenafil 5mg/kg**
Sniffing	7.60±2.56	6.33±0.48	12.00±.84	9.680±1.59	13.75±1.49
Licking	18.80±6.71	12.60±0.40	23.20±5.67	18.50±5.74	30.60±4.35*
Nongenital Grooming	8.80±2.46	15.67±1.20	24.26±3.94*	20.25±3.95	20.60±3.08*
Genital Grooming	16.00±9.59	70.66±5.45	70.00±15.08	104.00±16.03*	82.00±5.25*
Exploration	5.00±1.05	5.68±1.02	4.00±1.3	3.20±0.97	4.25±0.73
Rearing	27.40±6.19	20.67±8.03	18.00±4.43	11.25±4..21	11.00±2.90
Climbing	52.80±10.24	36.25±8.16	23.40±7.67	30.00±5.10	24.00±1.90

n=6, *=P< 0.05 when compared with control.

Table 2. Effects of methanolic root extract of *Waltheria indica* on sexual orientation parameters in male albino rats after daily administration for 7 days.

	Mean±SEM				
Parameters	**Normal saline 10ml/kg**	***W. indica* 50 mg/kg**	***W. indica* 100 mg/kg**	***W. indica* 200 mg/kg**	***Sildenafil* 5 mg/kg**
Sniffing	5.00±1.38	8.26±0.58	9.40±2.46	6.60±2.14	8.60±3.04
Licking	4.80±2.50	15.00±0.32	14.68±1.83	37.50±1.53*	38.80±7.19*
Nongenital Grooming	10.00±2.00	18.50±1.16	19.25±2.08	25.20±0.49*	19.80±2.67*
Genital Grooming	57.60±13.26	75.00±9.64	101.60±8.28	114.40±28.84*	118.40±17.48*
Exploration	4.00±1.64	3.76±0.80	2.00±0.55	4.00±1.34	4.60±0.51
Rearing	15.20±5.00	19.00±4.11	3.60±1.60	6.80±3.44	7.20±1.86
Climbing	22.80±5.74	19.50±4.55	9.00±3.29	22.40±9.06	24.60±8.72

n=6, *=P< 0.05 when compared with control.

The increase in mount frequency (MF) and intromission frequency (IF) following the administration of the aqueous extract of *Waltheria Indica* at 50, 100 and 200 mg/kg body weight suggests improved sexual vigor libido as shown in Table 3 and 4. Similar finding was also reported by Tajuddin *et al.* (2004). The dose dependent increase in frequencies of mount and intromission in the rats administered with the extract were indications that the extract has the potential to control erectile dysfunction and arousal disorders in males.

Table 3. Effects of methanolic root extract of *Waltheria indica* on male sexual behavioral parameters in male albino rats after day 1 of administration

	Mean ± SEM				
Parameters	**Normal saline**	***W. indica* 50 mg/kg**	***W. indica* 100 mg/kg**	***W. indica* 200 mg/kg**	**Sildenafil 5 mg/kg**
ML(Sec)	264.80±18.77	80.75±18.65*	96.75±24.18*	48.00±7.48*	103.00±16.38*
MF	7.00±1.27	19.50±2.69	30.68±3.86*	22.00±1.27	16.80±1.28
IL(sec)	408.20±56.55	172.00±51.69*	210.5±77.54	116.20±22.01*	54.80±7.05*
IF	5.73±0.49	20.7±0.48*	19.63±2.26*	24.5±1.12*	20.00 ±1.14*
EL(Sec)	380.8±12.49	307.6±1.07	331.0±0.31	1339.0±34.15*	671.00±137.48
EF	0.60±0.40	4.05±0.32*	3.76±0.37*	3.30±0.49*	1.80±0.49
PEI(sec)	375.00±23.72	150.72±0.66*	125.00±10.43*	79.60±6.49*	214.20±47.19*

n=6, *=P< 0.05 when compared with control. Mounting latency (ML), Mounting frequency (MF), Intromission latency (IL), Intromission frequency (IF), Ejaculation latency (EL), Ejaculation frequency (EF) Post ejaculation Interval (PEI).

Table 4. Effects methanolic root extract of *Waltheria indica* on male sexual parameters in male albino rats after daily administration for 7 days.

	Mean±SEM				
Parameters	**Normal saline**	***W. indica* 50 mg/kg**	***W. indica* 100 mg/kg**	***W. indica* 200 mg/kg**	**Sildenafil 5 mg/kg**
ML(Sec)	225.6±64.97	65.04±1.45*	58.73±11.91*	32.20±2.65*	43.20±7.79*
MF	9.80±0.735	34.80±4.59*	21.32±2.68	18.67±1.43	29.20±4.48
IL(sec)	511.00±154.34	628.66±88.66	39.25±11.32*	38.68±2.40*	43.25±2.60*
IF	6.00±1.45	16.00±1.38	26.60±16.00*	34.00±6.68*	35.60±5.96*
EL(Sec)	386.3±0.33	252.7±15.97*	205.74±12.36*	336.3±20.92	538.80±99.39
EF	1.20±0.800	3.80±0.200*	4.66±0.183*	5.25±0.487*	3.00±0.316
PEI(sec)	355.0±0.32	186.7±34.94*	115.4±26.7*	120.9.0±0.10*	130.80±6.01*

n=6, *=P< 0.05 when compared with control. Mounting latency (ML), Mounting frequency (MF), Intromission latency (IL), Intromission frequency (IF), Ejaculation latency (EL), Ejaculation frequency (EF) Post ejaculation Interval (PEI).

Mount latency and intromission latency are indicators of sexual motivation. Both parameters are inversely proportional to sexual motivation (Yakubu and Afolayan 2009). The reduced latencies of mount and intromission are indications of reduction in the hesitation time of the male rats towards the receptive females (Yakubu *et al.*, 2005). Therefore, the decrease in the mount and intromission latencies at the doses of 50, 100 and 200 mg/kg body weight in this study might imply stimulation of sexual motivation and arousal. It may also be an indication of enhanced sexual appetitive behavior in the male rats which further supports the sexual improvement effect of the extract. This agreed with the findings on *Massularia acuminata* root in male wistar rats at the concentrations of 25, 50 and 100 mg/kg body weight (Yakubu *et al.*, 2011). The significant increases in mount and intromission frequencies were indications of stimulation of sexual arousability, performance, motivation and vigor in the rats. An increase in intromissions frequency also suggests the enhancement of full erection of male organs (Ratnasooriya *et al.*, 2000). These showed that orally administered methanolic root extract of *Waltheria indica* at the doses of 50, 100 and 200 mg/kg weight may be useful in the management of erectile dysfunction and desire or arousal disorders in males.

Prolongation of the ejaculatory latency by itself suggests an aphrodisiac action. This also suggests that libido, sexual vigour and copulatory performance were enhanced during the aphrodisiac action (Fouche *et al.*, 2015). The significant increase in ejaculation latency (EL) of 200 mg/kg of methanolic root extract *Waltheria indica* as suggests that the extracts prolonged the duration of coitus, which is an indicator of increase in sexual motivation and improved sexual function.

Post-ejaculatory interval (PEI) is an index of potency, libido and rate of recovery from exhaustion after first series of mating (Tajuddin *et al.*, 2014). It is an important parameter for evaluating the effect of administered extracts on erectile function (Sharma *et al.*, 2010). In humans, it has been shown that penile sensitivity is altered in men suffering from premature ejaculation compared with controls. Decrement of post-ejaculatory interval is a reflection of the improvement of erectile function and the ability to perform better copulation (Rowland, 1998). The dose dependent Significant decreased in post-ejaculatory interval observed with the 50, 100 and 200 mg⁄kg body weight

of methanolic root extract of *Waltheria indica* indicates the enhanced potency and libido or less exhaustion in the first series of mating or both. Similar findings have been reported by Pattij *et al.* (2005) on the study of the differences in the ejaculatory behaviors of individual rats.

Table 5. Effects of fractions of methanolic root extract of *Waltheria indica* on sexual behavioral parameters in male albino rats.

Treatment (mg/kg)	ML(sec)	MF	IL(sec)	IF	EL(sec)	EF	PEI(sec)
Normal saline	382.40 ±221.93	14.60 ±3.36	325.20 ±191.74	12.60 ±4.43	544.00 ±65.85	1.20 ±0.80	642.00 ±90.79
EAF 12.5	151.80±50.19	7.20±2.65	217.60±50.79	12.20±0.66	797.00±134.09	1.80±0.20	587.00±21.19
EAF 25	145.00 ± 7.91	15.00 ±0.94	192.00 ±19.28	13.00 ±0.63	746.00 ±0.00*	1.00 ±0. 00	514.00 ±0. 00
NBF 12.5	101.80±36.86*	13.00±1.98	102.00±44.09	15.80±2.15	227.80±61.03*	3.80±0.489*	396.80±80.36*
NBF 25	103.80 ±37.74*	14.20 ±1.20	43.60 ±7.13*	22.20 ±2.22*	176.00 ±35.82*	3.20 ±0.37*	300.20 ±6.05*
MLF 12.5	43.80±3.71*	17.00±5.02	62.80±5.83*	23.75±0.487*	429.20±53.87*	3.20±0.20*	355.20±22.60*
MLF 25	23.80 ± 3.25*	8.80 ±1.32	55.00 ±14.12*	18.20 ±0.20	201.20 ±24.73*	3.80 ±0.20*	333.80 ±20.25*

n=6, *=P< 0.05 when compared with control.
Results were presents as Mean ± SEM EAF= Ethylacetate Fraction, NBF=N-Butanol Fraction, MLF= Mother Liquor fraction
Mounting latency (ML), Mounting frequency (MF), Intromission latency (IL), Intromission frequency (IF), Ejaculation latency (EL), Ejaculation frequency (EF) Post ejaculation Interval (PEI).

The purpose of fractionating the methanol extract is to partition the constituents present in the extract into non – polar and polar constituents (Kotta *et al.*, 2013). The fractions obtained include ethyl acetate, n-butanol and mother liquor fractions; however the n hexane fraction was negligible for any pharmacological study. From the result of the study of sexual behavioral activity, it can be deduced that the polar constituents present in the plant have a higher tendency to induce aphrodisiac effect in male rats compared to the non-polar constituents.

4. Conclusion

The aphrodisiac potential of Methanol root extract and fractions of *Waltheria indica* were evaluated in male albino rats. Results obtained showed enhanced Sexual behavioral parameters of mount, intromission, ejaculation and post ejaculation interval. Further work on the mechanism of action of the extract and determination of the active compounds is recommended.

5. References

Ajuogu, P.K., Ere R., Nodu, M.B., Nwachukwu, C.U. and Mgbere, O.O. 2020. The influence of graded levels of *Cyathula prostrata* (Linn.) Blume on semen quality characteristics of adult New Zealand white bucks. *Translational Animal Science*, 4:1134–1139.

Basiru, A. and Olayemi, F.O. 2014. Effects of aqueous extract of *Waltheria indica* leaves on blood profile of male albino rats. *Journal of Medicinal Plant Research*, 8(41): 1223-1229.

Chen, X. and Lee, T.J.F. 1995. Ginsenosides induced nitric oxide mediated relaxation of the rabbit carvenosum. *British Journal of Pharmacology*, 115(1): 15–18.

Corine, G.T., Pham, T.N. and Demougeot, C. 2015. Promise of plant-derived substances as inhibitors of arginase. Mini *Reviews in Medicinal Chemistry*, 15(10): 798–808.

Drewes, S.E., George, J. and Khan, F. 2003. Recent findings on natural products with erectile-dysfunction activity. *Phytochemistry*, 62: 1019–1025.

Enema, O.J., Umoh, U.F., Umoh, R.A. *et al.* 2018. Chemistry and Pharmacology of aphrodisiac plants: A review. *Journal of Chemical and Pharmaceutical Research*, 10(7): 70-98.

Evans, W. C. 2009. Trease and Evans Pharmacognosy. 16th edition Saunders Elsevier p. 350-450.

Ezebuiro, I., Obiandu, C., Tamuno-Opubo, A., Owhorji, B.I. and Obiandu, A.C. 2022. Improved reproductivefFunctions of male wistar rats administered with leaf extract of *Cnidoscolus aconitifolius*. *Saudi Journal of Biomedical Research*, *7*(5): 184-189.

Fouche, G., Afolayan, A.J., Wintola, O.A., Thrombi, T.E. and Senabe, J. 2015. Effect of the aqueous extract of the aerial parts of *Monsonia angustifolia* E. Mey. Ex A. Rich., on the sexual behaviour of male Wistar rats. *BMC Complementary and Alternative Medicine*, 15: 343.

Gakunga, N.J., Mugisha, K., Owiny, D. and Paul, W. 2014. Effects of crude aqueous leaf extracts of Citropsis *articulata* and *Mystroxylon aethiopicum* on sex hormone levels in male albino rats. *International Journal of Pharmaceutical Science Invention*, 3(1): 5–17.

Gauthaman, K. and Adaikan, P.G. 2008. The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction- an evaluation using primates, rabbits and rats. *Phytomedicine*, 15: 44–54.

Gauthaman, K., Adaikan, P.G. and Prasda, R. N.V. 2002. Aphrodisiac properties of *Tribulus terrestis* extract (protodioscin) in normal and castrated rats. *Life Science*, 71: 1385–1396.

Ghosh, D., Mondal, S. and Ramakrishna, K. 2019. Acute and sub-acute (30-day) toxicity studies of *Aegialitis rotundifolia* Roxb., leaves extract in Wistar rats: safety assessment of a rare mangrove traditionally utilized as pain antidote. *Clinical Phytoscience*, 5(13): 1-16.

Gotep, J.G., Sabo, S.Y., Makama, S., Uguru, M.O., Okwuasaba F.K. 2021. Aphrodisiac activity of ethanol extract and fractions of *Fadogia cienkowskii* Schweinf. Rubiaceae roots in albino rats. *Journal of Medicinal Plants Research*, 15(2): 86-95.

Graham, J.G., Quinn, M.L., Fabricant, D.S. and Farnsworth, N.R. 2000. Plants used against cancer an extension of the work of Jonathan Hartwell. *Journal of Ethnopharmacology*, 73: 347–377.

Hernandez-Gonzalez M (2000) Prepubertal genital grooming: Penile erection in relation to sexual behavior of rats. *Physiology and Behavior*, 71(1-2): 51-56.

Hossain, M.A., Al-Hdhrami, S.S., Weli, A.M., Al-Riyami, Q. and Al-Sabahi, J.N. 2014. Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of Mentha piperita L grown in Sultanate of Oman. *Asian Pacific Journal of Tropical Biomedicine*, 4(Suppl 1): S368-S372.

Jansen, O., Angenot, L and Tits, M. *et al.* 2010. In vitro antiplasmodial activity of ethnobotanically selected plants from Burkina Faso. *Journal of Ethnopharmacology*, 130: 143–150.

Kim, H.J., Woo, D.S., Lee, G and Kim, J.J. 1998. The relaxation effects of ginseng saponin in rabbit corporal smooth muscle, is it a nitric oxide donor. *British Journal of Urology*, 82: 744–748.

Kotta, S., Ansari, S.H. and Ali, J. 2013. Exploring scientifically proven herbal aphrodisiacs. *Pharmacognosy Reviews*, 7: 1-13.

Lorke, D. 1983. A new approach to practical acute toxicity testing. Archives of Toxicology 54(4):275–287.

Msiska, T., Mwakikunga, A., Tembo, D. and Lampiao, F. 2020. A phytochemical analysis and *in vivo* effects of an herbal aphrodisiac *Newtonia hildebrandtii* on male wistar rat reproductive system. *Pharmacognosy Research*, 12(3): 243-249.

Musa, Y., Musah, M., Yerima, H. and Erena, N.B. 2016. Study of Phytochemical and Antimicrobial Activities of *Waltheria indica*. *Journal of Advance Research in Applied Science*, 3(4): 30-39.

Njoga, U.J., Jaja, I.F., Onwuka, O.S *et al.* 2022. Reproductive effects of medicinal plant (Azadirachta indica) used as forage and for ethnoveterinary practices: New insights from animal models. *Challenge*, 13(40): 1-18.

Ofeimun, J.O. and Ayinde, B.A. 2017. Preliminary investigation of the aphrodisiac potential of the methanol extract and fractions of *Rhaphiostylis beninensis* Planch ex Benth (Icacinaceae) root on male rats. *Journal of Science and Practice of Pharmacy*, 4(1): 182-188.

Oigbochie, E.V., Omage, K. and Odiase, E.D. 2019. Aqueous root extract of *Chrysophyllum albidum* caused dose and duration dependent increases in some reproductive hormones and spermatogenic arrest in the testes of male wistar rats. *Clinical Phytoscience*, 5(3): 1-8.

Olajuyigbe, O.O., Babalola, A.E. and Afolayan, A.J. 2011. Antibacterial and phytochemical screening of crude ethanolic extracts of *Waltheria indica Linn*. *African Journal of Microbiology Research*, 5(22): 3760–3764.

Oluranti, A.C., Michael, U.O., Jane, U.C. and Ayembe, N.A. 2012. Ethno botanical studies of medicinal plants used in the management of Peptic ulcer disease in Sokoto State, North Western Nigeria. *International Research Journal of Pharmacy and Pharmacology*, 2(9): 225-230.

Padashetty, S.A. and Mishra, S.H. 2007. Aphrodisiac studies of *Tricholepis glaberrima* with supportive action from antioxidant enzymes. *Pharmaceutical Biology*, 45: 580-586.

Padhy, R.., Patro, S.K., Jena, M. and Dash, S.K. 2017. Toxicity evaluation of methanolic rhizome extracts

obtained from *Drynaria quercifolia* (Linn.) J. Smith in experimental animals. *The Saudi Journal of Life Sciences*, 2(7): 255-258.

Pattij, T., De-Jong, T.R., Uitterdijk, A. *et al.* 2005. Individual differences in male rat ejaculatory behaviour: searching for models to study ejaculation disorders. *European Journal of Neuroscience*, 22(3): 724–734.

Ratnasooriya, W.D., Dharmasiri, M.G. 2000. Effects of *Terminalia catappa* seeds on sexual behavior and fertility of male rats. *Asian Journal of Andrology*, 2: 213–219.

Rowland. D.L. 1998. Penile sensitivity in men: a composite of recent findings. *Urology*, 52(6): 1101–1105.

Sanders, S.A., Graham, C.A., Bass, J.L and, Bancroft, J. 2001. A prospective study of the effects of oral contraceptives on sexuality and well-being and their relationship to discontinuation. *Contraception*, 64(1): 51-58.

Sharma, V., Thakur, M., Chauhan, N.S. and Dixit, V.K. 2010. Effects of petroleum ether extract of *Anacyclus pyrethrum* DC on sexual behaviour in male rats. *Journal of Chinese Integrated Medicine*, 8: 767–773.

Singh, R., Ali, A., Gupta, G., Semwal, A. and Jeyabalan, G. 2013. Some medicinal plants with aphrodisiac potential: A current status. *Acute Disease*, 2: 179-188.

Tajuddin, A.S., Latif, A. and Qasmi, I.A. 2004. Effect of 50% ethanolic extract of *Syzygium aromaticum (L)* Merr. & Perry.(Clove) on sexual behaviour of normal male rats. *BMC Complementary & Alternative Medicine*, 4: 17–24.

Tang, X., Olatunji, O.J., Zhou, Y. and Hou, X. 2017. *In vitro* and *in vivo* aphrodisiac properties of the seed extract from *Allium tuberosum* on corpus cavernosum smooth muscle relaxation and sexual behavior parameters in male Wistar rats. *BMC Complementary and Alternative Medicine*, 17: 510.

Yakubu, M.T, Akanji, M.A. and Oladiji, A.T. 2005. Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heirn) stem in male albino rats. *Asian Journal of Andrology*, 7(4): 399–404.

Yakubu, M.T. and Afolayan, A.J. 2009. Effect of aqueous extract of *Bulbine natalensis* (Baker) stem on the sexual behaviour of male rats. *International Journal of Andrology*, 32: 629–636.

Yakubu, M.T., Awotunde, O.S., Ajiboye, T.O., Oladiji, A.T. and Akanji, M.A. 2011. Pro-sexual effects of aqueous extracts of *Massularia acuminata* root in male Wistar rats. *Andrologia*, 43: 334–340.

Zongo, F., Ribuot, C., Boumendjel, A. and Guissou, I. 2013. Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn.*Waltheria americana*): A review. *Journal of Ethnopharmacology*, 148: 14–26.