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APHRODISIAC EFFECTS OF METHANOLIC LEAF EXTRACT OF *PSEUDOPANAX ARBOREUS* (ARALIACEAE) (L.F. PHILLIPSON) IN NORMAL MALE RATS

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Abstract

Background: The leaves of *Pseudopanax arboreus* have been used traditionally for decades as aphrodisiac without scientific investigation. In this study, the effects of methanolic leaf extract of *P. arboreus* were evaluated on sexual behavior of normal male rats.
Materials and Methods: Twenty-eight adult male rats were randomly grouped into 4 groups of 7 rats each. Rats in group 1 were treated with 10 ml/kg body weight distilled water, group 2 rats received 6mg/kg body weight Viagra™, while the rats in groups 3 and 4 were given 46.5 mg and 93mg/kg body weight respectively of the methanolic extract of the leaves of *P. arboreus*. Female rats were made receptive by ovariectomy and subsequent hormonal treatment. Sexual behavior parameters were monitored on days 1, 7, 14 and 21 by pairing each male rat to a receptive female. Relative weight of sex organs and hormonal (FSH, LH and testosterone) profile were also determined.
Results: Doses of 46.5 mg/kg and 93 mg/kg, the extract significantly increased the mount and intromission frequencies, penile licking and relative weight of sex organs and enhanced testosterone production; and significantly reduced mount and intromission latencies, mean intromission interval, when compared to the distilled water group. The 93 mg/kg body weight dose prolonged ejaculation latency and reduced post-ejaculatory interval. However, the reference drug, Viagra™ proved more efficient than the extract.
Conclusion: The methanolic extract of the leaves of *P. arboreus* possesses aphrodisiac properties which may be due to the actions of bioactive compounds present in the extract.

Keywords: *Pseudopanax arboreus*; sexual behavior; methanolic extract; aphrodisiac.

Abbreviations: **DW:** distilled water: **ME:** methanolic extract: **ME1:** methanolic extract dose 1: **ME2:** methanolic extract dose 2: ***P.*:** *Pseudopanax:* **ML:** mount latency: **MF:** mount frequency: **IL:** intromission latency: **IF:** intromission frequency: **EL:** ejaculation latency: **PEI:** post ejaculatory interval: **MII:** mean intromission interval: **ICE:** inter-copulatory efficiency: **PL:** penile licking: **FSH:** follicle stimulating hormone: **LH:** luteinizing hormone: **NO:** nitric oxide: **eNO:** endothelial nitric oxide: **spp**: species: **CNS:** central nervous system : **ED**: erectile dysfunction: **MSD:** male sexual dysfunction : **W.H.O**.: World Health Organization: **ELIZA**: Enzyme-Linked Immuno-absorbent Assay: **SEM**: Standard Error of Mean: **µg**: micrograms : **kg**: kilograms: **ml**: millilitres : **mIU**: micro International Units : **ng**: nanograms : **UB-IACUC**: University of Buea Institutional Animal Care and Use Committee: **OECD:** Organization for Economic Development and Corporation: **s:** seconds.

Introduction

Sexual relationships are among the most important social and biological relationships in human life; and sexual health is an important component of an individual's quality of life and well-being (WHO, 2002). One of the main aims of marriage is procreation (reproduction) to ensure the continuity of an individual's lineage and, more importantly, for sexual fulfillment of both partners. For life to continue, an organism must reproduce itself before it dies (Yakubu et al., 2007).

In humans, reproduction is initiated by the mating of a male with a female in sexual intercourse which facilitates the coming together of sperm and egg for the purpose of fertilization (Fullick, 1994). In order to have a normal sexual intercourse and sexual fulfillment in males, the male sexual organs (the copulatory organ, that is, the penis) and factors relating to erection must function normally. The recurrent or repeated inability of the male to perform a satisfactory sexual function or any disorder that interferes with his full sexual response cycle is termed male sexual dysfunction (MSD) (Yakubu et al., 2007; Yakubu and Akanji , 2011). MSD is an important contributor of male infertility with about 30-50% of infertility cases attributed to problems with males alone (Ekwere et al., 2007). Apart from other underlying causes, MSD is a pathology that occurs naturally as age advances (Moreira et al., 2006; Wattanathorn et al., 2012). The prevalence of sexual dysfunction is still increasing. Also, despite advances in modern and orthodox medicines, its effective control by drugs or adjuvant therapies is affected by drug efficacy and safety, as well as cost (Jian et al., 2012). Continuous search for potent anti-MSD agents is, therefore, needed to develop new, safe and effective formulae for the treatment of MSD.

One of the approaches for the management of MSD is by the use of aphrodisiacs. An aphrodisiac can, therefore, be described as any substance that enhances sexual drive and/or sexual pleasure. Aphrodisiac can also be viewed as any food, drug, scent or device that can arouse or increase sexual drive or libido (Rosen and Ashton, 1993). Plants constitute an important source of medicines and play a key role in the health of a greater portion of the world's population. The use of plants or their products to treat sexual disorders or improve on sexual performance has a long history in most countries, and their investigations in animals have proven that they are effective in improving sexual desire and sexual behavior in male animals. Some of these include: *Mondia whitei* (Watcho et al., 2007), *Massularia accuminata* (Yakubu et al., 2008; Yakubu and Akanji, 2011), *Fadioga agrestris* (Yakubu et al., 2008), *Ficus capensis* (Njoku-Oji et al., 2015), *Garcinia kola* (Ralebona et al., 2012), *Arctium lappa* (Jian et al., 2012), *Monsonia angustifolia* (Fouche et al., 2015), *Caesalpinia bonduc* (Gbangkoto et al., 2015), *Moringa oleifera* (Thawatchai et al., 2012), and so forth.

Pseudopanax arboreus, commonly called "Five Finger" in Cameroon, is a New Zealand native tree belonging to the family Araliaceae. The family from tropical area origin is present in cooler climates too. They are found in the Americas, Eurasia, Africa (including Manyu Division, Cameroon), Australia, New Zealand, New Caledonia, and Pacific Islands. The Family Araliaceae is closely related to the Family Apiaceae and Family Pittosporaceae (Plunkett et al., 1997). Members of these Families have been shown to possess aphrodisiac potentials. Also, some Araliaceae contain essential oils, are resinous and heterophyllous. Members of the Family include "devil's walking-stick," (*Aralia spinosa*), the "devil's club", (*Oplopanax horridus*), *Hedera* spp. (e.g. *Hedera helix);* and herbs such as ginseng *Panax* spp., a native of Korea and used as medicinal herb. Apart from the folk claim that *P. arboreus* possesses sex stimulating potentials, there is no scientific investigation to support or refute this indigenous claim. Leaves of *P. arboreus* (Araliaceae) have been considered as aphrodisiac by the people of manyu division (Cameroon). For a long time, they have been employed traditionally by the indigenous males to ensure endurance during sexual activity, and to improve on their sexual performance. In a previous study (Egbe et al., 2017), we evaluated the effects of its leaf-aqueous extract on the sexual behavior of normal male rats. As a consequence, the present study was designed to investigate the effects of methanolic leaf extract of *P. arboreus* on sexual behavior of normal male rats.

Materials and Methods

Plant material

Fresh leaves of *P. arboreus* were harvested from the tropical rainforest of Mamfe, under the guide of a local traditional practitioner and poacher, who confirmed the plant's identity based on its local vernacular name. A full branch and an attached flower of the plant were carefully preserved in a local newspaper and taken to the National Herbarium in Yaounde for authentication, where a voucher number 2734/SFRK (YA) of the specimen was given. Meanwhile, the fresh leaves were chopped into smaller pieces, air-dried under shade for about two months and ground using an electric grinder. The ground powder (300g) was added to 3000 ml of methanol, kept for 72 hours with mechanical agitation. It was thereafter filtered using the Laboratory test sieve (ENDOCOTTS LTD, ENGLAND) of 38 µm aperture. This was followed by solvent evaporation in a rotavapor under reduced pressure to yield 47.54 g of paste giving a 15.85% yield of extraction. Part of the extract was submitted to the Department of Chemistry, University of Buea for phytochemical screening. Meanwhile, administrative doses were determined following the traditional practitioner's directives and screening tests.

Chemicals

Products used in this study included sildenafil citrate (Viagra™) (Pfizer Inc, USA), diclofenac, estradiol and progesterone (Sigma Chemicals, USA) as well as bioassay kits for FSH (DRG Diagnostics, Germany), LH (DRG Diagnostics, Germany) and testosterone (Omega Diagnostics LTD, Scotland, UK), which were all purchased from BIOPHARCAM Douala, Cameroon. All Chemicals and reagents were transported and stored under the recommended conditions until used.

Animals

Animals used were Wistar strain rats of either sex, raised in the Animal House of the Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Cameroon, under standard conditions of temperature, humidity and a 12H light/12H dark cycle. They were given free access to water and laboratory diet.

Ovariectomy and induction of estrus in females

This was done according to the methodology described by Cariton (1986) and in our previous study (Egbe et al., 2017). Briefly, a total of 30 females rats obtained from the Animal House of the Department of Zoology and Animal Physiology, Faculty of Science of the University of Buea, Cameroon, were starved for 24 hours and prepared for surgical operation. They were weighed and given an intra-peritoneal injection of 0.02 ml/100 g (equivalent to 10 mg/kg body weight) diazepam, followed by 0.01 ml/100 g (equivalent to 5mg/kg body weight) ketamine, as anaesthesia; the two injections were separated by 5 minutes interval and after the onset of anaesthesia, the right and left dorsal lumbar of each female rat was shaved and the exposed skin prepared for aseptic surgery (97% alcohol wipe). This was followed by incising the dorsal flank to penetrate the abdominal cavity and attain each ovary. The par-ovarian fatty tissue was identified and retracted and the exposed ovary and its associated oviduct severed, after making a ligature at the anterior zone to prevent bleeding. The peritoneum and skin were then stitched followed by an intramuscular injection of 0.2 ml of penicillin-G to prevent any post-surgical infection, and oral administration of diclofenac capsule at the dose of 30 mg/kg as analgesia.

About 14 days following surgery and complete healing of the wounds, each ovariectomised female rat was given 66.67 µg of estradiol benzoate solution subcutaneously to bring them to estrus. This was followed 48 hours later by another subcutaneous injection of 600 µg progesterone solution. The progesterone was administered 6 hours before pairing each female rat with a sexually-experienced, normal (non-experimental) male rat. Only those rats exhibiting good sexual receptivity (solicitation behaviour and lordosis in response to mount) and no rejection behaviour were employed in the experiment (Ratnasooriya and Dharmasiri, 2000; Watcho et al., 2007).

Experimental design
Animal grouping and extract administration

A total of 28 adult Wistar male rats obtained from our breed and weighing 180-200 g each were randomly divided into 4 groups of 7 rats each. Rats in group 1 (control) were administered 10 ml/kg body weight distilled water; group 2 received 6 mg/kg body weight Viagra™ (standard drug); while those in groups 3 and 4 were given 46.5 and 93 mg/kg body weight respectively of the leaf-methanolic extract of *P. arboreus*. Each male rat was housed in a separate polypropylene cage.

Mating behavior test

This was done following the method described by Agmo (1997), and modified by Watcho et al. (2007), and as in our previous study (Egbe et al., 2017). Briefly, 30 minutes after the administration of the test substance, an estrous female was introduced into respective cages and observed for mating performance. Observations were conducted in the dark phase (as from 20:00 local time) of the light-dark cycle under dim light and very quiet conditions. Treatment lasted for 21 days and observations were done on days 1, 7, 14 and 21. Each test session was considered ended when Mount latency (ML) and Post Ejaculatory Interval (PEI) was 20 minutes.

The following performance parameters were assessed

Mount (when the male rat raised the forelimbs and gripped the female followed by the movement of its pelvic region towards the vagina of the female rat aimed at introducing his penis into the female's vagina); Intromission (the thrusting of the pelvic region of the male rat into pelvic region of the female followed by the penetration of the erect penis into the female's vagina); penile licking (when the male bent and licked the penis without mounting or intromission; and ejaculation (when the male griped the female with the latter raising its snout in an upward direction). In rats, this often comes after a series of successive mounts and intromissions. From these parameters, the following indices were determined and/or calculated: Mount latency (ML) (the time interval from the introduction of the female into the cage until the first mount); Mount Frequency (MF) (the total number of mounts preceding ejaculation); Intromission latency (IL) (the time interval from the introduction of the female into the cage until the first intromission); Intromission frequency (IF) (the number of intromissions preceding an ejaculation); Ejaculation latency (EL) (the time from the first intromission to ejaculation); Post-Ejaculatory Interval (PEI) (the time interval between an ejaculation and the next first mount); and Mean Intromission Interval (MII) or Inter-Copulatory Efficiency (ICE) (computed as: MII (ICE)= ejaculation latency/intromission frequency) (Jian et al., 2012; Fouche et al., 2015).

Assessment of relative weight of sex organs

On day 22 following beginning of treatment, all treated groups were starved, sacrificed under ethyl-ether as anesthesia, dissected, and the following organs were isolated: testes, epididymis, vas deferens, prostate, seminal vesicle and the penis. Each isolated tissue/organ was rinsed thoroughly and wiped with clean absorbent paper, carefully freed from all connective tissues, and then weighed using an electronic balance (NVT 1601/1, OHAUS Corporation, USA). Their individual weights were then expressed as a percentage of the total body weight.

Hormonal profile assessment

Alongside the sex organs, blood was collected using a 5-ml syringe through cardiac puncture and immediately transferred into heparinized test-tubes. The blood was kept for 24 hours after which the supernatant was collected and put into test-tubes. It was then centrifuged for 15 minutes at 2500 rpm. At the end of this, the supernatant was again collected. Plasma concentrations of FSH, LH and testosterone were determined using enzymatic kits and standardised reagents, and following the protocol prescribed by the manufacturer. In each case, a blank solution was prepared to help calibrate or standardise the ELIZA reader (Tietz, 1995; Egbe et al., 2017).

Ethical considerations

The experimental animals were handled in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for testing chemicals 423 and 425 [OECD, 2008 (a and b)]. The research protocol was approved by the University of Buea Institutional Animal Care and Use Committee (UB-IACUC) on the 30th of May 2018 and an ethical clearance number (UB-IACUC No 003/2018) was given.

Statistical Analysis

Values were expressed as Means (±SEM). Mean values were calculated for each animal and quantitative comparison between groups established from these means. One way Analysis of Variance (ANOVA) followed by Duncan test using SPSS for windows version 20.0 was done to test for significance level at $P<0.05$.

Results

Effects of leaf-methanolic extract of *Pseudopanax arboreus* on sexual behaviour of male rats

Effects on Mount (ML) and Intromission (IL) Latencies

Treatment of normal male rats with the leaf-methanolic extract of *P. arboreus* at 46.5 mg/kg and 93 mg/kg doses (ME1 and ME2, respectively) resulted in significant decreases in mount (ML) and intromission (IL) latencies, compared to the distilled water-treated (control) animals (Table 1).

Table 1: Effects of leaf-methanolic extract (ME) of *P. arboreus* on Mount (ML) and Intromission (IL) Latencies.

		Treatment			
Parameter	**Day**	**DW**	**Viagra™**	**ME1**	**ME2**
ML (s)	1	109.20±27.17^{ad}	81.80±9.52^{bd}	92.20± 11.45^{cd}	92.60 ±18.90^{cd}
	7	96± 30.80^{ad}	77.80 ±12.81^{ad}	83 ±15.65^{ad}	82.80± 6.98^{ad}
	14	82.40±57.74^{ae}	75 ±13.85^{ad}	79.40 ±16.30^{ae}	76.0±8.17^{ae}
	21	77.51 ±33.14^{ae}	70.4 ±14.01^{ae}	76.20 ±8.87^{ae}	73.4± 30.8^{ae}
IL (s)	1	121.20± 24.64^{ad}	87.80± 8.26^{bd}	99.20 ±11.61^{cd}	97.60± 17.63^{cd}
	7	106.33 ±27.48^{ae}	86± 14.47^{bd}	89.40 ±9.84^{bd}	88.60 ±7.37^{bd}
	14	94.17 ±48.07^{ae}	85.60± 15.93^{ad}	87 ±16.75^{ad}	86.80± 12.01^{ad}
	21	85.2 ±13.44^{af}	71.4 ±32.82^{ae}	79.6± 9.83^{ae}	77.4± 9.93^{ae}

Values are presented as Means (±SEM); DW: distilled water; ME1: leaf-methanolic extract dose1 (46.5 mg/kg); ME2: leaf-methanolic extract dose 2 (93 mg/kg); s: seconds; On the same row, values with same letter (a-c) are not significantly different; on the same row, values with different letters are significantly different; on the same column, values with the same letters (d-f) are not significantly different; on the same column, values with different letters are significantly different; $P<0.05$.

Effects on Mount (MF) and Intromission Frequencies (IF)

Like with the ML and IL, subjection of normal male rats to a 21-day treatment with the leaf-methanolic extract of *P. arboreus* at either dose (ME1 or ME2) resulted in an increase in mount frequency (MF) with significant ($p<0.05$) values obtained on day 21 of treatment, compared to the control animals; whereas both extract failed to induce significant ($p<0.05$) effects on intromission frequency (IF) throughout the treatment period compared to the control rats (Table 2).

Table 2: Effects of leaf-methanolic extract (ME) of *P. arboreus* on mount (MF) and intromission frequencies (IF) of normal male rats.

Parameter	Day	Treatment			
		DW	**Viagra™**	**ME1**	**ME2**
MF	1	10.55± 4.32^{ad}	20.1± 4.12^{bd}	12.85± 2.8^{ad}	10.81± 6.67^{ad}
	7	11.90 ±8.1^{ad}	21.05± 2.6^{cd}	13.95± 5.1^{ad}	12± 3.48^{ad}
	14	13.55± 3.51^{ad}	22.25± 3.1^{cd}	18.35± 6.66^{cd}	14.3± 9.87^{ad}
	21	14.15± 5.02^{ad}	24.60± 2.98^{cd}	20.05± 5.24^{bce}	17.95± 6.36^{be}
IF	1	10.15± 3.51^{ad}	16.65± 3.72^{bd}	11.35± 2.69^{ad}	10.2± 6.1^{ad}
	7	11.05± 7.02^{ad}	18.15 ±2.80^{bd}	12.45 ± 3.80^{ad}	10.75± 2.21^{ad}
	14	11.85± 2.63^{ad}	19.8± 3.15^{bd}	13.5± 5.97^{ad}	11.35± 6.93^{ad}
	21	12.7± 3.57^{ad}	22.25± 3.19^{cd}	16.5± 4.67^{bd}	13.65 ±4.39^{ad}

Values are presented as Means (±SEM); DW: distilled water; ME1: leaf-methanolic extract dose1 (46.5 mg/kg); ME2: leaf-methanolic extract dose 2 (93 mg/kg); s: seconds; On the same row, values with same letter (a-c) are not significantly different; on the same row, values with different letters are significantly different; on the same column, values with the same letters (d-f) are not significantly different; on the same column, values with different letters are significantly different ($p<0.05$).

Effects on Ejaculation latency (EL) and Post ejaculatory interval (PEI)

Treatment of normal male rats with the leaf-methanolic extract of *P. arboreus* at the doses of 46.5 mg/kg and 93 mg/kg (ME1 and ME2 respectively) induced contrasting effects on both EL and PEI. Rats treated with the ME1 dose witnessed a significant ($p<0.05$) incease in EL and a significant decrease in PEI from day 1 (613.35 ±194.76) through to day 21 (659.44± 220.82) of treatment compared to distilled water-treated (control) group; whereas those that received the ME2 dose showed a significant increase in EL (606.60± 192.83) on day 1, and (465.45± 264.73) on day 21; and a significant decrease in PEI (512.6± 336.62) on day 1, and (351.21± 173.44) compared to the distilled water-treated animals (Table 3).

Table 3: Effects of leaf-methanolic extract of *P. arboreus* on EL and PEI of normal male rats.

Parameter	Day	Treatment			
		DW	**Viagra™**	**ME1**	**ME2**
EL (s)	1	501.55 ±100.92^{ad}	548.45± 168.78^{abd}	613.35 ±194.76^{cd}	606.60± 192.83^{cd}
	7	486.65 ±135.13^{ad}	578.13± 140.68^{bd}	628.75± 189.17^{cd}	577.45± 264.73^{bd}
	14	477.82 ±200.92^{ad}	648.23± 155.37^{be}	647.31 ±194.76^{bd}	526.20± 192.83^{ae}
	21	435.71 ±105.13^{ae}	686.41± 165.38^{be}	659.44± 220.82^{bd}	465.45± 264.73^{af}
PEI (s)	1	380.8 ±282.86^{bd}	280.95± 175.82^{ad}	512.6± 336.62^{cd}	288.5± 191.6^{ad}
	7	215.85±117.05^{ad}	251.95± 164.1^{ad}	468.15±175.3^{cd}	389.9±165.44^{be}
	14	369.65± 152.67^{bd}	249.40± 113.3^{ad}	416.4± 274.99^{ce}	453.85± 330.49^{cf}
	21	372.75± 168.09^{bd}	237.1± 157.86^{ad}	351.21± 173.44^{bf}	476± 185.1^{cf}

Values are presented as Means (±SEM); DW: distilled water; ME1: leaf-methanolic extract dose1 (46.5 mg/kg); ME2: leaf-methanolic extract dose 2 (93 mg/kg); s: seconds; On the same row, values with same letter (a-c) are not significantly different; on the same row, values with different letters are significantly different; on the same column, values with the same letters (d-f) are not significantly different; on the same column, values with different letters are significantly different; $P<0.05$.

Effects on Penile licking (PL) and Mean intromission interval (MII)

Following treatment of normal male rats with either dose of the leaf-methanolic extract of *P. arboreus*, there was a non-significant ($p<0.05$) increase in PL induced by both doses from day 1 (3.5±1.05 and 4.75±1.37) to day 21 (4.75± 1.92 and 5.70± 1.98) (for ME1 and ME2 respectively), compared to the control animals. Similarly, a non-significant decrease in MII was observed from day 1 (45.38±13.89 and 45.73± 14.02) to day 21 (34.33± 7.09 and 37.24± 8.47), respectively for animals receiving ME1 and ME2, compared to the control group which showed a non-significant increase in this parameter.

Table 4: Effects of leaf-methanolic extract (ME) of *P. arboreus* on PL and MII of normal male rats.

Parameter	Day	Treatment			
		DW	**Viagra™**	**ME1**	**ME2**
PL	1	2.55± 1.93^{a}	5.55± 1.70^{a}	4.75±1.37^{a}	3.50± 1.05^{a}
	7	3.3±1.13^{a}	5.90± 1.12^{a}	5.20± 1.32^{a}	3.75± 2.20^{a}
	14	3.35± 1.23^{a}	6.25± 2.33^{a}	5.45± 1.19^{a}	4.55±1.57^{a}
	21	3.85± 1.04^{a}	7.30±2.15^{b}	5.70± 1.98^{a}	4.75± 1.92^{a}
MII (s)	1	49.28± 15.73^{a}	37.05±11.62^{a}	45.38±13.89^{a}	45.73± 14.02^{a}
	7	49.99±20.33^{a}	36.26± 29.08^{a}	40.06± 11.16^{a}	38.53±19.37^{a}
	14	53.49± 22.71^{a}	34.33± 7.09^{a}	34.35± 15.75^{a}	38.29± 16.99^{a}
	21	55.32± 29.3^{a}	23.96± 18.43^{a}	34.33± 7.09^{a}	37.24± 8.47^{a}

Values are presented as Means (±SEM); DW: distilled water; ME1: leaf-methanolic extract dose1 (46.5 mg/kg; ME2: leaf-methanolic extract dose 2 (93 mg/kg); s: seconds; On the same row, values with same letter (a-c) are not significantly different; on the same row, values with different letters are significantly different; on the same column, values with the same letters (d-f) are not significantly different; on the same column, values with different letters are significantly different; $p<0.05$.

Effects of leaf-methanolic extract of *P. arboreus* on hormonal profile of normal male rats.

Plasma concentrations of the male reproductive hormone, testosterone, increased significantly ($p<0.05$) in animals that received either dose of the leaf-methanolic extract of *P. arboreus* compared to the control animals. However, greater values were recorded in animals that received the standard drug (Viagra™) than in the extract-treated animals (Table 5). Meanwhile, effects of both doses of the extract on FSH and LH plasma levels were non-significant, compared to the control group.

Table 5: Effects of leaf-methanolic extract of *P. arboreus* on plasma concentrations of FSH, LH and Testoste**rone**

Hormone	Treatment			
	DW	**Viagra™**	**ME1**	**ME2**
FSH (mIU/ml)	2.31±0.72[a]	3.33±0.15[a]	2.57±0.84[a]	2.01±0.63[a]
LH(mIU/ml)	1.77±0.21[a]	2.94±0.32[a]	2.11±0.66[a]	1.46±0.58[a]
Testosterone (ng/ml)	2.71±0.66[a]	4.68±0.93[b]	4.04±1.02[b]	4.16±0.24[b]

Values are presented as Means (±SEM); DW: distilled water; ME1: leaf-methanolic extract dose 1 (46.5 mg/kg); ME2: leaf-methanolic extract dose2 (93 mg/kg); FSH: follicle stimulating hormone; LH: luteinizing hormone; on the same row, values with the same letter are not significantly different; on the same row, values with different letters are significantly different; $p<0.05$.

Effects of leaf-methanolic extract of *P. arboreus* on relative weight (% of body weight) of reproductive organs of normal male rats

Compared to the control group (Table 6), treatment of normal male rats with the leaf-methanolic extract of *P. arboreus* at either dose for a 21-day period provoked a non-significant ($p>0.05$) increase in the relative weight of reproductive organs: testes, epididymis, vas deferens, seminal vesicles, prostate and penis.

Table 6: Effects of leaf-methanolic extract of *P. arboreus* on relative weights of the reproductive organs of normal male rats.

Organ	Treatment			
	DW	**Viagra™**	**ME1**	**ME2**
Testes	1.32± 0.01[a]	1.59 ±0.14[a]	1.62± 0.08[a]	1.57± 0.04[a]
Epididymis	0.480 ±0.02	0.557± 0.06	0.516± 0.04	0.573 ±0.23
Vas deferens	0.09± 0.03[a]	0.11± 0.03[a]	0.11± 0.01[a]	0.12 ±0.04[a]
Sem. vesicle	0.32± 0.10[a]	0.49± 0.08[b]	0.41± 0.06[a]	0.34± 0.11[a]
Prostate	0.13± 0.05[a]	0.27± 0.05[b]	0.15± 0.01[a]	0.16± 0.06[a]
Penis	0.14± 0.02[a]	0.23±0.04[b]	0.18± 0.03[a]	0.18± 0.04[a]

Values are presented as Means (±SEM); DW: distilled water; ME1: leaf-methanolic extract dose 1 (46.5 mg/kg); ME2: leaf-methanolic extract dose2 (93 mg/kg); on the same row, values with the same letter are not significantly different; on the same row, values with different letters are significantly different; $p<0.05$.

Discussion

To the best of our knowledge, there is no scientific report on the aphrodisiac effects of the leaf extract of *Pseudopanax arboreus* in the literature. The present study was, therefore, designed to scientifically investigate the folk use of *Pseudopanax arboreus* as a sex stimulant. Overall, the leaf-methanolic extract of *P. arboreus* enhanced the sexual activity of male rats compared to the control rats, although its effects were less than those of Viagra™. There is no doubt that this drug is a standard sexual enhancer and the study confirms its wide use in treating most male sexual dysfunctions, especially erectile dysfunction (ED).

Phytochemical screening of the methanolic leaf extract of *P. arboreus* reveals the presence of alkaloids, flavonoids, saponins, steroids, tannins and triterpenoids. Studies in laboratory animals such as rats have attributed the sexual stimulant activity to many components of plant extracts as the possible bioactive agents increasing endogenous testosterone level and enhancing male sexual behavior. These include steroids and steroidal saponins, which may act as intermediaries in the steroidal pathway of androgen production. Saponins can bind to hormone receptors, resulting in conformational changes that can induce the physiological functions of the hormone; or can bind to hormones receptors

involved in the synthetic-pathway of the said hormones and as a consequence, promote their production (Drewes et al., 2003; Gauthaman et al., 2008). Also, flavonoids have been involved in altering androgen levels and may also be responsible for enhancing male sexual behavior either by promoting testosterone synthesis or inhibiting its metabolic degradation (Ratnasooriya and Fernando, 2008).

Both doses of the extract induced significant decreases in ML and IL, and increases in MF and IF, compared to the distilled water-treated control group. MF and IF are regarded as indicators of libido or sexual desire; while ML and IL are also regarded as indicators of sexual arousal. Mount Frequency and Intromission Frequency are useful indices of vigor, libido and potency. 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 (Agmo, 1997). The decreases in ML and IL and increases in MF and IF noted following administration of the leaf-methanolic extract of *P. arboreus* at both doses throughout the treatment period indicate that libido and arousal were enhanced by both doses of the plant extract (Tajuddin et al., 2004; Mbongue et al., 2005). At the 96 mg/kg body weight dose, the extract prolonged EL and increased PEI; effects that are similar to those of Viagra™. Prolonged ejaculation is an indicator of prolonged coitus. PEI is regarded as an indicator of potency, libido and potential to recover from exhaustion after the first ejaculatory series. Increment of PEI is a reflection of the improvement of erectile function and the ability to perform better copulation (Thakur et al., 2009). These increased values of PEI are indicators that *P. arboreus* has potential for use in erectile disorders, and further evaluation of *in vivo* effects on endothelial nitric oxide (eNO) production could provide insight towards the mechanism of action of *P. arboreus* on penile erection (Bivalacqua et al., 2007). This data clearly supports our findings in the previous study (Egbe et al., 2017) and thus further confirms the use of this plant in folk medicine.

PL and MII (ICE) are important indices for evaluating the effect of drug administration on erectile function (Thakur and Dixit, 2007). Both doses of the plant's – leaf methanolic extract induced an increase in PL and a decrease in MII (ICE) throughout the treatment period, compared to the distilled water-treated group, though to a lesser degree than the Viagra™-treated group. This further shows that the leaf-methanolic extract of *P. arboreus* increases sexual potency. The prolonged EL noticed with the 96 mg/kg dose, and the increased penile erection (PL) noticed with both doses suggest involvement of NO in the intervention (Du and Hull, 1999). Gingseng saponin has been shown to enhance libido and copulatory performance by acting directly on the CNS and gonadal tissues (Murphy and Lee, 2002), and evidence indicates its potential to facilitate penile erection by directly inducing the vasodilation and relaxation of the penile corpora cavernosa via an NO-dependent mechanism (Chen and Lee, 1995), including arginase inhibition (Corine et al., 2015).The improvement in sexual function observed in this study might, therefore, be as a result of the presence of such compounds in the leaf-methanolic extract of *P. arboreus*. However, further studies are necessary to identify the exact active constituent(s) responsible for the sexual function improvement activities, and the mechanisms through which these activities could be mediated.

There was an increase in plasma testosterone levels in rats treated with both doses of the extract, compared to the distilled water-treated, control rats. This major male androgen is synthesized and secreted by the Leydig cells of the testis under the influence of LH (Luteinizing Hormone), a gonadotrophin. Unfortunately, plasma levels of both LH and FSH were less significant; which means some phyto-constituents of the plant's leaves extract could mimic the role of LH to stimulate the Leydig cells. In the regulation of copulatory behavior, testosterone has been associated with an increase in sexual behavior (Mills et al., 1996; Murphy et al., 1998). Also, for normal sexual activity, penile tumescence and rigidity in addition to accessory muscles that help in improving penile rigidity and ejaculation, have been reported to be testosterone-dependent (Gauthaman et al., 2002). Furthermore, according to Hull et al. (1999) and Putnam et al. (2001), testosterone may enhance sexual behavior by increasing dopamine release in the medial preoptic area of the hypothalamus, and potentiating NO (nitric oxide) neurotransmission.

The effects of the extract on relative weight of reproductive and accessory organs, though non-significant, are a reflection of the plasma concentrations of testosterone recorded. Testosterone has been reported to be useful for the histo-morphometric development and maintenance of the testes, and ultimately, the biochemical process of sperm production (Njoku-Oji et al., 2012).The increase in the relative weight of these organs noticed in the extract-treated rats could, therefore, be attributed to the action of testosterone on them. The enhancements in the weights of accessory sexual organs of male rats are usually associated with androgenic activity and anabolic function. Androgens can stimulate the growth of accessory sexual organs (e.g., testis, seminal vesicles and prostate) and increase their weights (Chauhan et al., 2009). If certain drugs or natural compounds can increase the weights of accessory sexual organs, they are considered to possess androgenic properties (Luo et al., 2006).

Many bioactive components of plant extracts also exhibit aphrodisiac potentials by acting directly on the CNS to modulate the action of neurotransmitters and gonadal tissues in males, or through vasodilation and the generation of NO, which can also change sexual behavior. Independently of NO, alkaloids and saponins are known to increase dilation of blood vessels in the sexual organs (Zamble et al., 2008); through ROCK II enzyme (Rho-Kinase II) inhibition, hence, relaxation of smooth muscles of corpus cavernosum (Sumanta et al., 2012; Sumanta et al., 2013) and through inhibition of soluble epoxide hydrolase (sEH) (Jang et al., 2015). The results of this study suggest that the methanolic extract of the leaves of *P. arboreus* may be a potential new agent for the clinical management of MSD.

Conclusion

Overall, this study showed that the methanolic extract of the leaves of *P. arboreus* could enhance sexual behavior and performance in male rats and possess some anabolic potential. These actions could be as a result of its androgenic effects and other pathways through the actions of bioactive compounds such as alkaloids, flavonoids, saponins, steroids, tannins and triterpenoids present in the extract. The present laboratory findings support the folkloric use of this plant as an aphrodisiac by the people of Manyu Division in Cameroon.

Declaration of conflict of interest: The authors declare that this work has no conflict of interest with other people or organization.

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References

1. Agmo A. (1997). Male rat sexual behavior; Brain Research Protocols; 1:203–209.
2. Bivalacqua T.J., Liu T, Musicki B., Champion H.C. and Burnett A.L. (2007). Endothelial nitric oxide synthase keeps erection regulatory function balanced in the penis; European Journal of Urology; 51(6): 1732-1740.
3. Cariton A.E. (1986). Experimental Surgery of the Genital system. In: Gay William I, Heavner James E., editors. Methods of Animal Experimentation: Research Surgery and Care of the Research Animal; Part B Surgical approaches to organ systems. Volume 7. Orlando, Florida: Academic Press, Inc.; p.191.
4. Chauhan N.S., Sharma V. and Dixit V.K. (2009). Effect of *Asteracantha longifolia* seeds on the sexual behaviour of male rats; Natural Products Research; 14: 1-9.
5. Chen X and Lee T.J.F. (1995). Ginsenosides-induced nitric oxide mediated relaxation of the rabbit carvenosum; British Journal of Pharmacology; 115:15-18.
6. Corine G-T, T.-N. Pham and C. Demougeot (2015). The Promise of Plant-Derived Substances as Inhibitors of Arginase; Mini-Reviews in Medicinal Chemistry; 798-808.
7. Drewes S.E., George J. and Khan F. (2003). Recent findings on natural products with erectile dysfunction activity. Phytochemistry; 62:1019-1025.
8. Du J. And Hull E.M. (1999). Effects of testosterone on neuronal nitric oxide synthase and tyrosine hydrolase; Brain Research; 836:90-98.
9. Egbe B. Besong, Ateufack Gilbert and Albert Kamanyi (2017). Validating Sex-enhancing Potentials of Aqueous Extract of *Pseudopanax arboreus* (ARALIACEAE) on Normal Male Rats; International Journal of Multidisciplinary and Current Research; ISSN: 2321-3124; Research Article; Available at: http://ijmcr.com
10. Ekwere, P.D., Archibong, E. I., Bassey, E. E., Ekabua, J. E,Ekanem, E. I. and Feyi-Waboso, P. (2007). Infertility among Nigerian couples as seen in Calabar. Port Harcourt Medical Journal; 2: 35-40.
11. Fouche Gerda, Anthony J. Afolayan, Olubunmi A. Wintola, Tendani E. Khorombi and Jeremiah Senabe (2015). Effects 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.
12. Fullick A. (1994). Biology. Heinemann Advanced Science. Oxford. (1994) p. 332.
13. Gauthaman K., Adaikan P.G. and Prasad R.N. (2002). Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats; Journal of Life Science; 31:1385-1396.
14. Gauthaman K. And Adaikan P.G. (2008). The hormonal effects of *Tribulus terrestris* and its role in the management of erectile dysfunction - an evaluation using primates, rabbits and rats. Phytomedicine; 15:44-54.
15. Gbankoto Adam, Eugénie Anago, Pauline A. Houndjo, Dogbè clémentadjahouinou and Fernand gbaguidi (2015). Effect of Aqueous and Ethanolic Extracts of *Caesalpinia bonduc* Root on Sexual Behaviour of Male Wistar Rats; International Journal of Multidisciplinary and Current Research. 3; 2321-3124.

16. Hull E.M., Lorrain D.S., Du J., Matuszewich L., Lumley L.A., Putnam S.K. and Moses J. (1999). Hormone-neurotransmitter interactions in the control of sexual behaviour; Behaviour Brain Research; 105; 105-116.
17. Jang Hoon Kim, Abubaker Morgan and Bui Huu Tai (2015). Inhibition of soluble epoxide hydrolase activity by compounds isolated from the aerial parts of *Glycosmis stenocarpa;* Journal of Enzyme Inhibition and Medicinal Chemistry; 31(4):1-5.
18. Jian Feng Cao, Zhang PengYing, Xu ChengWei, Huang Tao Tao, Bai Yun Gui and Chen Kao Shan (2012). Effects of aqueous extract of *Arctium lappa* L. (burdock) roots on the sexual behavior of male rats; BMC Complementary and Alternative Medicine; 12:8.
19. Luo Q, Li Z, Huang X, Yan J, Zhang S and Cai YZ (2006). *Lycium barbarum* polysaccharides: protective effects against heat-induced damage of rat testes and H2O2-induced DNA damage in mouse testicular cells and beneficial effect on sexual behavior and reproductive function of hemicastrated rats; Journal of Life Science, 79(7): 613-621.
20. Mbongue F.G.Y., Kamtchouing P., Essame O.J.L., Yewah P.M., Dimo T. and Lontsi D. (2005). Effects of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats. Indian Journal of Pharmacology; 37:30-32.
21. Mills T.M., Relly C.M. and Lewis R.W. (1996). Androgens and penile erection: a review. Journal of Andrology; 17: 633-638.
22. Moreira, E.D., S.C. Kim, D. Glasser and C. Gingell (2006). Sexual activity, prevalence of sexual problems and associated help-seeking patterns in men and women aged 40-80 years in Korea: data from the Global Study of Sexual Attitudes and Behaviors (GSSAB). Journal of Sexual Medicine; 3: 201-211.
23. Murphy L.L., Cadena R.S., Chavez D. and Ferraro J.S. (1998). Effects of American Ginseng (*Panax quinquefollum*) on male copulatory behaviour. Physiology and Behaviuor. 64; 445-450.
24. Murphy L.L. and Lee T.J.F. (2002). Ginseng, sex behaviour and nitric oxide. New York Academy of Sciences. 962: 372-377.
25. Njoku-Oji N.N, Osaji D. E, IfegwuN.O, Uchefuna R.C, Ezejindu DN (2015). Effects of aqueous extract of *Ficus capensis* leaf on some reproductive parametersin normal adult male wistar Rats; International Journal of Multidisciplinary Research and Development; 2(2): 577-583.
26. Organization for Economic Cooperation and Development (2008a). Guidelines for the Testing of Chemicals/Draft Updated Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents.
27. Organization for Economic Cooperation and Development (2008b). OECD guidelines for testing of chemicals, Test No. 425, Acute toxic class method.
28. Plunkett, G.M., Soltis, D.E. & Soltis, P.S. (1997). Clarification of the relationship between Apiaceae and Araliaceae based on MATK and RBCL sequence data. American Journal of Botany 84: 565-580.
29. Putnam S.K., Du J., Sato S. and Hull E.M (2001). Testosterone restoration of copulatory behaviour correlates with medial preoptic dopamine release in castrated male rats; Hormone and Behaviour; 39: 216-224.
30. Ralebona N., Sewani Rusike C.R. and Nkeh-Chungag B.N. (2012). Effects of ethanolic extract of *Garcinia kola* on sexual behaviour and sperm parameters in male Wistar rats. African Journal of Pharmacy and Pharmacology; 6(14): 1077-1082.
31. Ratnasooriya, W.D., and Dharmasiri, M.G. (2000). Effects of *Terminalia catappa* Seeds on Sexual Behaviour and Fertility of Male Rats. Asian Journal of Andrology; 12; 56-62.
32. Ratnasooriya WD and Fernando TSP (2008). Effect of black tea brew of *Camellia sinensis* on sexual competence of male rats. Journal of Ethnopharmacology; 118: 373-377.
33. Rosen and. Ashton (1993). Prosexual Drugs: Empirical Status of New Aprhodisiacs. Archives of Sexual Behaviour; 22: 521-543.
34. Sumanta Kumar Goswami, Manoj Kumar Pandre, Rohitash Jamwal, Shekhar Dethe, Amit Agarwal and Mohammed Naseeruddin Inamdar (2012). Screening of Rho-kinase 2 inhibitory activity of Indian medicinal plants for management of erectile dysfunction. Journal of Ethnopharmacology; 144: 483–489.
35. Sumanta Kumar Goswami, Mohammed Naseeruddin Inamdar, Manoj Kumar Pandre, Rohitash Jamwal and Shekhar Dethe (2013). Erectogenic and Aphrodisiac Effects of Butea frondosa Koenig ex Roxb. in Rats: Involvement of Enzyme Inhibition. Evidence-Based Complementary and Alternative Medicine; 10pages.
36. Tajuddin A, Ahmad S and Latif A, Qasmi IA (2004). Effect of 50% ethanolic extract of *Syzygium aromaticum* (L.) Merr. & Perry. (clove) on sexual behaviour of normal male rats. BMC Complementary and Alternative Medicine; 4:17–24.
37. Tajuddin Ahmad S., Latif A., Qasmi I. A. and Amin K. M.Y. (2005). An experimental study of sexual function improving effect of *Myristica fragrans* Houtt. (nutmeg); BMC Complementary and Alternative Medicine; 5:16.23.
38. Thakur M. And Dixit M.K. (2007). Aphrodisiac activity of *Dactylorhiza hatagirea* (D.Don) Soo in male Albino rats; BMC Complementary and Alternative Medicine; 4:29-31.
39. Thakur M., Chauhan N.S., Bhargava S. and Dixit V.K. (2009). A comparative study on aphrodisiac activity of some ayurvedic herbs in male albino rats. Archives of Sexual Behaviour; 38(6): 1009-1015.

40. Thawatchai Prabsattroo,Jintanaporn Wattanathorn, Sitthichai Iamsa-ard, Supaporn Muchimapura and Wipawee Thukhamme (2012). *Moringa Oleifera* Leaves Extract Attenuates Male Sexual Dysfunction. American Journal of Neuroscience; 3 (1): 17-24.
41. Tietz N.W. (1995). *Clinical Guide to Laboratory Tests*. 3rd edition. Philadelphia, Pa, USA: W. B. Saunders.
42. Watcho Pierre, Fabien Zelefack, Telesphore Benoit Nguelefack, Silvere Ngouela, Phelix Bruno Telefo, Pierre Kamtchouing, Etienne Tsamo and Albert Kamanyi (2007). Effects of the aqueous and hexane extracts of *Mondia whitei* on the sexual behavior and some fertility parameters of sexually inexperienced male rats. African Journal of Traditional, Complementary and Alternative Medicine; 4 (1):37-46.
43. Wattanathorn, J., P. Pangphukiew, S. Muchimapura, KSripanidkulchai and B. Sripanidkulchai (2012). Aphrodisiac activity of *Kaempferia* parviflora. American Journal of Agriculture and Biological Science, 7: 114-120.
44. World Health Organisation working document on sexual and reproductive health (2002).
45. Yakubu M.T. and Akanji M.A. (2011). Effect of Aqueous Extract of *Massularia acuminata* Stem on Sexual Behaviour of Male Wistar Rats. Evidence-Based Complementary and Alternative Medicine; 1: 738103.
46. Yakubu, M. T., Akanji, M. A. and Oladiji, A. T. (2007). Male Sexual Dysfunction and Methods used in Assessing Medicinal Plants with Aphrodisiac Potentials. Pharmacognosy Reviews; (1): 49-56.
47. Yakubu M.T., Akanji M.A., Oladiji A.T. and Adesokan A.A. (2008). Androgenic potentials of the aqueous extract of *Massularia accuminata* (G.don) Bullock ex Hoyl. Stem in male Wistar rats. Journal of Ethnopharmacology; 118: 508-513.
48. Yakubu Musa Toyim , Musbau Adewumi Akanji and Adenike Temidayo Oladiji (2008). Effects of oral administration of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem on some testicular function indices of male rats. Journal of Ethnopharmacology; 115: 288–292.
49. Zamble A., Sahpaz S., Brunet C. And Bailleul F. (2008). Effects of *Microdesmis keayana* roots on sexual behaviour of male rats. Phytomedicine; 8:625-629.