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Validating Sex-enhancing Potentials of Aqueous Extract of *Pseudopanax arboreus* (ARALIACEAE) (L.F. Phillipson) on Normal Male Rats

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Abstract

Leaves of Pseudopanax arboreus (ARALIACEAE) (L.F. Phillipson) have been considered as aphrodisiac by the people of Manyu Division and other parts of Cameroon for a long time, without scientific investigation. The present study was designed to investigate the effects of the aqueous extract of leaves of Pseudopanax arboreus (L.F. Phillipson) on sexual behavior in normal male rats. Twenty-eight (28) adult male Wistar rats were randomly partitioned into 4 groups of 7rats each. Rats in gropu1 were administered 10ml/kg distilled water, group 2 received 6mg/kg Viagra, while those in groups 3 and 4 were given 75 and 150mg/kg body weight respectively of the aqueous extract of the leaves of P. arboreus. Sexual behavior parameters were monitored on days 1, 7, 14 and 21 by pairing each male to a receptive female. Relative weight of sex organs and hormonal profile were also determined. Both doses increased mount and intromission frequencies, ejaculation latency, penile licking, testosterone production and relative weight of sex organs; but significantly reduced mount and intromission latencies, post-ejaculatory interval and mean intromission interval. The aqueous extract of P. arboreus is a sex enhancer and could constitute a potential alternative to treatment of male sexual dysfunction.

Keywords: *Pseudopanax arboreus; aphrodisiac; sexual behavior; male sexual dysfunction*

Introduction

In human life, sexual relationships remain the most important of all human, social and biological relationships; and since time immemorial, men and women have employed all means to develop, preserve or regain their own sexual capacities and to stimulate their partner's desire. However, some men still face the problem of repeatedly being unable to perform a sexual function effectively or have a disorder that interferes with their full sexual response cycle (A. Gbankoto *et al*., 2015).This pathology termed male sexual dysfunction (MSD) could come from various origins including personal life styles (chronic alcohol abuse, cigarette smoking), androgenic deficiency, ageing, psychological disorders, side effects of some psychiatric medications, antidepressants and chronic medical conditions like diabetes and pulmonary cancer (M.T. Yacubu *et al*., 2007). MSD could take different forms, such as disorders of desire (libido); erectile dysfunction (ED); disorders of ejaculation marked by delayed ejaculation and recurrent ejaculation with minimum sexual stimulation that occur before, during, or shortly after the penetration; and disorders of orgasm (M.T. Yacubu and M.A. Akanji, 2011). Several pharmacologic substances are available especially in developed countries to correct these disorders. In the developing countries on the contrary, particularly in Africa South of the Sahara, the high cost of these treatments makes them inaccessible and unaffordable for the majority of the populations (J. Bekker, 1996). In addition, these substances are non-tolerable to most sufferers and the secondary effects they induce are not easily endurable; which motivate these populations to prefer the traditional medicine using local plants or plant parts (P. Archana *et al*., 2005).

This has caused plant-derived chemicals that have sex-enhancing potentials for animals to gain more attention and notoriety and to become worldwide known as a treatment (A. Adimoedja, 2000). These phytochemicals increase libido (sexual desire and arousal), sexual potentiality (effectiveness of erection) and sexual pleasure or orgasm (P. Sandroni, 2001). Nowadays, medicinal plants are commonly used in the treatment of various sex related affections, amongst others. With the growing practice of the self-medication with natural products as aphrodisiacs, there is likely an increased risk of diseases induced by their usage (F.B. Beach, 1976). Some of these effects on man could be ache in the penis,

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burning of urethra, infections, pains and bleeding (M.T. Yacubu and M.A. Akanji, 2007). It is therefore of the utmost best to undertake investigations on the use of the natural aphrodisiacs. Apart from the folk claim of *P. arboreus* as an aphrodisiac, no other therapeutic properties of the plant have been reported. In a similar study (unpublished data), we evaluated the effects of its leaves methanolic extract on the sexual behavior of normal rats. The present study was therefore designed in an attempt to validate the folk usage of the plant as aphrodisiac by evaluating the effects of its leaves-aqueous extract on the sexual behavior of normal male rats.

Materials and methods

Plant material

Fresh leaves of *P. arboreus* were harvested from the tropical rainforest of Mamfe, South-West Region of Cameroon, under the guide of a local tradi-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 Yaounde for authentication, where a voucher of the specimen is found. Meanwhile, the fresh leaves were chopped into smaller pieces, air-dried under shade for about two months and ground using an electric grinder. 300g of the powder were introduced into 3000ml of distilled water and kept for 72 hours, while occasionally agitating mechanically. This was followed by filtration using the Laboratory test sieve (ENDOCOTTS LTD, ENGLAND) of 38µm aperture and the subsequent evaporation of the filtrate in an oven at 45°-50°C to obtain 63.27g of a black dry residue, giving a 21.09% yield of extraction.

Part of the extract was submitted to the Laboratory for Plant and Organic Chemistry of the Department of Chemistry, University of Buea for phytochemical screening. Meanwhile, administrative doses were determined following the tradipratitioners directives and screening tests.

Chemicals

Products used in this study included sildenafil citrate (Viagra) (Pfizer Inc, USA), estradiol (Sigma Chemicals, USA) 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, transported and stored under the recommended conditions until used.

Animals

Raising of Animals

Animals used were rats of the Wistar Strain of either sex raised in the Animal house of the Zoology and Animal Physiology Department of the Faculty of Science, University of Buea (Cameroon) under standard conditions of temperature, humidity and light(12H cycle). They were given free access to water and laboratory diet. International guidelines for the use of laboratory animals in biochemical research as recommended by the University of Buea council of Animal health (UBAC, 1985) were respected.

Ovariectomy and induction of estrus in females

A total of 30 females obtained from the breed of the Animal House of the Department of Animal Biology, Faculty of Science of the University of Buea, were starved for 24 hours and prepared for surgical operation. Briefly, they were weighed and given an intra-peritoneal injection of 0.02ml/100g body weight diazepam followed by 0.01ml/100g body weight ketamine, as anaesthesia. The two injections were separated by 5 minutes interval and after the onset of anaesthesia, the right and left lumbar dorsa of each female was shaved and the exposed skin prepared for aseptic surgery (97% alcohol wipe); next, a 3/4 cm dorsal flank incision penetrating the abdominal cavity was made for each ovary; the par-ovarian fatty tissue was identified and retracted; 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, after which an intramuscular injection of 0.2ml of penicillin-G was given to prevent any post-surgical infection (A.E. Cariton, 1986).

To bring them to estrus, each ovariectomised female was given 100µg of estradiol benzoate solution subcutaneously. This was followed 48 hours later by another subcutaneous injection of 600µg of progesterone solution. The progesterone was administered 6 hours before pairing each female with a male (W.D. Ratnasooriya and M.G. Dharmasiri, 2000). They were then screened for receptivity by pairing each with a sexually experienced non-experimental male and only those exhibiting good sexual receptivity (solicitation behaviour and lordosis in response to mounting) and no rejection behaviour were employed in the experiment.

Experimental design

Animal grouping and extract administration

A total of 28 adult Wistar male rats obtained from our bred and each weighing 180-200g were randomly divided into 4 groups of 7 rats each. Rats in group1 (negative control) were administered 10ml/kg body weight distilled water, group 2 rats received 6mg/kg body weight Viagra (sildenafil citrate), while those in groups 3 and 4 were given 75 and 150mg/kg body weight respectively of the aqueous extract of the leaves of *P. arboreus* (L.F. Phillipson). Each male was housed in a separate cage.

Mating behavior test

This was conducted as described by M.T. Yacubu and M.A. Akanji (2011), J.F. Cao *et al*. (2012), G. Fouche *et al*.

(2015) and like in our previous studies (unpublished data). 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 griped the female followed by the movement of its pelvic region towards the vagina of the female 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 nor intromission); and Ejaculation (when the male griped the female with the latter raising its snout in an upward direction. In rats, it often comes after a series of successive mounts and intromissions). From these parameters, the following indices were determined 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 (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); Mean Intromission Interval (MII) or Inter-Copulatory Efficiency (ICE) calculated as ejaculation latency divided by intromission frequency.

Sex organ relative weight assessment

On day 22, the animals were starved and then sacrificed under ethyl ether as anaesthesia. The following organs were isolated: testes, epididymis, vas deferens, prostate, seminal vesicle and the penis. Each was rinsed thoroughly and wiped with clean absorbent paper and carefully freed from all connective tissue and then weighed. Their individual weights were then expressed as a percentage of the total body weight.

Hormonal profile assessment

This was done as described by N.N. Njoku-Orji *et al*. (2015). Alongside the selected organs, blood was collected using a 5ml syringe through cardiac puncture and immediately introduced into heparinized test-tubes. It was kept for 24 hours after which the supernatant was collected and put into test tubes. It was then centrifuged for 15 minutes at 2500rpm in the Eppendorf Centrifuge. At the end of this, the supernatant was again collected. The plasma FSH, LH and testosterone concentrations of the animals were determined using the procedure outlined in the manufacturer's instruction manual. This was based on the principle of competitive binding between the hormone in the plasma and hormone-HRP conjugate for a constant amount of rat anti-hormone (N.W. Tietz, 1995). In each case, a blank solution was prepared to help calibrate or standardise the ELIZA reader.

FSH assay procedure consisted in dispensing 25 μL of FSH reference standards (0, 5, 10, 20, 50 and 100mIU/mL), plasma and FSH controls 1 and 2 into IgG-coated microtitre wells after which 100μL of FSH-enzyme conjugate reagent (blue color) was added. The resulting solution was thoroughly mixed by placing on an electric shaker for 10 seconds and incubated at room temperature for 30 minutes. This was to allow a fixed amount of HRP-labelled FSH to compete with the endogenous FSH in the standard and samples (plasma) for a fixed number of binding sites of the specific FSH antibody (since the amount of FSH peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of FSH in the specimen increases). The microwells were rinsed and flicked 5 times with aqua desk solution (400 μL/well) (to remove the unbound FSH peroxidase conjugate) before dispensing 100 μL of substrate solution into each well. The resulting solution was incubated at room temperature for another 10 minutes for blue color to develop. The color development was stopped with the addition of 50 μL of Stop Solution to each well and when gently mixed, the color changed from blue to yellow. The absorbance was read within 10 minutes at 450 nm with a microtitre well (ELIZA) reader. The intensity of the color formed was proportional to the amount of enzyme present and was inversely proportional to the amount of unlabeled FSH in the samples. The FSH concentration in the plasma of the animals was calculated from a calibration curve (obtained by plotting the concentration of the standard against the absorbance) using the following expression:

FSH concentration (ng/mL)= Cs×F,

(where C_s is Corresponding FSH concentration from the calibration curve and *F* is Dilution factor)

The procedure for the assay of LH was similar to that of FSH. 50μL each of the reference standards (0, 5, 15, 50, 100, and 200mIU/mL) and the plasma were dispensed into coated microtitre wells after which 00μL of LH-enzyme conjugate reagent (blue colour) was added. The resulting solution was thoroughly mixed for 30 seconds by placing on an electric shaker and incubated at room temperature for 45 minutes. This was to allow a fixed amount of labeled LH to compete with the endogenous

LH in the standard and samples (plasma) for a fixed number of binding sites of the specific LH antibody (since the amount of LH peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of LH in the specimen increases). The microwells were emptied and rinsed 3 times with distilled water (300 μL/well) (to remove the unbound LH peroxidase conjugate). They were again emptied by striking on absorbent paper and rinsed 5times with distilled water. This was followed by dispensing 100 μL of substrate solution into each well. The resulting solution was mixed gently for 5seconds and incubated in the dark at room temperature for another 20 minutes for blue colour to develop. The colour development was stopped with the addition of 100 μL of Stop Solution to each well and when gently mixed for 30 seconds, the colour changed from blue to yellow. The absorbance was read within 10 minutes at 450 nm with a microtitre well (ELIZA) reader. The intensity of the colour formed was proportional to the amount of enzyme present and was inversely proportional to the amount of unlabeled LH in the sample. The plasma concentration of LH of the animals was calculated from a calibration curve (obtained by plotting the concentration of the standard against the absorbance) using the following expression:

LH concentration (mIU/mL)= Cs×F,

(where C_s is Corresponding testosterone concentration from the calibration curve and *F* is Dilution factor)

Testosterone assay procedure entailed dispensing 25 μL of testosterone reference standards (0, 0.2, 0.5, 1.0, 2.0, 6.0 and 16.0 ng/mL), plasma and testosterone controls 1 and 2 into IgG-coated microtitre wells after which 200μL of testosterone-enzyme conjugate reagent (blue colour) was added. The resulting solution was thoroughly mixed for 10 seconds and incubated at room temperature for 60 minutes. This was to allow a fixed amount of HRP-labelled testosterone to compete with the endogenous testosterone in the standard and samples (plasma) for a fixed number of binding sites of the specific testosterone antibody (since the amount of testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of testosterone in the specimen increases). The microwells were rinsed and flicked 3 times with diluted wash solution (400 μL/well) (to remove the unbound testosterone peroxidase conjugate) before dispensing 200 μL of substrate solution into each well. The resulting solution was incubated at room temperature for another 15 minutes for blue colour to develop. The colour development was stopped with the addition of 100 μL of Stop Solution (1N HCl) to each well and when gently mixed, the colour changed from blue to yellow. The absorbance was read within 10 minutes at 450 nm with a microtitre well (ELIZA) reader. The intensity of the colour formed was proportional to the amount of enzyme present and was inversely proportional to the amount of unlabeled testosterone in the sample. The testosterone concentration in the plasma of the animals was calculated from a calibration curve (obtained by plotting the concentration of the standard against the absorbance) using the following expression:

Testosterone concentration (ng/mL)= Cs×F

(where C_s is Corresponding testosterone concentration from the calibration curve and *F* is Dilution factor)

Statistical Analyses

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

Results

1. Eeffects of the aqueous extract of leaves of *Pseudopanax arboreus* (Araliaceae) (L.F. Phillipson) on the mating parameters of normal male wistar rats.

1.1. Effects on Mount (ML) and Intromission (IL) Latencies (s)

The effects of the aqueous extract of leaves of *P. arboreus* on normal male rats are summarized in Table1 and illustrated in Figures 1a&1b. Subjection of normal male rats to a 21 day treatment period with the aqueous extract of leaves of *P. arboreus* at 75 and 150mg/kg (AE1 and AE2 respectively) induced a significant (p<0.05) reduction in mount (ML) and intromission latencies (ML) in a dose-dependent manner, compared to the distilled water group, from day 1 to day 21. The lowest values were recorded in rats treated with 6mg/kg of sildenafil citrate.

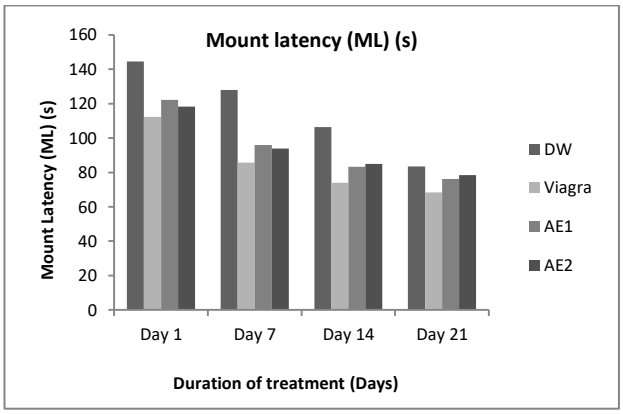

Figure 1a: Effects of the aqueous extract (AE) of *P. arboreus* on the Mount Latency (ML) (s) of normal male rats; DW=Distilled Water; AE1=Acqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg; s: seconds

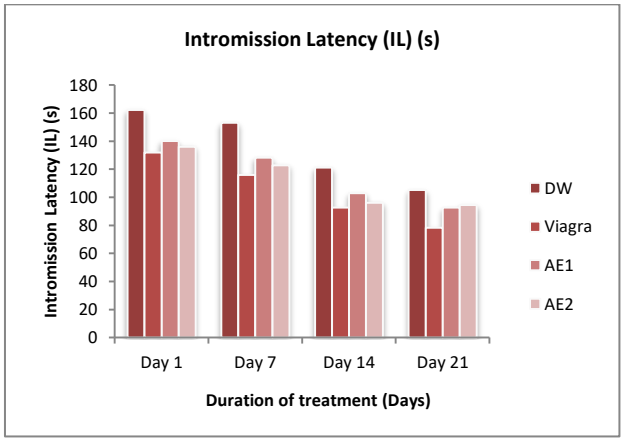

Figure 1b: Effects of the aqueous extract (AE) of *P. arboreus* on the Intromission Latency (IL) (s) of normal male rats; DW=Distilled Water; AE1= Aqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg; s: seconds

Table 1: Effects of the aqueous extract (AE) of *P. arboreus* on the Mount (ML) and Intromission Latencies (IL) (s) of normal male rats

Parameter	Day	Treatment			
		DW	Viagra	AE 1	AE2
ML (s)	1	144.44±22.17^{ae}	112.33±9.52^{be}	122.10± 14.45^{ce}	118.21±16.90^{ce}
	7	128± 31.80^{af}	85.61±12.81^{bf}	96±15.65^{bf}	93.82± 6.98^{bf}
	14	106.26±17.74^{ag}	74 ±13.85^{bf}	83.18 ±14.30^{bf}	85.0±8.17^{bf}
	21	83.4 ±13.14^{ah}	68.4 ±12.01^{afg}	76.2 ±8.87^{ag}	78.41± 10.8^{ag}
IL (s)	1	162.20± 24.64^{ae}	131.82±8.26^{be}	140.22 ±11^{be}	136.0± 17.63^{be}
	7	153.21 ±22.48^{ae}	116± 13.47^{bf}	128.31±19.84^{cde}	122.70±21.37^{bde}
	14	121.20 ±18.07^{af}	92.60± 15^{bg}	103±16.75^{bf}	96.17± 12.01^{bf}
	21	105.2 ±23.44^{ag}	78.4 ±32.82^{bh}	92.6± 19.83^{bcf}	94.4± 19.93^{bcf}

Values presented as Mean±SEM; DW: distilled water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/kg; s: seconds; On the same row, values with same letter (a-d) are not significantly different; on the same row, values with different letters are significantly different; on the same column, values with the same letters (e-h) are not significantly different; on the same column, values with different letters are significantly different

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

Values obtained in these parameters are presented in Table 1 and illustrated on Figures 2a&2b.

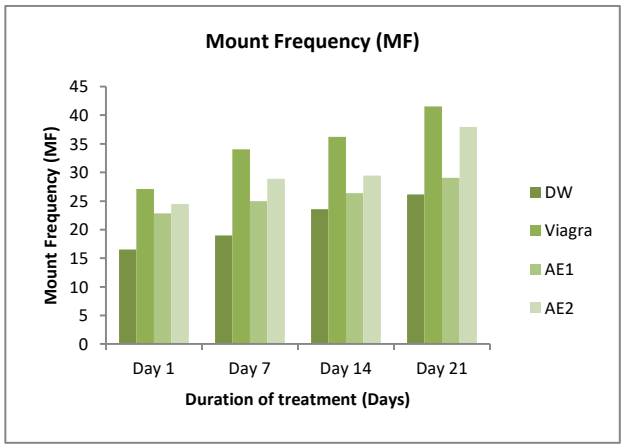

Figure 2a: Effects of the aqueous extract (AE) of *P. arboreus* on Mount Frequency (MF) of normal male rats; DW=Distilled Water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/kg;

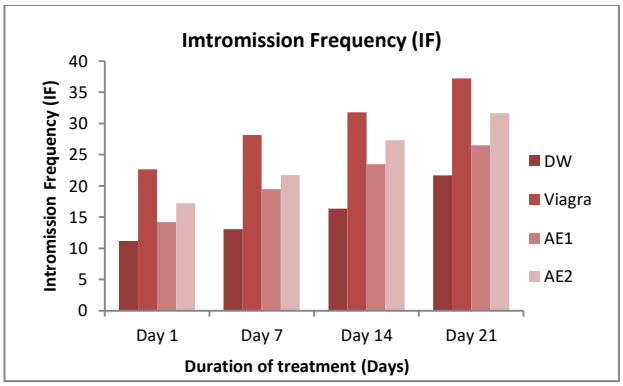

Figure 2b: Effects of the aqueous extract (AE) of *P. arboreus* on Intromission Frequency (IF) of normal male rats; DW=Distilled Water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/kg;

Treatment of normal male rats with both doses of the extract caused an increase in mount (MF) and intromission (IF) frequencies dose-dependently from day 1 to day 21 with significant ($p<0.05$) values recorded throughout the treatment period, compared to the negative control group. Like in the latencies, the reference product induced a more significant effect.

Table 2: Effects of the aqueous extract (AE) of *P. arboreus* on Mount (MF) and Intromission Frequencies (IF) of normal male rats

Parameter	Day	Treatment			
		DW	Viagra	AE1	AE2
MF	1	16.55± 6.32^{ae}	27.1± 4.12^{be}	22.85± 2.8^{be}	24.45± 6^{be}
	7	19.0 ±8.1^{ae}	34.05± 2.6^{bce}	24.95± 5.1^{ade}	28.9± 3.48^{bde}
	14	23.55± 3,51^{ae}	36.25± 3.1^{bce}	26.35±6.66^{ade}	29.46±9.87^{ade}
	21	26.15± 5.02^{af}	41.52±2.98^{bcf}	29.05±5.24^{ade}	37.95±6.36^{bcf}
IF	1	11.15±2.77^{ae}	22.65± 3.72^{be}	14.17± 2.69^{ace}	17.2± 3.1^{ace}
	7	13.05± 4.02^{ae}	28.15 ±2.80^{bce}	19.45 ± 3.80^{bde}	21.75±2.21^{bde}
	14	16.32± 1.63^{ae}	31.8± 3.15^{bce}	23.5± 5.97^{ade}	27.35±6.93^{bde}
	21	21.7± 3.57^{af}	37.25± 3.19^{bcf}	26.5± 4.67^{bdf}	31.65 ±4.39^{bdf}

Values presented as Mean±SEM; DW: distilled water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/kg; s: seconds; On the same row, values with same letter (a-d) are not significantly different; on the same row, values with different letters are significantly different; on the same column, values with the same letters (e-g) are not significantly different; on the same column, values with different letters are significantly different

1.3. Effects on Ejaculation latency (EL) (s) and Post Ejaculatory Interval (PEI) (s)

The extract produced contrasting effects on EL and PEI (Table 3 and Figures 3a&3b).

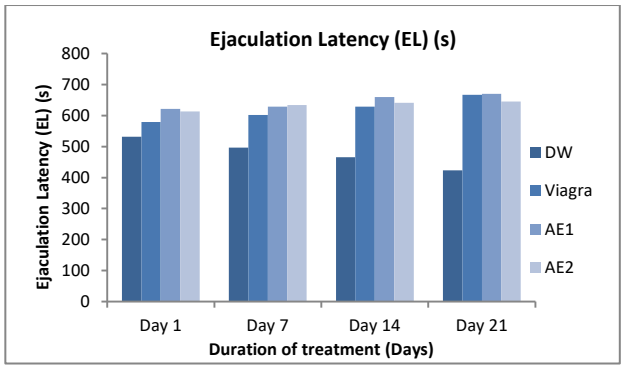

Figure 3a: Effects of the aqueous extract (AE) of *P. arboreus* on Ejaculation Latency (EL) (s) of normal male rats; DW=Distilled Water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/kg

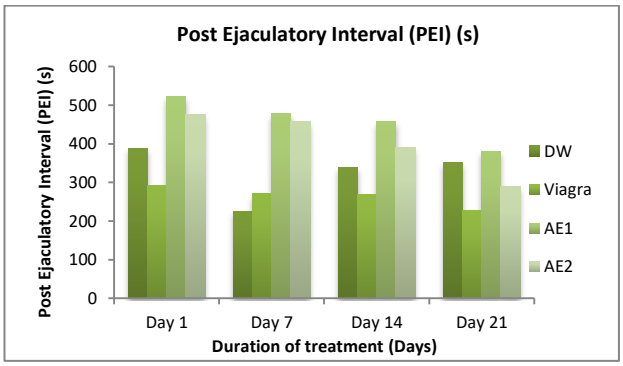

Figure 3b: Effects of the aqueous extract of *P. arboreus* on Post Ejaculatory Interval (PEI) (s) of normal male rats; DW=Distilled Water; AE1= Aqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg; s: seconds

Like in viagra-treated rats, rats treated with both doses of the extract showed a significant ($p<0.05$) increase in ejaculation latency (EL) contrarily to the distilled water-treated rats where a decrease in EL was noticed; meanwhile, they induced a decrease in PEI throughout the treatment period.

Table 3: Effects of the aqueous extract (AE) of *P. arboreus* on Ejaculation Latency (EL) (s) and Post-Ejaculatory Interval (PEI) (s) of male rats

Parameter	Day	Treatment			
		DW	Viagra	AE1	AE2
EL (s)	1	531.25±100.92^{ae}	578.45± 168.78^{bce}	621.35 ±194.76^{bde}	612.60±192.83^{bde}
	7	496.65 ±135.13^{af}	601.13± 122.68^{be}	628.22± 220.82^{de}	633.45± 234.73^{ce}
	14	465.82±200.92^{ag}	628.23± 178.78^{bf}	659.31 ±194.76^{bf}	641.20± 192.83^{af}
	21	422.71±105.13^{ah}	666.41± 144.68^{bg}	669.44± 220.82^{bf}	645.± 164.73^{af}
PEI (s)	1	388.8 ±82.86^{be}	290.95± 155.82^{ae}	522.6± 236.62^{de}	476.52 ± 77^{ce}
	7	225.85±77.05^{af}	271.95± 164.1^{be}	478.15±135.3^{df}	457 63.21^{ce}
	14	339.65± 102.67^{bf}	269.40± 88.3^{ae}	456.4± 204.99^{cf}	389.9±165.44^{bf}
	21	352.75±128.09^{bfg}	227.1± 97.86^{af}	381.21± 143.44^{bg}	288.5± 191.6^{ag}

Values presented as Mean±SEM; DW: distilled water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/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-g) are not significantly different; on the same column, values with different letters are significantly different

1.4. Effects on Penile Licking and Mean Intromission Interval (MII) or Inter Copulatory Efficiency (ICE) (s)

Though non-significant ($p<0.05$), an increase in PLs was recorded in rats treated with either dose of the extract, compared to those that received distilled water. Table 4 and Figures 4a&4b also indicate that rats treated with either dose of the extract (AE1 or AE2) or the reference product recorded significant effects on MII or ICE, compared to those that received distilled water. It was noticed that MII or ICE values dropped from 45.88±9.89 on day 1 to 24.33± 7.09 on day 21 and from 40.73± 11.02 on day 1 to 27.24± 8.47 on day 21 for the 75 (AE1) and 150mg/kg (AE2) doses, respectively.

Table 4: Effects of the aqueous extract (AE) of *P. arboreus* on Penile licking (PL) and Mean Intromission interval (MII) or Inter-Copulatory Efficiency (ICE) (s) of male rats

Parameter	Day	Treatment			
		DW	Viagra	AE1	AE2
PL	1	2.88± 1.2^{a}	6.55± 1.33^{bc}	4.20±1.12^{b}	5.11± 1.05^{b}
	7	3.74±0.76$_{a}$	7.95± 1.2^{bc}	5.48± 0.87^{b}	6.0 ± 0.63^{b}
	14	4.32± 1.23^{a}	8.75± 0.77^{bc}	6.11± 1.02^{b}	6.22±1.24^{b}
	21	4.44± 1.04^{a}	9.36±2.15^{b}	6.24± 1.98^{a}	6.75± 1.92^{a}
MII (s)	1	55± 15.73^{a}	33.05±6.62^{bc}	45.88±9.89^{b}	40.73±11.02^{b}
	7	59.28±18.33^{a}	29.26±11.08^{bc}	37.06± 11.16^{b}	38.27±8.37^{b}
	14	61.17± 24.71^{a}	24.33± 7.09^{b}	29.35± 15.75^{b}	28.29±16.99^{b}
	21	64.53± 19.3^{a}	23.96± 8.43^{b}	24.33± 7.09^{b}	27.24± 8.47^{b}

Values presented as Mean±SEM; DW: distilled water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/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

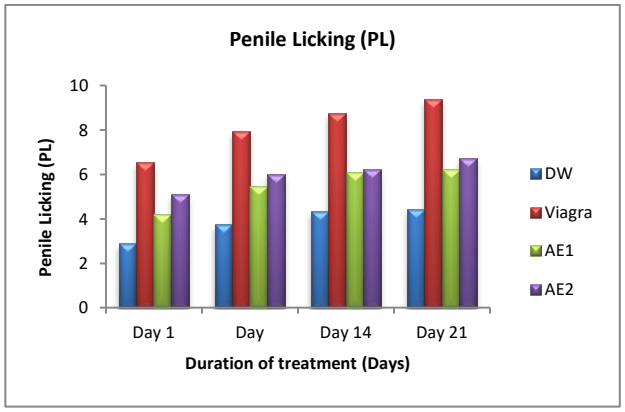

Figure 4a: Effects of the aqueous extract of *P. arboreus* on Penile Licking (PL) of normal male rats; DW=Distilled Water; AE1=Aqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg

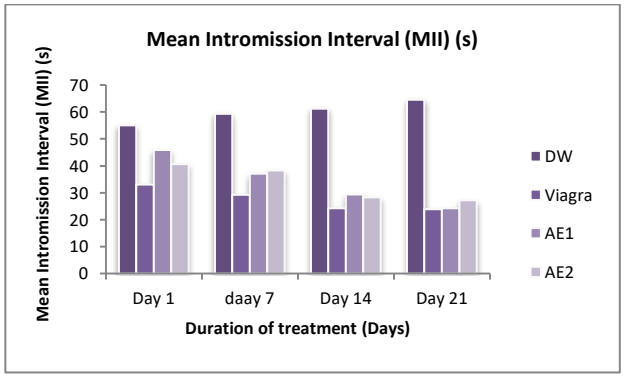

Figure 4b: Effects of the aqueous extract of *P. arboreus* on Mean Intromission Interval (MII) or Inter Copulatory Efficiency (ICE) (s) of normal male rats; DW=Distilled Water; AE1= Aqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg; s: seconds

1.2. Effects of the aqueous extract of *Pseudopanax arboreus* (Araliacea) (l.f. Phillipson) on the relative weight (%) of reproductive organs of normal male rats.

As seen in Table 5 and Figure 5, treatment of normal male rats with the aqueous extract of *P. arboreus* at 75 and 150mg/kg body weight doses (AE1 and AE2 respectively) for a period of 21 days induced an increase in relative organ weight (%), compared to the distilled water rats, with a significant ($p<0.05$) effect recorded in the testes in rats treated with the 150mg/kg (AE2) dose

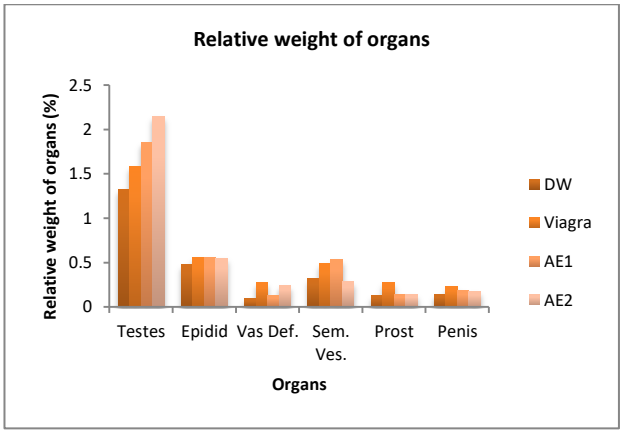

Figure 5: Effects of the aqueous extract of *P.arboreus* on relative weight of sex organs of normal male rats DW=Distilled Water; AE1= Aqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg;

Table 5: Effects of the aqueous extract of *P. arboreus* on the relative weight of reproductive organs of normal male rats

Organ	Treatment			
	DW	Viagra	AE1	AE2
Testes	1.32±0.11^{a}	1.58± 0.14^{a}	1.85 ±0.08^{a}	2.14 ±0.06^{b}
Epididymis	0.48 ±0.02^{a}	0.56± 0.06^{a}	0.56 ±0.06^{a}	0.55± 0.03^{a}
Vas deferens	0.09± 0.02^{a}	0.27± 0.044^{b}	0.13± 0.01^{a}	0.24± 0.03^{a}
Sem. Vesicles	0.32± 0.10^{a}	0.49± 0.10^{a}	0.53± 0.09^{a}	0.28± 0.06^{a}
Prostate	0.13± 0.0^{a}	0.27± 0.05^{a}	0.14± 0.03^{a}	0.14± 0.02^{a}
Penis	0.14± 0.02^{a}	0.23± 0.04^{a}	0.18± 0.01^{a}	0.17± 0.02^{a}

Values presented as Mean±SEM; DW: distilled water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/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

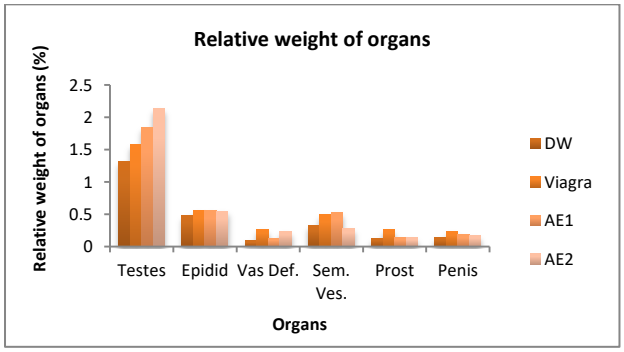

Figure 5: Effects of the aqueous extract of *P.arboreus* on relative weight of sex organs of normal male rats DW=Distilled Water; AE1= Aqueous extract 75mg/kg; AE2=Aqueous extract 150mg/kg

1.3. Effects of the aqueous extract of P. arboreus on hormonal profile of normal male rats

Table 6: Effects of the aqueous extract of leaves of *P. arboreus* on the plasma concentrations of FSH, LH and Testosterone

Hormone	Treatment			
	DW	Viagra	AE1	AE2
FSH (mIU/ml)	1.44±0.72[a]	3.55±0.15[a]	2.57±0.64[a]	2.11±0.13[a]
LH(mIU/ml)	2.72±0.21[a]	2.94±0.32[a]	2.11±0.66[a]	1.56±0.28[a]
Testosterone (ng/ml)	1.71±0.66[a]	4.68±0.93[b]	3.04±1.02[b]	3.25±0.24[b]

Values presented as M±SEM; DW: distilled water; AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/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

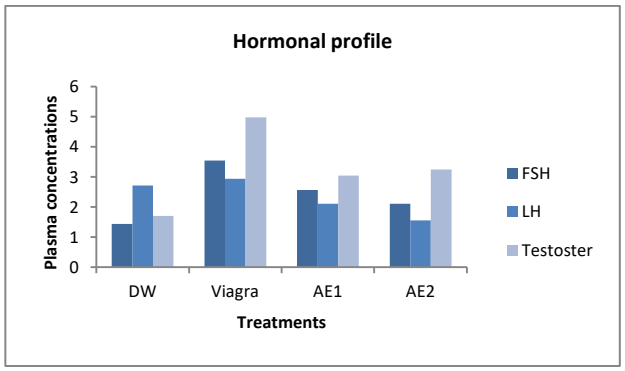

Figure 6: Effects of the aqueous extract of *P. arboreus* on the plasma concentrations of FSH, LH and Testosterone of normal male rats. AE1: aqueous extract 75mg/kg; AE2: aqueous extract 150mg/kg; FSH: follicle stimulating hormone; LH: luteinizing hormone; testoster: testosterone

Continuous administration of the plant-leaves extract at both doses (AE1 and AE2) for a 21-day period, resulted in a significant ($p<0.05$) increase in plasma concentrations of the male reproductive hormone, testosterone, though greater values were obtained with viagra than the extract, compared to the distilled water-group. Meanwhile, effects of both doses of plant-leaves extract on FSH and LH plasma levels were statistically non-significant compared to the negative control group (Table 6 and Figure 6).

Discussion

Pseudopanax arboreus belongs to the Family Araliaceae, which is closely related to the Family Apiaceae and Family Pittosporaceae (G.M. Plunkett *et al*., 1997). Members of these Families have been demonstrated to possess aphrodisiac potentials. Leaves of *Pseudopanax arboreus* are renowned in their action on male sexual activity amongst the people of Manyu Division (Cameroon). In efforts to provide insight to this claim, we conducted a similar study (unpublished data) with the methanolic extract of the plant leaves. Overall, it was noticed that the methanolic extract of the leaves of *P. arboreus* (L.F. Phillipson) could enhance sexual behavior and performance in male rats; findings that supported the folk use of this plant by the people of Manyu Division, Cameroon.

In the present investigation, we were interested in evaluating the actions of the leaves-aqueous extract on the sexual behavior of normal male rats. Results of this study revealed the prosexual stimulatory potentials of *P. arboreus* in normal male rats. The copulatory behavior of normal male rats when paired with an estrous female consists of repeated series of mounts and intromission, accompanied by penile licking and culminating with ejaculation, followed by a refractory period called post ejaculatory interval (A.K. Slob and T.B.J.J.Van der Werff, 1997; P. Watcho *et al*., 2007). The results of our phytochemical screening agree with those found in aqueous extract of *Fadogia agrestis* (M.T. Yakubu *et al*., 2008); *Massularia acuminate* stem (M.T. Yacubu and M.A. Akanji, 2011); *Caesalpinia bonduc* (A. Gbankoto *et al*., 2015); *Ficus capensis* (N.N. Njoku-Oji *et al*., 2015); *Monsonia angustifolia* (G. Fouche *et al*., 2015), etc, which all entailed an accelerator effect on the sexual behavior of the male Wistar rats. Our plant extract is rich in sterols, polyterpens, flavonoids, saponin, tannins, reducing sugars, coumarins and alkaloids. The observations in this experiment are similar to those noted with the methanolic extract (unpublished data). Upon administration of the aqueous extract at 75 and 150mg/kg body weight doses, the animals showed a significant reduction in the hesitation time of males towards receptive females as indicated by a significant ($p<0.05$) decrease in mount (ML) and intromission latencies (IL), compared to the distilled water group, though the difference between both doses was not statistically significant. The Polyterpens revealed in our extract could be responsible for the pro-erectile actions of *P. arboreus*. These bioactive principles probably act by inducing changes in levels of neurotransmitters,

modulating the actions of these neurotransmitters on their target cells or by increasing androgen levels (P.K. Suresh *et al*., 2000). Similarly in a dose-dependent manner, both doses induced a significant increase in mount (MF) and intromission frequencies (IF).These increases are of physiological interest since these sexual performance parameters constitute important criteria for the determination of libido or sexual desire (G.Y.F. Mbongue *et al*., 2003). 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 (A. Agmo, 1997).

PEI is an important parameter for evaluating the effect of administered extract on erectile function. PEI is regarded as an indicator of potency, libido and potential to recover from exhaustion after the first ejaculatory series. Both doses of the plant extract prolonged ejaculation and showed a decreased PEI, effects that were similar to those of Viagra. Decreased PEI could be as a result of enhanced potency and libido, and/or reduced exhaustion in the first ejaculatory series; while prolonged ejaculation indicates prolonged coitus. This data clearly supports the role of leaves of *P. arboreus* in enhancing male sexual function and thus confirms is folk use by males to ensure endurance during sexual activity (T.J. Bivalacqua *et al*., 2007).

Both doses of the plant-leaves aqueous extract induced an increase in PL and a decrease in MII (ICE) throughout the treatment period compared to the distilled water-treated group, actions that were similar to the Viagra-treated group. PL and MII (ICE) are important indices for evaluating the effect of drug administration on erectile function (M. Thakur and V.K. Dixit , 2007). This further shows that the aqueous extract of *P. arboreus* leaves increases potency. The prolonged EL and increased penile erection noticed with both doses suggest the involvement of NO in the intervention (J. Du and E.M. Hull,1999).

An increase in plasma testosterone levels was noticed in rats treated with both extracts compared to the distilled water-treated rats. Testosterone 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 phytoconstituents of the plant-leaves extract could mimic the role of LH to stimulate the Leydig cells. In the regulation of copulatory behavior, this main male androgen has been associated with an increase in sexual behavior (T.M. Mills *et al*., 1996; L.L. Murphy *et al*., 1998). Also, 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-dependant (K. Gauthaman *et al*., 2002). Furthermore, according to E.M. Hull *et al*. (1999) and S.K. 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.

In this study, we determined the weights of testis, epididymis, vas deferens, seminal vesicles, prostate glands and the penis in both controls and extract-treated rats. Like Viagra treatment, the extract treatment could increase the weights of the accessory sexual organs in rats, as compared with the control group rats, thus exhibiting androgenic effect. 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 (D. Morton, 1988; W.H. Walker, 2010), low serum levels may have adverse effect on fertility. 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 (N.S. 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 (Q. Luo *et al*. 2006). These effects on relative weight of reproductive and accessory organs are a reflection of the plasma concentrations of testosterone recorded.

Conclusion

Our findings show that the aqueous extract of the leaves of *P. arboreus* (L.F. Phillipson) enhances sexual behavior in male rats and also possesses anabolic functions. The aphrodisiac properties of plant extract may be due to the actions of bioactive compounds such as alkaloids, flavonoids, steroids, saponins, tannins and triterpenoids working probably through androgenic and other pathways. Further findings are required to determine the exact mechanism of action of the extract.

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