

Original Research Article

Aphrodisiac Potentials of the Methanol Extract of *Homalium Letestui* Root in Male Rats

Comment [PM1]: Just or n-butanol, dichloromethane, ethyl acetate, petroleum ether and aqueous too ?

Abstract

Aims: In this study, the methanol extract of *Homalium letestui* root was investigated with a view to ascertaining the scientific basis for its ethnobotanical use, and establishing, if any, the mechanisms of its effects, as an aphrodisiac.

Methodology: The crushed root of *Homalium letestui* was soaked in methanol for 72h and filtered with Whatman paper No.4; the extract was stored at -4°C . -Mature male rats were divided into three groups (active, sluggish, and impotent) and administered with various doses of the extract. The rats were, thereafter, observed for the following: mount latency, intromission latency, ejaculation latency, post ejaculation latency, mount frequency, intromission frequency, erection frequency, and penile erection latency.

Result: The result showed that the *Homalium letestui* root extract and fractions (n-butanol, dichloromethane, ethyl acetate, petroleum ether and ~~aqueous~~aqueous) caused and maintained erection in the subjects, and their effects on the rats were dose-dependent and statistically significant ($p < 0.05-0.001$). In addition, the aphrodisiac properties of the *Homalium letestui* root may in part be predicated on the properties of its phytochemical constituents which include alkaloids, saponins, flavonoids, tannins and cardiac glycosides.

Comment [PM2]: No analysis in this article?

Conclusion: These findings, therefore, justify the *Homalium letestui* root's folkloric use as a sexual performance enhancer and demonstrate that the plant possesses aphrodisiac properties.

Keywords: *Homalium letestui*; aphrodisiac; rodents; methanol extract; medicinal plants.

1. INTRODUCTION

Since the onset of human existence, plants have been dependable sources of sustenance and medications to human beings. For example, some modern medicines today are produced from active compounds isolated from plants [1]. Nevertheless, no scientific studies have been done to prove the efficacies of most of the medicinal plants used worldwide [2].

Globally, about 21,000 plant species have the potential for use as medicinal plants [3], some of which are used for congestive heart failure (digitalis), for hypertension (*Rawwolfia serpentina*), and for inflammation (*Asparagus pubescence* and *Uvaria chamae*) [4]. In addition, sexual dysfunctions are managed by the use of orthodox aphrodisiacs [5].

Derived from plants, animals, or minerals, aphrodisiacs are any food or drug that arouses the sexual instinct and increases pleasure and performance [6]. The search for aphrodisiacs capable of increasing libido, potency and sexual pleasure has been the passion of man since time immemorial. In folk medicine of different cultures, some of the aphrodisiacs derived from animals and plants have been confirmed pharmacologically to enhance sexual performance [7,8]. -In Nigerian, traditional medicines are used to treat numerous health problems such as mental disorders, insomnia, broken bones, infertility, and other reproductive health conditions [9]. -For instance, *Homalium letestui* is popularly used as a sexual enhancer among the people of Ibiono Local Government of Akwa Ibom State, Nigeria.

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Homalium letestui is traditionally known as Otong idim by the Annang/ Ibibios, abo-ako by the Yoruba's and akputukwu by the Igbo's of Nigeria; it belongs to the family flacourtiaceae, commonly found in the rain forest of West Africa and known as African homalium [10]. It is widely used in traditional medicine. For example, its root and stem bark are used in various decoctions traditionally

by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, malaria, and other inflammatory diseases [11]. Reports of antiplasmodial [11], antidiabetic [12], cellular antioxidant, anticancer and anti-leishmanial [13] activities of the plant have been published. There is no information, however, regarding the aphrodisiac property of its root extract and fraction in rodents. The aim of this study was to evaluate the aphrodisiac potentials of the methanol root extract and fractions of *Homalium letestui* on sexual behaviours in male rats in order to ascertain its ethnobotanical use as a sex enhancing plant and establishing, if any, the mechanisms of its effects, as an aphrodisiac.

Comment [PM3]: ?

Comment [PM4]: why just MeOH extract ? explain more.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The plant material was obtained in Uyo Local Government of Akwa Ibom State, Nigeria. A branch of the plant with leaves and flowers was presented for identification, authentication, and voucher specimen referencing by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The plant was identified as *Homalium letestui* and deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Nigeria under a voucher specimen reference number UUPH A69(i).

2.2 Extraction and Phytochemical Analysis

The fresh roots of *Homalium letestui* were harvested from the wild, washed and drained. Afterward they were chopped into small pieces and air-dried for three weeks. The dried roots were pulverized to coarse powder using mortar and pestle. The powdered root was weighed. 1kg was macerated in 99% methanol (Sigma Chemical, USA) for 72h. The liquid methanolic extract was obtained by filtration using Whatman filter paper No.4 and subsequently evaporated to dryness in a water bath regulated at 40°C. The extract was stored at -4°C for further experiment. The phytochemical screening of the extract was done according to the standard methods by [14, 15,16]. Tests for alkaloids, saponins, flavonoids, tannins and cardiac glycosides were carried out.

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2.3 Experimental Animals

Adults Wistar rats of both sexes (120-200g) and mice of both sexes (18-23g) were obtained from the Department of Pharmacology and Toxicology Animal House, University of Uyo, Nigeria. The male and female rats/mice were kept separately, quarantined, and acclimatized for 2 weeks, during which they were given free access to feed (Grower Marsh, Grand Bendel Ltd, Edo State) and water *ad libitum*. They were maintained under standard conditions (12-hours light/dark cycle), randomly selected, identified, and kept in their cages prior to dosing. Strict care was taken to ensure that the experimental subjects were available in the appropriate size and weight range for the entire study. Approval for the use of the animals for the study was obtained from the Animal Ethics Committee of the faculty of Pharmacy, University of Uyo, Nigeria. All animal experiments were conducted in accordance with internationally accepted Laboratory Animal Use and Care of Laboratory Animals (1996) as adopted and promulgated by the National Institute of Health (NIH publication No. 85(23), revised (1996), based on Helsinki convention and guidelines and rules of faculty of Pharmacy, University of Uyo, Nigeria for animal experimentation.

2.4 Determination of Median Lethal Dose (LD50)

The method of Miler and Tainter, (1944), was used to determine the median lethal dose (LD50) (Randhawa, 2009). Sixty-six healthy albino mice weighing (20-25g) were divided into 11 groups of 6 mice per group. Different doses (200-7000mg/kg) of the extract were administered, intraperitoneally (IP). The Median lethal dose (LD50) was calculated to be 335.0±183.33mg/kg. This median lethal dose (LD50) was used in this study.

Comment [PM5]: What is the interest ?

2.5 Sexual Behaviour Testing Procedure

The sexual behaviour of male rats was tested in a sound-attenuated, air conditioned room lit with a dim red light, during the dark cycle (17.00-20.00 GMT). After a 10 minutes' adaptation period of male rats in the copulation cage (rectangular glass cage), a sexually receptive female rat was presented to each male by dropping gently into the cage and the copulatory test started. The following parameters

of sexual behaviour were measured [17]-: mount latency (ML), time from the introduction of the female until the first mount; intromission latency (ML), time from introduction of the female to the first intromission (vaginal penetration); ejaculation latency (EL), time from the first intromission to ejaculation; post-ejaculatory interval (PEI), time from ejaculation to the first intromission of the second copulatory series; penile erection latency (PEL), the time interval from ejaculation to the first penile erection; erection frequency (EF), the number of erection from ejaculation to post ejaculatory intromission; mount frequency (MF), number of mounts preceding ejaculation; and intromission frequency (IF), number of intromissions preceding ejaculation. Male rats were trained with sexually receptive female rats for 30min at seven different occasions, with a gap of 5 days between each exposure. After the seventh pre-experimental training test, rats achieving ejaculation in any three of the test was defined as sexually potent. The remaining rats who failed to achieve ejaculation during any of the last three exposures were considered to be sexually impotent. The female rats allow mating only during the oestrus phase. Thus, they were artificially brought into oestrus (heat) by the method of [18]. They were administered with suspension of 17- β - estradiol at the dose of 100 μ g/100g of the animal 48h prior to the pairing plus progesterone injected subcutaneously, at the dose of 0.5mg/100g bw animal 4h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and standard animals. The most receptive female was selected for the study. -The experiment was carried out on the 7th day after commencement of the treatment of the male animals. The experiment was conducted at 19.00h in the same laboratory and under the light of same intensity. -The receptive female animals were introduced to the male animals, one female to one male (F/M).

2.6 Equipotent Aphrodisiac Effects of the Methanol Extract and its Fractions

Sexually active male rats were used for this experiment. A total of forty-two rats were used to determine the equipotent aphrodisiac effect of the extract and its fractions. These animals were randomly divided into seven (~~7~~) groups of six (~~6~~) rats each. Group 1 received 10ml/kg of normal saline per oral (PO) and served as the negative control; Groups 2-7, middle dose of the methanol extract and its fractions (Dichloromethane, Ethyl acetate, n-butanol, Aqueous and Petroleum Ether), respectively; and Group 8, testosterone (1mg/kg) serving as the positive control. Treatments in all the groups were done through intraperitoneal (IP) route of drug administration. The experiment was repeated with low and high doses of the methanol extract, while maintaining constant doses of negative and positive groups. Parameters monitored include mount latency, intromission latency, penile erection latency, ejaculation latency, post ejaculatory interval, mount frequency, intromission frequency, and erection frequency.

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Comment [PM6]: Explain more

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2.7 Biochemical Parameters

Blood samples were collected into each plain sample tube (centrifuge tubes) and centrifuged immediately at 2500rpm for 15min at room temperature to separate the serum. With the serum obtained, cholesterol, triglyceride and high density lipoprotein (HDL) levels of the experimental rats were measured using standard colorimetric methods. The low and very low- density lipoprotein (LDL and VLDL) were estimated from the formular of [19].

Comment [PM7]: Reformulate

2.8 Statistical Analysis and Data Evaluation

Results were expressed as multiple comparisons of Mean \pm SEM. Significance was determined using one-way ANOVA followed by Tukey –Kramer multiple comparison post-test. A probability level of less than 5% was considered significant.

3. RESULTS

3.1 Phytochemical Screening

The result of the phytochemical screening as shown in Table1 showed that *Homalium letestui* root contains alkaloids, saponins, flavonoids, tannins and cardiac glycosides.

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Comment [PM8]: Why not identify all compounds ?

Table 1: Phytochemical screening of *Homalium letestui* root extract

Metabolites	Test	Observation	Inference
Alkaloids	Dragendorff's	Orange precipitate	+
Saponins	Frothing Test	Persistent frothing	+
	Heamolytic Test	Clear red liquid formed	+
Tannins	Ferric chloride	Dark green precipitate	+
Flavonoids	Ammonia test	Two layers formed with yellow colouration at ammonium layer	+
	Sodium hydroxide test	Yellow colouration	+
Cardiac glycosides	Salkowski's test	Redish brown ring at interphase	+
	Keller Killiani test	Brown ring at interphase	+
	Lieberman's test	Violet to blue to green colour change	+
Anthraquinones	Borntrager's test	No reddish colour	-
	Combined anthraquinone test	No red or pink colour	-

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Key: + = present; - = absent

3.2 Effect of Extract on Sexually- Active Male Rats

The effects of the extract on sexually active male rats are as shown in Table 2. The extract demonstrated a dose-dependent decrease in mount latency, intromission latency, post ejaculatory intromission and penile erection latency. These decreases were statistically significant ($P < 0.01$) relative to control. In addition, the extract increased ejaculation latency, mount frequency, intromission frequency, and erection frequency in a dose-related manner, these increases being statistically significant ($P < 0.01$). In the presence of testosterone, the effects of the extract were enhanced in some of the parameters.

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Table 2: Effect of extract on sexually- active male rats

Dose (Mg/Kg)	ML (Min)	IL (Min)	EL (Min)	PEI (Min)	MF	IF	EF	PEL (Min)
Control	53.33±1.54	1.31±0.03	7.21±0.20	11.1±0.49	19.55±0.99	11.5±0.76	4.833±0.60	5.53±0.20
250mg/kg	37.33±1.50 ^c	0.82±0.11 ^c	11.95±0.15 ^c	8.35±0.14 ^c	31.17±1.33 ^c	20.67±0.88 ^a	10.5±0.76 ^c	4.33±0.16 ^b
500mg/kg	28.17±0.95 ^c	0.40±0.03 ^c	13.54±0.06 ^c	6.99±0.17 ^c	37.67±1.26 ^c	26.5±0.89 ^c	11.83±0.33 ^c	3.98±0.16 ^c
750mg/kg	19.17±0.87 ^c	0.33±0.04 ^c	15.25±0.06 ^c	6.17±0.31 ^c	42.67±1.63 ^c	39.17±2.26 ^c	13.67±0.33 ^c	3.18±0.14 ^c
Testosterone	20.00±1.18 ^c	0.35±0.04 ^c	14.95±0.15 ^c	5.60±0.13 ^c	48.67±2.57 ^c	32.00±1.39 ^c	14.5±0.34 ^c	3.46±0.12 ^c
Testosterone +500mg/kg	17.17±0.70 ^c	0.35±0.31 ^c	11.36±1.63 ^c	5.60±0.22 ^c	37.67±1.94 ^c	24.83±1.01 ^c	13.00±0.26 ^c	3.65±0.17 ^c

Values represent Mean± S.E.M.
 Significance relative to control: ^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$;
 n=6
 Min= Minutes
 ns= not significant.

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Key words: ML= Mount Latency; IL= Intromission Latency; EL= Ejaculation Latency; PEI= Post Ejaculatory Intromission; MF= Mount Frequency; IF= Intromission Frequency; EF= Erection Frequency; PEL= Penile Erection Latency.

3.3 Effect of Extract on Sexual Behaviour of Sexually-Impotent Male Rats

The effects of the extract on sexually impotent male rats are as shown in Table 3. The extract exhibited a dose-dependent decrease in mount latency, intromission latency, post ejaculatory intromission and penile erection latency. The decreases were statistically significant ($P<0.05$). The extract also caused a dose-related increase in ejaculation latency, mount frequency, intromission frequency and erection frequency. These increases were statistically significant ($P<0.01$). In the presence of testosterone, the effects of the extract were enhanced in some of the parameters.

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Table 3: Effect of extract on sexual behaviour of sexually-impotent male rats

Comment [PM9]: Group table 2, 3 and 4

Dose (Mg/Kg)	ML (Min)	IL (Min)	EL (Min)	PEI (Min)	MF	IF	EF	PEL (Min)
Control	36.72±1.52	41.26±1.09	4.94±0.47	10.54±0.35	13.17±0.06	9.00±0.05	7.16±0.303	9.13±0.03
250mg/kg	14.14±0.51 ^c	15.89±0.22 ^c	9.73±0.23 ^c	9.75±0.21 ^a	15.33±0.22 ^{ns}	11.33±0.06 ^c	9.67±0.01 ^{ns}	7.88±0.20 ^b
500mg/kg	11.76±0.43 ^c	12.48±0.30 ^c	11.57±0.20 ^c	7.72±0.30 ^{ns}	19.67±0.55 ^c	14.83±1.45 ^c	13.83±0.47 ^c	7.07±0.30 ^a
750mg/kg	8.08±0.30 ^c	8.59±0.19 ^c	13.74±0.36 ^c	7.62±0.20 ^{ns}	26.00±0.81 ^c	19.17±1.02 ^c	17.50±0.61 ^c	5.04±0.20 ^a
Testosterone	1.74±0.19 ^c	5.53±0.19 ^c	7.63±0.23 ^b	6.86±0.21 ^c	28.50±1.72 ^c	21.00±0.77 ^c	20.00±0.57 ^c	3.31±0.21 ^c
Testosterone +500mg/kg	5.39±0.28 ^c	8.23±0.65 ^c	7.60±0.28 ^b	5.42±0.20 ^c	20.17±0.47 ^c	20.50±0.76 ^c	19.67±1.11 ^c	5.25±0.20 ^c

Values represent Mean± S.E.M.
Significance relative to control: ^a $P<0.05$; ^b $P<0.01$; ^c $P<0.001$;

Min= Minutes

n=6

ns= not significant.

Key words: ML= Mount Latency; IL= Intromission Latency; EL= Ejaculation Latency; PEI= Post Ejaculatory Intromission; MF= Mount Frequency; IF= Intromission Frequency; EF= Erection Frequency; PEL= Penile Erection Latency.

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3.4 Effect of Extract on Sexual Behaviour of Sexually Sluggish Male Rats

The effects of the extract on sexually sluggish male rats are as shown in Table 4. The extract caused a dose-dependent decrease in mount latency, intromission latency, post ejaculation intromission and penile erection latency. These increases were statistically significant ($P<0.05$). The extract increased ejaculation latency, mount frequency, intromission frequency, and erection frequency in a dose-dependent manner, and these increases were statistically significant ($P<0.01$). In the presence of testosterone, the effects of the extract were enhanced in some parameters.

Table 4: Effect of extract on sexual behaviour of sexually sluggish male rats

Dose (MG/KG)	ML (Min)	IL (Min)	EL (Min)	PEI (Min)	MF	IF	EF	PEL (Min)
Control	23.40±2.86	26.26±2.83	7.32±0.14	16.18±0.01	9.66±0.10	6.16±0.04	3.33±0.02	12.62±0.02
250	15.83±1.99 ^a	19.71±2.45 ^{ns}	10.97±0.16 ^c	14.77±0.10 ^c	13.66±0.04 ^{ns}	9.33±0.11 ^c	3.83±0.01 ^{ns}	9.56±0.12 ^c
500	10.77±0.58 ^c	11.19±0.41 ^c	12.28±0.31 ^c	12.44±0.12 ^c	18.66±1.47 ^b	13.00±0.06 ^c	4.00±0.20 ^b	8.78±0.10 ^c
750	2.04±0.98 ^c	2.79±1.12 ^c	15.43±0.25 ^c	10.88±0.03 ^c	21.33±2.43 ^c	16.17±0.04 ^c	4.16±0.10 ^c	5.94±0.12 ^c

Testosterone	1.51±0.21 ^c	4.94±0.19 ^c	13.00±0.22 ^{ns}	13.69±0.01 ^c	27.50±2.02 ^c	18.83±0.07 ^c	5.16±0.10 ^c	3.97±0.10 ^c
500mg + Testosterone	16.30±0.30 ^a	17.75±0.63 ^a	7.93±0.31 ^c	5.41±0.11 ^c	21.83±1.16 ^c	16.17±0.01 ^c	3.50±0.15 ^{ns}	8.68±0.04 ^c

▲ Values represent Mean± S.E.M.

Significance relative to control: ^aP<0.05; ^bP<0.01; ^cP<0.001

Min= Minutes

n=6

ns= not significant.

Key words: ML= Mount Latency; IL= Intromission Latency; EL= Ejaculation Latency; PEI= Post Ejaculatory Intromission; MF= Mount Frequency; IF= Intromission Frequency; EF= Erection Frequency; PEL= Penile Erection Latency.

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3.5 The Equidosal Effect of Different Fractions on Sexual Indices of Male Rats

The effects of the fractions n-butanol 500mg/kg, dichloromethane 500mg/kg, petroleum ether 500mg/kg, ethyl acetate 500mg/kg and aqueous 500mg/kg on sexual behaviour of male rats are as shown in Table 5. The fractions showed a significance decrease in mount latency, intromission latency and penile erection latency ($P<0.01$). For ejaculation latency, except for aqueous fraction, all other fractions showed an increase in ejaculation latency which was statistically significant ($P<0.01$). All the fractions showed a statistically significant increase ($P<0.001$) in intromission frequency and erection frequency; the increase in mount frequency was not statistically significant. Petroleum ether, ethyl acetate and dichloromethane fractions showed a significant decrease ($P<0.001$) in post ejaculatory intromission.

Comment [PM10]: Origin of these fractions?

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Table 5: The Equidosal effect of different fractions on sexual indices of male rats

Dose	ML (Min)	IL (Min)	EL (Min)	PEL (Min)	M.F	I.F	E.F	PEI (Min)
500mg/kg								
Control	53.33±1.54	1.31±0.03	7.21±0.20	11.1±0.49	19.5±0.99	11.5±0.76	4.83±0.60	5.53±0.20
Aqueous	24.77±2.14 ^c	0.46±0.02 ^c	9.59±0.18 ^{ns}	9.04±0.27 ^c	31.0±1.71 ^c	29.33±2.17 ^c	10.5±0.22 ^c	5.69±0.17 ^{ns}
Ethyl Acetate	20.17±0.80 ^c	0.31±0.02 ^c	12.83±0.17 ^c	6.71±0.22 ^c	40.17±1.62 ^c	40.5±1.23 ^c	13.67±0.33 ^c	3.62±0.18 ^c
Butanol	29.67±3.21 ^c	0.39±0.02 ^c	10.20±0.73 ^a	9.45±0.19 ^b	25.17±1.78 ^{ns}	29.0±1.37 ^c	10.83±0.40 ^c	4.77±0.21 ^{ns}
Pet. Ether	24.17±1.85 ^c	0.40±0.03 ^c	13.74±0.18 ^c	7.66±0.14 ^c	36.5±1.38 ^c	39.17±2.61 ^c	13.67±0.56 ^c	3.01±0.26 ^c
DCM	40.67±0.99 ^c	1.02±0.10 ^b	10.03±0.24 ^c	9.64±0.40 ^a	22.67±1.69 ^{ns}	30.17±2.47 ^c	9.5±0.43 ^c	4.07±0.29 ^c
Testosterone	20.00±1.18 ^c	0.35±0.04 ^c	14.95±0.15 ^c	5.60±0.13 ^c	48.67±2.57 ^c	32.00±1.39 ^c	14.5±0.34 ^c	3.46±0.12 ^c

▲ Values represent Mean± S.E.M.

Significance relative to control: ^aP<0.05; ^bP<0.01; ^cP<0.001

Min= Minutes

(n=6)

ns= not significant

Key words: ML= Mount Latency, IL= Intromission Latency, EL= Ejaculation Latency, PEI= Post Ejaculatory Intromission, MF= Mount Frequency, IF= Intromission Frequency, EF= Erection Frequency, PEL= Penile Erection Latency

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3.6 The Effect of Extract on Some Biochemical Parameters of Treated Rats

The effects of the extract on biochemical parameters of treated rats are as shown in Table 6. Total cholesterol, total triacylglycerides, low density lipoprotein, high density lipoprotein and very low density lipoprotein were determined. The extract caused an increase in the total cholesterol levels of treated animals, though the increase was statistically not significant.

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Table 6: The effect of extract on some biochemical parameters of treated rats

Dose mg/kg	TC mmol/L	TG mmol/L	LDL mmol/L	HDL mmol/L	VLDL mmol/L
NS	2.43±0.15	1.65±0.07	1.05±0.10	0.68±0.04	0.70±0.04
250mg/kg	2.73±0.11 ^{ns}	1.58±0.03 ^{ns}	1.23±0.06 ^{ns}	0.83±0.06 ^{ns}	0.67±0.02 ^{ns}
500mg/kg	2.27±0.16 ^{ns}	1.57±0.03 ^{ns}	1.30±0.11 ^{ns}	0.73±0.06 ^{ns}	0.65±0.02 ^{ns}
750mg/kg	2.97±0.33 ^{ns}	1.62±0.09 ^{ns}	1.43±0.21 ^{ns}	0.80±0.12 ^{ns}	0.73±0.04 ^{ns}
Test. +500mg/kg	2.85±0.16 ^{ns}	1.58±0.03 ^{ns}	1.38±0.08 ^{ns}	0.75±0.10 ^{ns}	0.72±0.02 ^{ns}
Test. 1mg/kg	2.95±0.10 ^{ns}	1.57±0.04 ^{ns}	1.43±0.03 ^{ns}	0.82±0.06 ^{ns}	0.58±0.12 ^{ns}

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Values represent Mean± S.E.M. (n=6).

ns= not significant

Key words: TC= Total Cholesterol, TG= Total Glycerides, LDL= Low Density Lipoproteins, HDL= High Density Lipoproteins, VLDL= Very Low Density Lipoproteins, NS= Normal Saline, Test.= Testosterone

4. DISCUSSION

The indigenes of Ibiono Ibom local government area of Akwa Ibom State use *Homalium letestui* as a sexual enhancer. No scientific studies have, however, been done to prove its efficacy as an aphrodisiac. In this study, the effects of the methanol root extract of *Homalium letestui* and its fractions were investigated in order to understand the scientific basis behind this folkloric claim and to establish, if any, the mechanisms of its effects, as an aphrodisiac.

Comment [PM11]: Explain more

The result of the phytochemical screening of the extract revealed that *Homalium letestui* root contained alkaloids, saponins, flavonoids, tannins and cardiac glycosides. Phytochemicals are biologically active, naturally occurring chemical compounds [20], produced by plants to protect themselves, but recent researches demonstrate that these chemicals can also protect human against diseases [21]; they are found in different parts of plants - the roots, stems, leaves, flowers, fruits or seeds [22]. Numerous studies have shown that phytochemicals affect penile erection by different mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins. For example, alkaloids have ergogenic properties which induce vasodilation of the blood vessels, consequently resulting in erection [23], and saponin was reported to possess sexual enhancing properties by inducing the relaxation of smooth muscle Corpus cavernosum through the L-arginine/nitric oxide pathway [24]. Flavonoids, a constituent of the plant extract, with an antioxidant property have been reported to alter androgen levels in animals, thereby contributing to aphrodisiac effect [25]. It is a fact that an androgen, which may act both centrally and peripherally, induces sexual behaviour and erection [26]. The sexual behaviours of the male rats in this study may be due to the androgenic and gonadotropic activities of the extract, which could rationally be attributed to the flavonoid and saponin constituents of the plant, reported to alter androgen levels [25]. Furthermore, the steroidal nature of saponins may have facilitated its role as an intermediary in the steroidal pathway of androgen production [26]. It is, therefore, possible that through different mechanisms the extract might have crossed the blood-brain barrier of the animals to exert its aphrodisiac effect on the hypothalamic-pituitary-testicular axis [27].

Aphrodisiacs are substances which can stimulate sexual desire or libido [25] and can as well be used to revise impaired sexual functions [8]. In the male rats (active, sluggish, and impotent), the extract produced a clear modification in their sexual behaviours by causing a dose-dependent decrease in mount latency, intromission latency and post ejaculation latency and a dose-related increase in ejaculation latency, mount frequency, intromission frequency, and penile erection. Mount latency and intromission latency, indicators of sexual motivation, are inversely proportional to sexual motivation; mount frequency and intromission frequency are useful indices of vigour, libido and potency [28,29]. While the number of mount (MF) reflects sexual motivation, increase in the number of intromission (IF) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated [23]. Following the administration of the extract of *Homalium letestui* at all the doses, the increase in MF and IF, therefore, suggests enhanced libido. Furthermore, since intromission is not possible without adequate erection and coordinated activity of penile muscles [23], the increase in IF by the extract in this study suggests that the mechanism of penile erection was activated. *Homalium letestui* root extract may, therefore, increase potency by allowing or sustaining erection. The decrease in the mount and intromission latencies observed at the different doses of the extract investigated in this study might, therefore, imply stimulation of sexual motivation and arousability, and may also be an indication of enhanced sexual appetitive behaviour in the male rats. The significant increases in ejaculation latency, mount frequency, intromission frequency, and penile erection suggested an enhanced arousal and sexual vigour. These increases in the frequencies of mount, intromission and ejaculation, which suggested that libido, sexual vigour and sexual performance were enhanced, were in consonance with the work of [30]. The significant decrease in post ejaculation latency implied an enhanced arousal and sexual vigour. The decrease in intromission frequency indicated an increased copulation rate. These indicators of libido, when taken together, indicated that the extract might possess aphrodisiac properties.

The post ejaculatory interval is considered an index of potency, libido and the rate of recovery from exhaustion after first series of mating [31]. A post ejaculatory interval of more than 5400 sec indicates that the male is sexually exhausted and the intensity of sexual behaviour will be reduced in subsequent mating [23]. Therefore, the significantly decreased post ejaculatory interval of methanol extract of *Homalium letestui* root may be attributed to enhanced potency and libido or less exhaustion in the first series of mating or both. Additionally, the values of PEI obtained in this study are not up to or in any way close to the 90 min cut-off.

Cholesterol is a requirement for normal activity of the testicles and it is by far the major androgen secreted by the adult testes, and increase in testicular and/ or serum cholesterol levels have been shown to be linked to the aphrodisiac activity of medicinal plants [6]. This is because cholesterol is the precursor for the production of several physiologically important steroids including the bile acids, steroid hormones, and vitamin D amongst others [32,33]. Increase in cholesterol may lead to increase testosterone concentrations via steroidogenesis which should normally reflect in a corresponding increase in libido [6,34]. This study records increases, though not statistically significant, in serum cholesterol among the experimental groups; and such may have contributed to the enhanced libido seen in the male rats. The extract caused no statistical significance in the levels of triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein. All these support the sexual function improving effect of the extract at different doses.

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5. CONCLUSION

In conclusion, therefore, this research suggests that the methanol root extract of *Homalium letestui* could heighten indices that support sexual drive and energy by increasing mount and intromission frequencies and by decreasing mount and intromission latencies, thereby improving sexual behaviour in male rats - an indication of its aphrodisiac potentials. The aphrodisiac effect was dose dependent. The extract increased serum cholesterol concentrations which is a possible mechanism of action of its aphrodisiac potential. The aphrodisiac properties of the plant may also be predicted on the properties of its phytochemical constituents (alkaloids, saponins, flavonoids, tannins and cardiac glycosides), which may in part explain the mechanisms of its actions in this study. These findings, therefore, justify the *Homalium letestui* root's folkloric use as a sexual performance enhancer and demonstrate that the plant possesses aphrodisiac properties.

Comment [PM12]: May be others properties too, no?

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ETHICAL APPROVAL

Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Nigeria.

REFERENCES

Comment [PM13]: Add few recent references

- [1] Calixto, JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res.* 2000; 33(2):179-189.
- [2] Sandberg F, Corrigan D. *Natural Remedies: Their Origins and Uses*. Abingdon: Taylor & Francis; 2001.
- [3] World Health Organization. *World health statistics: Monitoring health for the SDGs, sustainable development goals*. World Health Organization; 2017
- [4] Nwafor, PA and Okwuasaba, FK. Antinociceptive and anti-inflammatory of methanolic extract of asparagus pubescens root in rodents. *Journal of Ethnopharmacology*. 2003;84: 125-129.
- [5] Bella, AJ and Shamloul, R. Traditional plant aphrodisiacs and male sexual dysfunction: Plant aphrodisiacs. *Phytotherapy Research*. 2014;28 (6): 831–835.
- [6] Yakubu, MT, Akanji, MA and Oladiji, AT. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (*Schweinf. Ex Hiern*) stem in male albino rats. *Asian J Androl*. 2005; 7:399–404.
- [7] Nwafor, AP, Idiong, JO, and Davies, K. Contraceptive and sexual behaviour effects of methanol extract of *Smilax Kraussiana* root in rodents. *Afr. Jour. of Pharma. and Therap.* 2013; 2(3): 83-87.
- [8] Essien, GE, Effiong, GS, Ebe, NU, Enoch, EN and Nwuzor, EO. Effect of ethanol leaf extract of *Telfairia occidentalis* on male reproductive activities. *GSC Advanced Research and Reviews*. 2020; 5(3):74–84.
- [9] Enwereji, E. Views on tuberculosis among the Igbo of Nigeria. *Indigenous Knowledge and Develop.* 1999; 7: 4–7
- [10] Hutchison, J and Daziel, M. *Flora of West Tropical Africa*. London: Common Agents; 1973.
- [11] Okokon, JE, Ita, BN and Udokpo, AE. The in-vitro antimalarial activities of *Uvaria chamae* leaves and *Hippocrates Africana*. *Annals of Tropical Medicine and Parasitology*. 2006; 100(7): 16-20.
- [12] Okokon, JE, Antia, BS and Ita, BN. Antidiabetic effects of *Homalium letestui* (*Flacourtiaceae*) in streptozotocin induced diabetic rats. *Research Journal of Medicinal Plants*. 2007; 1(4):134–138.
- [13] Okokon, JE, Okokon, PJ, Dar, A and Choudhary, MI. Anti-inflammatory and analgesic activities of *Homalium Letestui*. *Pharm Biol*. 2013; 51(11):1459 – 1466.
- [14] Harbone, J.B. *Phytochemical methods*. London: Chapman and Hall Ltd.; 1984: 49-188.
- [15] Trease, GE and Evans, WC. *Pharmacognosy*. 13th ed. London: Baillière Tindall; 1989: 683-684.
- [16] Sofowora, A. *Medicinal Plants and Traditional Medicine in Africa*. Ibadan: Spectrum Books Ltd; 1993: 134-156.
- [17] Carro-Juárez, M, Cervantes, E, Cervantes-Méndez, M and Rodríguez-Manzo, G. Aphrodisiac properties of *Montanoa tomentosa* aqueous crude extract in male rats. *Pharmacol Biochem Behav*. 2004; 78: 129–134.

- [18] Szechtman, H, Hershkowitz, M and Simantov, R. Sexual behavior decreases pain sensitivity and stimulates endogenous opioids in male rats. *European Journal of Pharmacology*. 1981; 70 (3): 279-285.
- [19] Friedewald, WT, Levy, RI and Fredrickson, DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972; 18:499–502.
- [20] Hasler CM, Blumberg JB. Symposium on phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*. 1999; 129:756S-757S.
- [21] Narasinga R. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific Journal of Clinical Nutrition*. 2003; 12 (1): 9-22
- [22] Costa MA, Zia ZQ, Davin LB, Lewis NG. Toward engineering the metabolic pathways of cancer-preventing lignans in cereal grains and other crops. In: Romeo JT (ed) *Recent advances in phytochemistry*. New York: Phytochemicals in Human Health Protection, Nutrition, and Plant Defense. 1999;33: 67–87
- [23] Agmo, A. Male rat sexual behaviour. *Brain Research Protocols*. 1997; 1: 203–209.
- [24] Kim, YC, Kim, JH, Davies, MG, Hagen, PO, and Carson, CC. Modulation of vasoactive intestinal polypeptide (VIP)-mediated relaxation by nitric oxide and prostanoids in the rabbit corpus cavernosum. *J. Urol*. 1995;153:807
- [25] Gauthaman, K, Adaikan, PG and Prasad, RNV. Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats. *Life Sci*. 2002; 71(12): 1385–1396.
- [26] Yakubu, MT and Afolayan, JA. Anabolic and androgenic activities of *Bulbine natalensis* stem in male Wistar rats. *Pharmaceutical Biology*. 2010; 48(5): 568-576.
- [27] Hull, EM, DU, J, Lorrain, DS and Matuszewich, L. Extracellular dopamine in the medial preoptic area: Implications for sexual motivation and hormonal control of copulation. *J. Neurosci*. 1995 ;15: 7465–7471.
- [28] Tajuddin SA, Shamshad A, Abdul L, and Iqbal A. Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragrans* Houtt. (nutmeg) and *Syzygium aromaticum* (L) Merr. and Perry (clove) in male mice: A comparative study. *Complement Altern. Med*. 2003; 3: 6.
- [29] Mbongue, FGY, Kamtchouing, P, Essame, OJL, Yewah, PM, Dimo, T and Lontsi, D. Effect of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats. *Indian J of Pharmac*. 2005; 37: 30–32.
- [30] Ratnasooriya, WD and Dharmasiri, MG. Effects of *Terminalia catappa* seeds on sexual behavior and fertility of male rats. *Asian Journal of Andrology*. 2002; 2: 213-226.
- [31] Tajuddin, A, Ahmad, S, Latif, A and Qasmi, IA. Effect of 50% ethanolic extract of *Syzygium aromaticum* (L.) (clove) on sexual behaviour of normal male rats. *BMC Complementary and Alternative Medicine*. 2004; 4: 17–24.
- [32] Bedwall, RS, Edwards, MS, Katoch, M, Bahuguna, A and Dewan, R. Histological and biochemical changes in testes of zinc deficient in BALD/C strain of mice. *Indian J of Experim Bio*. 1994; 32:243–247.
- [33] Watcho, P, Kamtchouing, P, Sokeng, SD, Moundipa, P, Tantchou, J and Essame JL. Androgenic effect of *Mondia whitei* roots in male rats. *Asian J Androl*. 2004; 6: 269-272.
- [34] Yakubu, MT, Akanji, MA and Oladiji, AT. Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacogn. Mag*. 2007; 3: 34-38.

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