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THE POTENTIAL OF *MILICIA EXCELSA* (WELW.) C. C. BERG (MORACEAE) ROOT BARK METHANOL EXTRACT AND FRACTIONS IN THE TREATMENT OF MALE INFERTILITY IN RATS

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ABSTRACT

Milicia excelsa root bark decoction is a traditional aphrodisiac and remedy for internal ailments like asthma, cough, heart disease, dysmenorrhoea, and externally for treating scabies, hair loss, sprains and wounds. Therefore, this study evaluated aphrodisiac potential of *M. excelsa* in male Wister rats by oral administration of graded doses: 100, 250 and 1000 mg/kg of crude methanol (MeOH) extract, and 100 and 200 mg/kg each of aqueous (AQ) and dichloromethane (DCM) fractions to male rats using standard procedure. Sildenafil citrate and distilled water served as positive and negative controls, respectively. Sexual behavioural parameters like mount, intromission and ejaculatory frequencies and latencies were recorded on day 7. Anti-lipid peroxidation effect and serum testosterone concentrations were also monitored. From the sexual behavioural study, MeOH extract (1000 mg/kg) and dichloromethane fractions (200 mg/kg) significantly ($p< 0.05$) increased intromission and mount frequencies, comparable to Sildenafil citrate. Ejaculatory frequency was increased ($p< 0.05$) by only the AQ fraction, and was comparable to standard drug. Intromission and mount latencies witnessed expected dose-dependent decreases by all three tested agents. Testosterone concentrations were also increased dose-dependently ($p< 0.05$) by all tested agents, and order of activity was: MeOH extract > DCM fraction > AQ fraction. Only the extract was comparable to standard drug in testosterone level. Lipid peroxidation was marginally inhibited in a concentration-dependent manner by MeOH extract and DCM fraction at 400 – 1000 *µg/mL,* but was not comparable to Vitamin E**.** *M. excelsa* root bark investigated for the first time for aphrodisiac activity increased serum testosterone and had positive effects on sexual behavioural indices, and this justifies its acclaimed local use in treating male infertility.

KEYWORDS: ***Milicia excelsa*; Anti-lipid peroxidation activity; Aphrodisiac activity; Rat; Root bark.**

INTRODUCTION

Erectile dysfunction (ED) is often attributed to physiological, mental and age factors leading to low sexual desire or poor sexual performance. Estimates of over 48.5 million infertile couples have been reported by World Health Organization [1]. A population based study in US revealed increasing prevalence of ED with age, 12% in those younger than 59 years, 22% for ages 60 to 69, and 30% in those older than 69 years [2]. According to global estimates, sexual dysfunction is more prevalent in women (25 – 63%) than in men (10 - 50%) [3]. The report of Ajao *et al*. [4] ranks Nigeria third in plants-based aphrodisiac research which is an indication of high prevalence of sexual dysfunction as

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corroborated by Oyelade *et al.* [5] in a hospital survey involving male population.

Many herbal drugs have been used by men with ED with varying degrees of success [2, 6, 7]. In the sub-Saharan Africa, 206 plant aphrodisiacs which included 28 Nigerian plants were documented by Ajao *et al*. [4]. Furthermore, over 700 aphrodisiac plants were published in a global review by Sin *et al* [8]. Several ethnobotanical surveys on aphrodisiac plants have also been published. The list includes those of Katsina State [9], Bauchi local government area [10], Akwa Ibom state [11], and other parts of northern Nigeria [9, 10], and elsewhere in Africa, Mali [12] and DR Congo [3]. In spite of the documentation of several plant-based aphrodisiacs, only a small proportion, 47 (22.8%) have been scientifically investigated [8], and this supports the need for continuous research in this field.

African teak, *Milicia excelsa* (Welw.) C. C. Berg (Moraceae), is a large deciduous tree growing in West Africa, Sudan and Uganda, up to 50 m in height, with a straight trunk and dark brown bark reaching a girth of 3.50 m [13]. The plant has a variety of uses in African traditional medicine. Specifically, root bark decoction is employed in female sterility, and a decoction of root and stem bark is an aphrodisiac [13]. Bark preparations are also traditional remedies for treating a wide range of conditions like asthma, cough, heart trouble, lumbago, dysmenorhoea and rheumatism. Externally, bark is applied to treat scabies, hair loss, sprains and wounds.

A variety of biological studies such as anti-diarrhoeal [14], antihypoxic [15], sedative-hypnotic [16] and anti-inflammatory [17] activities amongst others of *M. excelsa* have been reported. Since there is no literature information on aphrodisiac potential of *M. excelsa,* we therefore investigated its root bark using both *in vitro* and *in vivo* models to justify its folkloric importance in the management of increasing male sexual dysfunction.

MATERIALS AND METHODS

Plant material and extraction

Root bark peels *M. excelsa* were collected from trees growing in Bode-Osi forest reserve, Ola-Oluwa Local Government Area, Osun State, Nigeria in February 2021, and authenticated (voucher no. IUO/17/149) at Department of Pharmacognosy herbarium, IUO, Nigeria. They were cut into small pieces, air-dried for seven days and mechanically powdered to obtain a coarse powder which was exhaustively extracted (1 kg) in a Soxhlet apparatus with methanol. Crude extract was concentrated on a water bath (40^{o}C) to give a residue which was fractionated with dichloromethane (DCM) to yield DCM and aqueous (AQ) fractions. Dried crude extract and fractions were weighed and refrigerated until required.

Phytochemical screening

Basic phytochemical screening was carried out on the crude methanol extract of the plant according to Sengar *et al*. [18] and the presence of secondary metabolites recorded.

Anti-lipid peroxidation assay (TBARS)

Lipid peroxidation assay was carried out using slightly modified method described by Adedokun *et al*. [19]. Liver homogenate was prepared from commercially available goat liver. The liver was washed several times in ice–cold saline solution. A 10% of liver homogenate was prepared using ice-cold KCL (0.15 M) in a blender. Lipid peroxidation was initiated in 1 mL of tissue homogenate incubated with varieties concentrations of extracts (20-100 mg/mL), by the addition of 0.1 mL ferric sulphate (25µM), 0.1 mL ascorbate (100 µM) and 0.1 mL KH_2PO4 (10 mM). The volume was made up to 3 mL with distilled water and incubated at 37 ˚ C for 1 h. followed by the addition of 1 mL 5% trichloroacetic acid (TCA) and 1 mL 0.67% TBA. Reaction mixture tubes were boiled for 30 min in an electric water bath, cooled and centrifuged at 3500 rpm for 10 min. Extent of inhibition of lipid peroxidation was evaluated by measuring thiobarbaturic acid reactive substance (TBARs) levels.

Animals maintenance

Wister rats of both sexes (male 150-300 g and females 120-180 g) were used. Male and female rats were kept separately in cages in the animal house of Department of Pharmacology, Igbinedion University Okada at 28-35°C under artificial (12 h. light/12 h. dark) lighting system, and maintained on grower mash feed (Bendel Foods and Flower Meal, Edo state, Nigeria) and water *ad libitum*.

Male rat sexual behaviour procedure

This experiment, performed as described by Yakubu and Akanji [20], was initiated after the approval of the Animal Ethics Committee (approval no. EC/FP/021/02 of 10th March, 2021). Male Wister rats were fasted for 3 h. prior to study, initial weights determined and they were divided into five groups of five animals each. Positive control group A received Sildenafil citrate (100 mg/kg) orally, negative control group B received distilled water (1 mL/kg), while

groups C - E received 100, 250 and 1000 mg/kg of crude MeOH extract, respectively for 7 days. The experiment was repeated separately with 100 mg/kg and 200 mg/kg of AQ and DCM fractions each. The Guide for the Care and Use of Laboratory Animals published by the National Academies Press [21] was conformed with in this study.

Induction of oestrus cycle in female rats

Following the procedure of Yakubu and Akanji [20], acclimatized female Wister rats were weighed on the 5th day, and then artificially brought into oestrus phase (heat) by oral administration of Tween 80 solutions of estradiol benzoate (10 µg/100 g) daily for 48 h., and progesterone (0.5 mg/100 g) 4 h. prior to physical study of sexual behaviour. The experiment was conducted between 19:00 and 22:00 h at the Postgraduate Research Laboratory of the College of Pharmacy.

Assessment of sexual parameters

The receptive female animals were introduced into the cages of male animals in the ratio 1 female to 1 male thrice daily for 4 days after a 30 min adaptation period. The observation for mating behaviour (proceptive and precopulatory) commenced immediately and continued for 1 h., and on days 1, 3 and 7 afterward. The occurrence of events and phases of mating was recorded using digital video recorder, and frequency determined from video transcriptions. Various male sexual behaviour indices were assessed according to Tang *et al.* [22] as follows: Mount frequency (MF, the number of mounts at a specified period of time without intromission from the time of introduction of the female), Intromission frequency (IF, the number of intromissions from the time of introduction of the female until ejaculation), Ejaculation frequency (EF, the number of ejaculations from the time of introduction of the female rats to the male within a given time frame), Mount latency (ML, the time interval between the introduction of the female and the first mount by the male), Intromission latency (IL, the time interval from the time of introduction of the female to the first intromission by the male), Ejaculation Latency (EL, the time interval between the first intromission and ejaculation). This is usually characterized by pelvic thrusting and springing dismount.

Estimation of serum testosterone concentration

The animals were anaesthetized in a jar containing cotton wool soaked in diethyl ether. When rats became unconscious, their neck region was quickly cleared of fur and skin to expose their internal jugular veins [20]. The veins were slightly displaced (to prevent contamination of the blood with interstitial fluid) after which they were cut sharply with a sterile blade. The rats were then held head downwards, allowed to bleed into clean, dry centrifuge tubes. Blood samples were allowed to clot for 10 min at room temperature and subsequently centrifuged at 22 × g for 10 min with Uniscope Laboratory Centrifuge (model SM800B, Surgifriend Medicals, Essex, England). The sera were aspirated with Pasteur pipette and used for the determination of testosterone concentration within 12 h. of preparation [20].

Statistical analysis

Results of assays were expressed as a mean ± standard deviation. The differences between the negative control or positive control, as well as tested agents were determined by analysis of variance (one-way ANOVA). Difference in means were considered significant at $^{*}P<0.05$.

RESULTS

As shown in Table 1, low yield of MeOH extract (4.80%), and higher yields of AQ (23.68%) and DCM (13.00%) fractions were recorded. Secondary metabolites detected in the crude extract were alkaloids, steroids, saponins, flavonoids, anthraquinone glycosides (Table 2). Among the tested agents, MeOH extract (39.13-49-32 %) and DCM fraction (38.81-49.40 %) were equipotent in inhibiting lipid peroxidation, and gave marginal concentration-dependent response at 400 – 1000 µg/mL (Figure 1). DCM fraction was twice as active ($^{*}p<0.05$) as AQ fraction at 600 – 1000 µg/mL. Activities of all tested agents were incomparable to that of standard drug, Vitamin E.

Considering the frequency parameters, only the AQ fraction (1.70 – 1.90 ejaculations) gave the expected dose-dependent increase in ejaculation frequency (EF) (Table 3) and was comparable to Sildenafil citrate. However, all tested samples responded positively in increasing intromission frequency (IF) dose-dependently. DCM fraction was more active at 200 mg/kg (20.11 intromissions) than both doses of AQ fraction, and comparable to MeOH extract and standard drug. Mount frequencies (MF) witnessed dose-dependent increases for extract and fractions. MeOH was comparable at 1000 mg/kg (23.82 mounts/h) to Sildenafil citrate and with higher doses (200 mg/kg) of AQ and DCM fractions. Furthermore, DCM fraction (21.01 mounts/h) was more active than AQ fraction in MF activity at higher dose. As regards

Table 1: Profile of *Milicia excelsa* and yield of extract and fractions

Plant	Voucher number	Morphological part	Aspect	Location
Milicia excelsa	IUO/17/149	Root bark	Tree	Bose-Osi, Osun State
		Yield		
Crude MeOH extract		AQ fraction+		DCM fraction+
4.80%		23.68%		13.00%

+Relative to weight of crude extract used.

Table 2: Phytochemical screening of crude methanol extract of *Milicia excelsa* root bark

Phytocmetabolites	Result
Alkaloids	+
Anthraquinone glycosides	+
Cardiac glycosides	+
Flavonoids	+
Terpenoids	-
Tannins	-
Steroids	+
Saponins	+

Present (+), Absent (-)

Table 3: Effect of crude extract and fractions of *Milicia excelsa* root bark on sexual behaviour in rats after 7 days

Treatment	Mount Latency (ML, sec)	Mount Frequency (MF, mount/h)	Intromission Latency (IL, sec)	Intromission Frequency (IF)	Ejaculation Latency (EL, sec)	Ejaculation Frequency (EF)
Negative control (Distilled water) 10 mL/kg	34.75±9.72	11.00±0.82	47.00±7.38	5.50±0.29	64.75±8.47	n. d.
Positive control (Sildenafil citrate) 100 mg/kg	22.50±3.57	19.00±1.29	33.00±4.45	16.75±1.11*	96.50±4.99	1.50±0.28
Crude MeOH extract 100 mg/kg	28.82±2.23	13.51±1.28	31.54±0.25	10.11±3.02	66.74±12.40	2.20±0.24
Crude MeOH extract 250 mg/kg	21.47±0.52	16.73± 2.63	25.01±1.50*	14.21±2.02	74.43±0.51	1.72±0.22
Crude MeOH extract 1000 mg/kg	18.08±3.25*	23.82±3.07*	23.55±3.33*	20.50±5.11*	104.06±6.21*	1.05±0.45*
AQ fraction 100 mg/kg	31.35±35.31	14.31±3.92	37.08±8.09	11.59±6.03	74.56±7.82	1.70±0.81
AQ fraction 200 mg/kg	27.16±1.62	16.81±0.24	35.81±5.02	13.89±6.24	83.09±3.11	1.90±0.12
DCM fraction 100 mg/kg	25.60±1.15	13.61±11.21	32.31±34.00	12.00±0.26	86.00±1.80	2.00±0.30
DCM fraction 200 mg/kg	23.89±1.12	21.01±8.24*	28.81±83.70	20.11±8.02*	94.04±0.92*	1.57±0.59

Values above are mean of six replicates. n=6 (±SEM). Values with superscripts* indicate significant difference at *P<0.05 when compared to negative control, using ordinary One –way analysis (ANOVA). n. d, not determined.

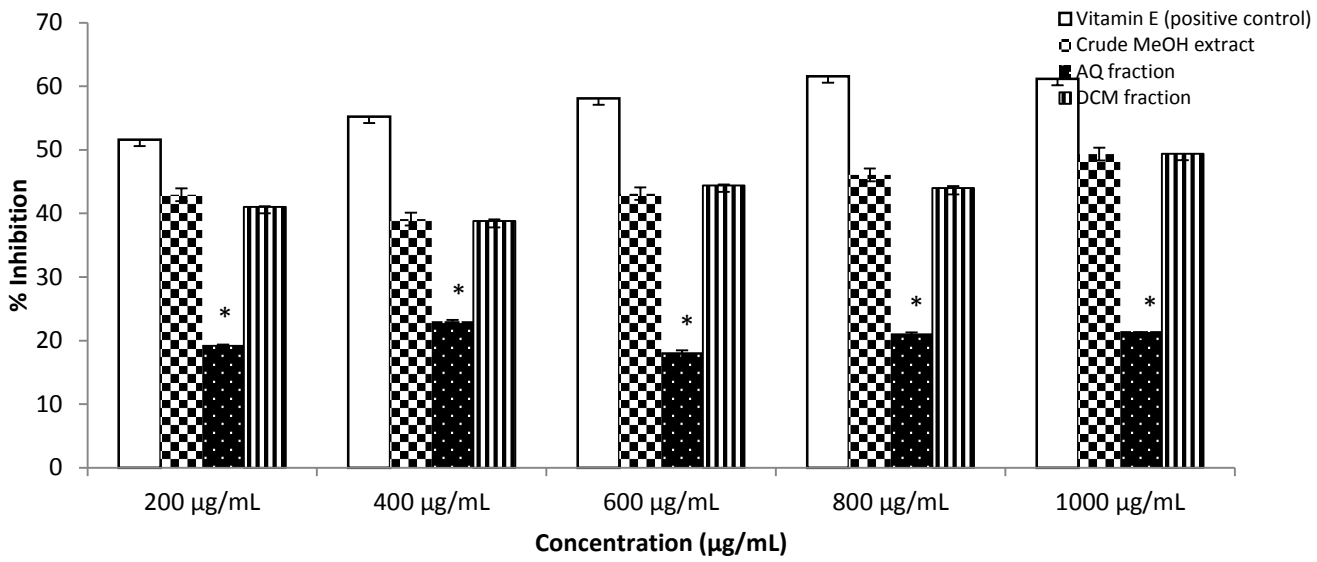

Figure 1: Anti-lipid peroxidation (TBARS) activity (% inhibition) of *Milicia excelsa* root bark extract.

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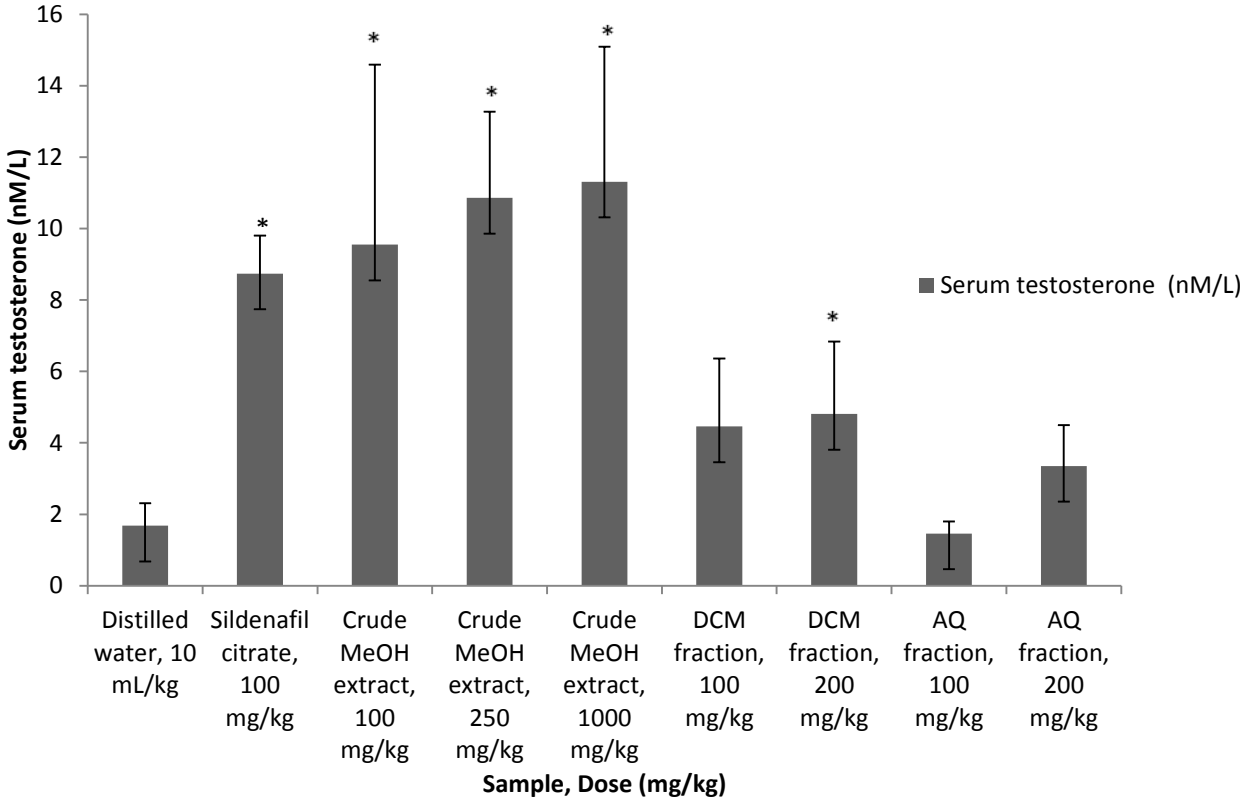

Figure 2: Effect of extract and fractions of *Milicia excelsa* root bark on blood testosterone level.

latency indices, the expected dose-dependent decreases were evident with all tested agents on mount and intromission latencies (IL). Lower doses of extract (100 and 250 mg/kg), were comparable to Sildenafil citrate (21.47 sec) and with both doses of DCM fraction in mount latency (ML) (Table 3). This latter fraction was more active (25.60-23.89 sec) than AQ fraction in ML activity. Comparable IL activity was recorded with both fractions (37-28 sec), Sildenafil citrate (33 sec) and extract (Table 3). Furthermore, observations on ejaculation latency (EL) were at variance with normal responses. Increases rather than decreases in EL were recorded in all cases.

Dose-dependent increases in testosterone levels were observed with all the tested agents. MeOH extract gave the highest increase (9.55-11.31 nM/L) at tested doses of 100 – 1000 mg/kg which was comparable to standard drug (Figure 2). It also exhibited 2-fold activity ($p<0.05$) of DCM at 100 and 200 mg/kg, and 3-fold activity ($p<0.05$) of AQ fractions at 200 mg/kg. Comparing the fractions, DCM fraction (4.46-4.81 nM/L) at both doses was thrice as active ($p<0.05$) as 100 mg/kg of AQ fraction (1.46 nM/L) in enhancing blood testosterone.

DISCUSSION

Results of sexual parameters from this study are in agreement with the inverse relationship of ML to sexual motivation proposed by some workers [20, 23, 24]. The observed decrease in the ML in this study, might imply stimulation of sexual motivation and arousability [20] leading to enhanced sexual appetitive behaviour in the male rats. Decreased EL activity in this study is in agreement with findings of Tang *et al.* [22] on *Allium tuberosum*. Overall, DCM fraction which appears more potent than AQ fraction in all frequency and latency parameters, as well as in enhancing blood testosterone, will qualify as a potential antifertility agent for further investigation to isolate active compounds. Findings of sexual performances in this study are in tandem with the report of other workers [24, 25], and the recent publications on antifertility property of *Khaya grandifoliola* stem bark [23] and *Khaya grandifoliola* leaf [26].

The results presented here agreed with those of other workers [20, 23, 24, 26], and suggest the aphrodisiac potential of the plant.

Yakubu and Akanji [20] postulated that the increase in IF as observed in this study, may be due to activation of the mechanism of penile erection. Other mechanisms of aphrodisiac action of plant extracts have been published [27, 28, 29]. MF and IF are useful indices of vigour, libido and potency, and the increases observed by both fractions in this study, suggest [20, 30] enhanced libido probably due to elevation several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behaviour. It may therefore be logical to attribute these behaviours to flavonoid and or saponin constituents of the plant since they have been reported [22] to alter androgen levels. In, summary, and with reference to all indices of sexual behaviour, MeOH extract and DCM fraction followed the traditional increases in MF, IF, EF, and decreases in IL and ML, and thus have clearly demonstrated potential as aphrodisiac agents.

Aphrodisiac mechanism of plant extracts has also been linked to elevated serum testosterone concentrations in sexually impaired animals [20, 22, 30, 31]. Sexual enhancement has also been attributed to dehydroepiandrosterone (DHEA), a major circulating steroid in the plasma and a common precursor for both androgen and estrogen synthesis, that is subsequently converted to testosterone and its metabolites [22].

CONCLUSION

Ability of methanol extract, DCM and AQ fractions to stimulate testosterone concentration and positively affect sexual behavioural indices supports its folkloric usage in African traditional medicine in treating male infertility. Activity appears to reside in the non-polar DCM fraction which will be a subject of future phytochemical investigation to isolate active compounds.

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