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Aphrodisiac properties of aqueous roots extract of *Pycnocomma macrophylla* Benth (Euphorbiaceae) in rat

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ABSTRACT

Erectile dysfunction (ED) is a real health problem for men worldwide. This work investigated the effects of aqueous roots extract of *Pycnocomma macrophylla* (Euphorbiaceae) on sexual behavior in rats. Twenty-five adults' rats divided into five groups were treated orally for fourteen days with distilled water (5 ml/kg, control), sildenafil citrate (5 mg/kg) and the aqueous extract of *P. macrophylla* (100, 200 and 400 mg/kg b.w) respectively. Sexual behavior parameters such as mount latency (ML), intromission latency (IL), ejaculation latency (EL), post-ejaculatory intervals (PEI), mount frequency (MF), intromission frequency (IF), ejaculation frequency (EF) and mean interval of copulation (MIC) were evaluated in treated animals mated with receptive female at days 1, 7 and 14 of treatment. At day 14, serum testosterone was measured. The results indicated that, the aqueous extract of *P. macrophylla* and especially the dose of 200 mg/kg significantly ($p < 0.01-0.001$) decreased mount and intromission latencies as well as the post-ejaculatory interval on day 14 compared to the control and initial values (day1) respectively while this extract significantly ($p < 0.01-0.001$) increased the frequencies of mounts intromissions and ejaculations, and the mean intervals of copulation. Moreover, serum testosterone significantly ($p < 0.05$) increased in rats treated with *P. macrophylla* (200 mg/kg b.w) when compared to the control. *P. macrophylla* possess pro-sexual and aphrodisiac effects in relation with the androgenic potential of particular bioactive components present in the aqueous root extract of plant. These outcomes could be applied for the management of sexual debilities in patients.

Keywords: *Pycnocomma macrophylla*, Sexual behavior, Testosterone, Erectile dysfunction.

INTRODUCTION

Erectile dysfunction (ED) also called male impotence is considered as the continuously inability to obtain and/or maintain a sufficient penile erection during sexual intercourse^[1]. Several works indicated that, the most prevalent male sexual problems are focused on problem concerning erection and ejaculation^[2]. Sexual debilities can be found among men of all cultural backgrounds, ages and ethnicities. In 1995, research has shown that, an estimation of about 152 million men over the world have gotten erectile disorder. This number will rich by 170 to 322 million by the year 2025^[3, 4]. The origins of ED can be divided into psychogenic including (stress, anxiety depression...) ^[5-7]. ED is responsible for psychosocial, well-being, personal relationships and quality of life impairments in many couples^[8]. It is therefore urgent to establish veritable strategies which can respond to different demands, in order to prevent and/or enhance the sexual dysfunction on male. In developed countries, several molecules, technics and protocols have been developed for the treatment of ED according to the nature and severity of the disease with type 5 phosphodiesterases (sildenafil, tadalafil and vardenafil) (PDE5Is) being the first-line therapy^[9]. Concerning the second and the third way of therapy, we have the injection in the cavernous corpus, exogenous testosterone implant, and medication in transurethral domain, prosthesis of penis, sex therapy and vacuum construction device^[9]. However, these different forms of treatment have many side effects and are sometime uncomfortable for the patients and are generally difficult to access for patients of low-income countries^[10-12]. Therefore, the use of medicinal plants as an alternative for the treatment of sexual disturbances has gained a great reputation in most of sub-Saharan african populations. Many plants are now scientifically recognized for their pro-sexual activities. These include *Montanoa tomentosa*^[13], *Tribulus alatus*^[14], *Dracaena arborea*^[15], *Massularia acuniminata*^[16], *Kaempferia parviflora*^[17], *Rauvolfia vomitoria*^[18], *Carpolobia alba*^[19] and Purple Corn^[20]. Among others. *P. macrophylla* Benth is a unisex shrub about 3m high with a hairless stem. It is present in garden^[21] and in the undergrowth of forests where all parts of the plant are purgative^[22]. Phytochemical studies revealed the presence of terpenoids and scopoletin^[23]. In southern Cameroon, the roots of *P. macrophylla* (family Euphorbiaceae)

locally called "Ekock Mbim" is used to treat sexual related debilities particularly erectile dysfunction. However, a scientific verification of this therapeutic potential of *P. macrophylla* has not yet been done. The present work was then undertaken to determine the prosexual and aphrodisiac potential of the aqueous roots extract of *P. macrophylla* in rat.

MATERIALS AND METHODS

Plant collection and preparation of the aqueous extract *P. macrophylla*

Fresh roots of *P. macrophylla* were collected in Cameroon, in the Ebondi village (Ocean division), in May and authenticated at the National Herbarium of Cameroon under the voucher number 15219/HNC. These roots were dried and cut into pieces, then kept at room temperature for several days. 1 kg of crushed roots were brought to a boil in 6 L of distilled water for 45 mn. The obtained solution was filtered using a Whatman filter paper No. 1 and then lyophilized to obtain a yield of 34.07 g (3.40 %). The working solution was prepared to obtain 138.8 mg/ml. The doses of 100, 200 and 400 mg/kg were used in our study. These doses were chosen based on the calculated therapeutic dose of the traditional healer which was 200 mg/kg.

Animals care

Fifty adults Wistar rats (25 females and 25 males) (90 days old, 250-270 g) obtained from the vivarium room of the Department of Animal Sciences, Faculty of science, University of Douala, Cameroon, were used in this study. They were housed in collective clean cages (five rats per cage, using wood shaving as bedding material) at room temperature, under a light-dark cycle of 12:12 hrs, with free access to water and food. Every two days, the bedding material was renewed after cleaning and drying the cages to maintain animals in good health. All the experiments were approved by the ethic committee of the University of Douala in accordance with the guidelines for laboratory animal use and care of the European Communities [24].

Experimental design

Twenty five rats were divided randomly into five groups of five animals each and treated as follows: Group 1, rats receiving distilled water (10 mL/kg, control); group 2, rats treated with sildenafil citrate (5 mg/kg, positive control); groups 3, 4 and 5, rats administered with the aqueous extract of *P. macrophylla* at doses of 100, 200 and 400 mg/kg respectively. After 12 hours of fasting, these rats were treated once a day (between 7 a.m. and 9 a.m.) for 14 consecutive days using endogastric cannula.

Female preparation

Ovariectomy of females

Ovariectomy was performed following the technique of Cariton [25], but Watcho *et al.* [15] did some modifications.

Estrus induction in females

Two weeks after the ovariectomy, females became receptive using subcutaneous injection of estradiol benzoate (30 μ g) and progesterone (600 μ g), 48 and 6 hrs respectively, using the technique described by Wankeu-Nya *et al.* [26]. Furthermore, the receptivity of these females were tested with non-experimental sturdy male rats and only those that were not rejected and exhibiting very well sexual receptivity behavior were used in the experiments.

Evaluation of sexual behavior parameters

This test was performed based on the methodology described by Wankeu-Nya *et al.* [26]. Immediately after the treatment, each rat was isolated in a copulatory cage for acclimation. One hour later, one receptive ovariectomized female was gently introduced into each cage and the copulatory parameters were evaluated for thirty minutes on days 1, 7 and 14 of treatment. This test was performed in a calm and quiet milieu and recorded parameters were: Mount latency (ML) - the time elapsed from the introduction of the female into the cage until the first mount; Intromission latency (IL) - the time elapsed from the introduction of the female into the cage until the first intromission; Ejaculation latency (EL) - the time from the first intromission until the first ejaculation; Post-ejaculatory interval (PEI) - time interval from the ejaculation until the first intromission in the next series; Mount frequency (MF) - the total number of mounts recorded during the period of observation; Intromission frequency (IF) - the total number of intromissions recorded during the 30 min of observation; Ejaculation frequency (EF) - the total number of ejaculations during the 30 min of observation; Mean interval of copulation (MIC) - time from the first mount until the last mount of the observation period.

Blood collection and serum testosterone evaluation

One day after the last treatment (6 am, local time) and after 10 hours of fasting, animals were sacrificed and the blood collected through the jugular vein was centrifuged for 20 min at 3000 rpm. The obtained serum was directly stored in the freeze at -20°C for subsequent testosterone determination by the ELISA method (Enzyme-Linked Immunosorbent Assay).

Statistical analysis

Data are expressed in mean \pm SEM. The duration's effect of treatment on each sexual behavior parameter was analyzed using the two ways ANOVA repeated measures followed by Bonferroni all pair comparism test when necessary. Within the same day of treatment, the two ways ANOVA followed by Bonferroni all pair comparism test was used. This was to compare the treated groups to control one. The evaluation of testosterone level was obtained using a one-way ANOVA followed by the Bonferroni all pair comparism test. A value of $P < 0.05$ was considered as statistically significant. The Graph-pad Prism version 5.1 was used to perform all these analysis.

RESULTS

Effects of *P. macrophylla* aqueous roots extract on sexual behavior parameters

Effects on mount, intromission and ejaculation latencies (ML, IL, EL) and post ejaculatory interval (PEI)

Although mount, intromission and ejaculatory latencies (ML, IL, EL) have significantly $p < 0.01-0.001$ increased in rats receiving distilled water throughout the treatment period when compared to their respective initial values (Day 1), the post ejaculatory interval (PEI) remained significantly ($p > 0.05$) unchanged. However, the oral administration of *P. macrophylla* aqueous extract have decreased the ML, IL and PEI (13.79%, 14.95% and 10.25% respectively), these decrease were more significant ($p < 0.01-0.001$) at dose of 200 mg/kg on day 14 of treatment when compared to rats receiving distilled water and positive control (Table 1).

Table 1: Effects of aqueous roots extract of *P. macrophylla* on ML, (IL), {EL} and [PEI] in rat

Groups	Observation Days		
	Day 1	Day 7	Day 14
DW (5 ml/kg) n=5	45.20 ± 0.53 (47.80 ± 0.78) {450.40 ± 0.54} [539.20±0.78]	48.60 ± 0.74 ^c (51.60 ± 0.61) ^b {500.80 ± 0.60} ^c [539.80±0.78]	51.4 ± 0.46 ^c (55.0 ± 0.50) ^c {489.8 ± 0.60} ^c [519.75±0.69] ^a
Sildenafil C. (5 mg/kg) n=5	37.20 ± 0.60 ^{***} (45.40 ± 0.46) {801.80 ± 0.53} ^{***} [240.80±0.60] ^{***}	39.20 ± 0.60 ^{***} (41.60 ± 0.68) ^{***b} {814.40 ± 0.61} ^{***c} [200.60±0.74] ^{***}	37.60 ± 0.54 ^{***} (40.20 ± 0.53) ^{***c} {809.60 ± 0.61} ^{***c} [310.00±0.76]
PMAE 100 mg/kg n=5	44.0 ± 0.64 ^{###} (49.6 ± 0.68) ^{##} {580.6 ± 0.61} ^{#####} [409.20±0.67] ^{*** ##}	47.8 ± 0.53 ^{###c} (50.4 ± 0.54) ^{###} {599.8 ± 0.60} ^{##### c} [402.40±0.61] ^{*** ##}	49.4 ± 0.68 ^{###c} (52.2 ± 0.78) ^{###} {610.8 ± 0.53} ^{#####a} [310±0.76] ^c
PMAE 200 mg/kg n=5	40.6 ± 0.61 ^{** #} (42.8 ± 0.60) ^{***} {700.2 ± 0.60} ^{#####} [400±0.76] ^{#####}	37.0 ± 0.64 ^{***b} (38.4 ± 0.46) ^{***c} {720.6 ± 0.74} ^{##### c} [360.40±0.74] ^{#####}	35.0 ± 0.64 ^{***c} (36.4 ± 0.46) ^{*** ##} {738.8 ± 0.60} ^{##### c} [359±0.64]
PMAE 400 mg/kg n=5	43.4 ± 0.68 ^{###} (49.4 ± 0.68) ^{##} {599.8 ± 0.78} ^{#####} [415.20±0.60] ^{#####}	47.8 ± 0.34 ^{###c} (51.2 ± 0.73) ^{###} {520.6 ± 0.61} ^{#####c} [409.40±0.46] ^{#####}	45.4 ± 0.46 ^{#####} (50.4 ± 0.74) ^{#####} {524.4 ± 0.54} ^{#####c} [419.60±0.46]

All values are Mean ± SEM. n=number of rats per group. DW= Distilled water. PMAE= *P. macrophylla* aqueous extract. ML= mount latency, (IL)= intromission latency, {EL}= ejaculation latency, [PEI]= post ejaculatory interval. During the period of treatment and in the same line, ^ap<0.05; ^bp<0.01; ^cp<0.001: significantly different compared to the initial day (day 1) (ANOVA Repeated Measures+ Bonferroni all pair comparism test). In the same period of treatment, *p<0.05 **p< 0.01; ***p<0.001: significantly different compared to control group (distilled water). #p<0.05; ##p<0.01; ###p<0.001: significantly different compared to the positive control (Sildenafil citrate) (Two ways ANOVA+Bonferroni all pair comparism test).

Effects on mount, intromission, and ejaculation frequencies (MF, IF, EF) and mean interval of copulation (MIC)

The mount, intromission and ejaculatory frequencies (MF, IF, EF) and mean interval of copulation (MIC) remained statistically (p>0.05) unchanged in control rats during the treatment period. However, all these parameters of *P. macrophylla* aqueous extract at all doses significantly (p<0.05) increased when compared to control and initial

value (day 1) respectively. These increases (34.91%, 27.64%, and 42.11% respectively) and (12.44%) were more significant (p<0.01-0.001) on day 14 in rats treated at the dose of 200 mg/kg of the plant extract. Except the MIC which was significantly higher in animals receiving PMAE (200 mg/kg), the values obtained in other parameters were quite similar to those recorded in rats treated with sildenafil citrate. (Table: 2).

Table 2: Effects of aqueous roots extract of *P. macrophylla* on MF, (IF), {EF} and [MIC] in rat

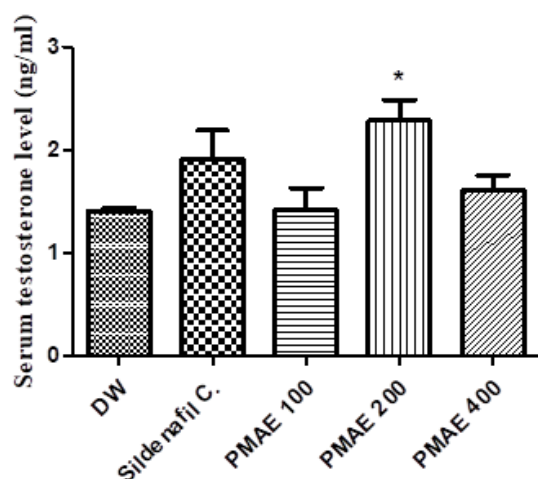
Groups	Observation days		
	Day 1	Day 7	Day 14
DW (5 ml/kg) n=5	23.40 ± 0.68 (19.60 ± 0.74) {1.20 ± 0.18} [1010.80±0.78]	24.20 ± 0.60 (19.2 ± 0.60) {1.60 ± 0.36} [1039.80±0.78] ^c	22.0 ± 0.76 (16.6 ± 0.61) {1.60 ± 0.36} [1104.80±0.73] ^c
Sildenafil C. (5 mg/kg) n=5	43.60 ± 0.54 ^{***} (39.6 ± 0.46) ^{***} {2.8 ± 0.34} [1550.0±0.64] ^{***}	45.20 ± 0.53 ^{***} (41.40 ± 0.74) ^{***} {3.0 ± 0.64} [1610.20±0.78] ^{***c}	45.6 ± 0.61 ^{***} (42.6 ± 0.68) ^{***} {3.5 ± 0.45} [*] [1614.80±0.60] ^{***c}
PMAE 100 mg/kg n=5	25.4 ± 0.46 ^{###} (20.8 ± 0.67) ^{###} {1.6 ± 0.36}	28.2 ± 0.60 ^{#####a} (21.6 ± 0.68) ^{###} {1.2 ± 0.18}	30.6 ± 0.54 ^{*** ##c} (25.0 ± 0.76) ^{#####b} {1.6 ± 0.3} [#]

	[1200.60±0.79]***###	[1100.20±0.73]*** ###c	[1114.60±0.74]*** ###c
PMAE 200 mg/kg n=5	30.2 ± 0.60***### (28.80± 0.78)***### {2.20 ± 0.34}	40.2 ± 0.53*** ###c (34.8 ± 0.60) ***### c {2.8 ± 0.34}	46.4 ± 0.68***c (39.8 ± 0.73) ***c {3.8 ± 0.34}**
PMAE 400 mg/kg n=5	[1444.60±0.74]***### 29.0 ± 0.76*** ### (23.6 ± 0.61)**### {1.6 ± 0.54}	[1499.80±0.73]***###c 29.6 ± 0.68***### (21.4 ± 0.79) ### {1.2 ± 0.18}	[1649.80±0.78]***###c 25.2 ± 0.73###c (25.2±0.53) ***###c {1.2 ± 0.18}##
	[1109.60±0.61]*** ###	[1004.60±0.61]*** ###c	[999.60±0.46]*** ###c

All values are Mean ± SEM. n=number of rats per group. DW= Distilled water. PMAE= *P. macrophylla* aqueous extract. MF= mount frequency, (IF)= intromission frequency, {EF}= ejaculation frequency, [MIC]= mean interval of copulation. During the period of treatment and in the same line, ^ap<0.05; ^bp<0.01; ^cp<0.001: significantly different compared to the initial day (day 1) (ANOVA Repeated Measures+ Bonferroni all pair comparism test). In the same period of treatment, *p<0.05; **p< 0.01; ***p<0.001: significantly different compared to control group (distilled water). #p<0.05; ## p<0.01; ### p<0.001: significantly different compared to the positive control (Sildenafil citrate) (Two ways ANOVA+Bonferroni all pair comparism test).

Effects of *P. macrophylla* on serum testosterone level

The Figure 1 shows that, after 14 consecutive days of oral administration of *P. macrophylla* aqueous extract, the testosterone level significantly increased (P < 0.01) at dose of 200mg/kg when compared to control. Although at that dose the testosterone level was not significant (P > 0.05) when compared to sildenafil citrate, we noticed an increase of 38.33%.



Each bar represents the mean±ESM. n=number of rats per group= 5. DW= Distilled water. Sildenafil c.= Sildenafil citrate 5 mg/kg. PMAE 100= *P. macrophylla* aqueous extract 100 mg/kg. PMAE 200= *P. macrophylla* aqueous extract 200 mg/kg. PMAE 400= *P. macrophylla* aqueous extract 400 mg/kg. MF= mount frequency, (IF)= intromission frequency, {EF}= ejaculation frequency, [MIC]= mean interval of copulation. *p<0.05 vs distilled water.

Figure 1: Variation of serum testosterone level after treatment with *P. macrophylla*.

DISCUSSION

The albino rat is the best studied mammalian species for sexual behavior [27]. Male rats usually begin a sexual encounter by anogenital sniffing followed by a repeated series of mount, intromission and ejaculations followed by a post-ejaculatory interval or refractory period [28]. The main goal of this work was to determine the copulatory performance of the aqueous roots extract of the *P. macrophylla* in the rat through the evaluation of some sexual behavioral parameters. It was interesting to find in this study that when compared to the control group and the respective initial values (day1), the oral administration of *P.*

macrophylla aqueous extracts significantly decreased ML, IL and PEI without affecting significantly the EL, and increased MF, IF, EF and MIC respectively throughout the treatment. Moreover, these effects were more pronounced on day 14 in rats treated with the dose of 200 mg/kg of the plant extract. ML, IL and PEI are indicators of sexual motivation while MF, IF, EF and MIC represent parameters of sexual performance [15]. This result thus confirms the stimulating potential of *P. macrophylla* on sexual motivation sexual performance parameters in rats as previously reported by Beach [29]. The results obtained in this study with *P. macrophylla* are in harmony with those of Ang *et al.* [30], Carro-Juárez *et al.* [13, 31] and Watcho *et al.* [15] with extracts of *Eurycoma longifolia*, *Montanoa tomentosa*, *Litsea chinensis* and *Dracaena arborea* respectively. Since it is well demonstrated that following each ejaculation, rats cease copulatory activity for a certain period called post ejaculatory interval (PEI) or refractory period which may last for 6 to 10 minutes [27, 32], the ability for the plant extract to decrease this PEI could be attributed to some bioactive components present in this extract, capable to increase the motivation in male rats and reinitiate contact successfully between male and female during copulation. Moreover, the enhancement of sexual pleasure in the extract-treated rats was illustrated in this study by the significant increase in performance parameters such as MF, IF, EF and MIC. The increase in MIC results from the increase in MF, IF and EF. The duration of the copulation (MIC) observed in the current study could be a consequence of the action of the bioactive principle at the peripheral level by direct stimulation of the cavernous body to produce NO, capital molecule responsible for maintaining the erection and prolonging the copulatory activity [33]. Since testosterone and its metabolites (Estradiol and dihydrotestosterone) are the main hormones contributing to the activation and modulation of mating in rats [27, 34], the pro-sexual effects of *P. macrophylla* extract observed in this study could derived from the activation of testosterone production at the level of Leydig cells. To verify this hypothesis, the evaluation of serum testosterone concentration was performed at the end of the two weeks of treatment in rats and results obtained indicated an increase in serum testosterone level observed on day 14 in rats treated with *P. macrophylla* at the dose 200 mg/kg compared to control rats. This results thus confirmed the implication of testosterone in the pro-sexual effects of *P. macrophylla* and this hormone would act by facilitating the activity of neuronal and endothelial nitric oxide synthase (nNOS and eNOS) in erectile tissue and/or by stimulating the release of dopamine in the median preoptic hypothalamic zone [35-37]. Everitt [38] demonstrated the mechanisms underlying appetitive and copulatory response in male rat and in 1956, Beach [29] showed that the decrease in the post-ejaculatory interval was inversely proportional to the sexual excitation induced by a pharmacological substance. Therefore, the pro-sexual and aphrodisiac properties of the aqueous roots extract of *P. macrophylla* Benth could be attributed to phytochemical components presents in this plant extract. Even though the phytochemical screening of *P. macrophylla* has not yet been done, that of some plants belonging to the same species

such as *Swietenia macrophylla* and *Gentiana macrophylla* indicated the presence of flavonoids, alkaloids, steroids, terpenes, tannins, glycosides, phenols and saponins [39, 40]. The implication of these bioactive components on sexual function and reproductive parameters have been widely documented [41-45].

These results justified the aphrodisiac properties of *P. macrophylla* extract and corroborated the results of Carro-Juárez *et al.* [13], Kpomah *et al.* [46] and Kenmogne *et al.* [21] who demonstrated the similar effects using *Zanthoxylum leprieurii*, *Piper guineense*, *Montanoa tomentosa* and *Carpolobia alba* respectively. These findings are consistent with those of Sandroni [47] and Alok *et al.* [48] who state that aphrodisiacs are substances that can increase libido, sexual potency and/or sexual pleasure. However, for the better understanding of the aphrodisiac properties of *P. macrophylla*, other parameters of the sexual function need to be investigated.

It can be concluded that, the aqueous extract of *P. macrophylla* possess pro-sexual and aphrodisiac effects in relation with the androgenic potential of particular bioactive components present in the aqueous root extract of plant. These outcomes which justified the traditional use of *P. macrophylla* as aphrodisiac, could be applied in the local folkloric medicine, for the management of sexual debilities in male patients.

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REFERENCES

1. NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. *JAMA* 1993; 270: 83-90.
2. Akre C, Berchtold A, Gmel G, Suris JC (2014). The evolution of sexual dysfunction in young men aged 18-25 years. *J Adolesc Health* 2014; 55:736-743.
3. Ayta IA, McKinlay JB, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. *Br J Urol Int* 1999; 84:50-56.
4. Buvat J, Glasser D, Neves RC, Duarte FG, Gingell C, Moreira ED Jr. Sexual problems and associated help seeking behavior patterns: Results of a population-based survey in France. *Int J Urol* 2009; 16(7):632-638.
5. Corona G, Ricca V, Bandini E, Mannucci E, Petrone L, Fisher A, *et al.* Association between psychiatric symptoms and erectile dysfunction. *J Sex Med* 2008; 5:458-568.
6. Rizvi SJ, Kennedy SH, Ravindran LN, Giacobbe P, Eisfeld BS, Mancini D, *et al.* The relationship between testosterone and sexual function in depressed and healthy men. *J Sex Med* 2010; 7:816-825.
7. Seftel AD, Sun P, Swindle R. (2004). The prevalence of hypertension, hyperlipidemia, diabetes mellitus and depression in men with erectile dysfunction. *J Urol* 2004; 171: 2341-2345.
8. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J Urol* 1994; 151(1):54-61.
9. Chan PTK. Update on erectile dysfunction treatment for aging men. *Sexologies* 2008; 17:184-189.
10. Nony P, Boissel JP, Lievre M, Leizorovicz A, Haugh MC, Fareh S, *et al.* Evaluation of the effect of phosphodiesterase inhibitors on mortality in chronic heart failure patients: a meta-analysis. *Eur J Clin Pharmacol* 1994; 46:191-196.
11. Corona G, Vignozzi L, Sforza A, Maggi M. Risks and benefits of late onset hypogonadism treatment: an expert opinion. *World J Mens Health* 2013; 31(2):103-125.
12. Rastrelli G, Dicuio M, Reismann Y, Sforza A, Maggi M, Corona G. Cardiovascular impact of testosterone therapy for hypogonadism. *Expert Review of Cardiovascular Therapy* 2018; 16(9):617-625.
13. Carro-Juárez M, Cervantes E, Cervantes-Mendez M, Rodriguez-Manzo G. Aphrodisiac properties of *Montanoa tomentosa* aqueous crude extract in male rats. *Pharmacol Biochem Behav* 2004; 78:129-134.
14. El-Tantawy WH, Temraz A, El-Gindi OD, El-Gindi A. Free serum testosterone level in male rats treated with *Tribulus alatus* extract. *Int Braz J Urol* 2007; 33:554-559.
15. Watcho P, Wankeu-Nya M, NGuelefack TB, Tapondjou L, Teponno R, Kamanyi A. Pro-sexual effects of *Dracaena arborea* (Willd) Link (Dracaenaceae) in sexually experienced male rats. *Pharmacologyonline* 2007; 1:400-419.
16. Yakubu, MT, Akanji MA, Oladiji AT, Adesokan AA. Androgenic potentials of aqueous extract of *Massularia acuminata* (G. Don) Bullock ex Hoyl. Stem in male Wistar rats. *J Ethnopharm* 2008; 118(3):508-513.
17. Chaturapanich G, Chaikyul S, Verawatnapakul V, Pholpramool C. Effects of *Kaempferia parviflora* extracts on reproductive parameters and spermatid blood flow in male rats. *Reproduction* 2008; 136:515-522.
18. Massoma DL, Koloko BL, Bend EF, Domkam J, Oundoum Oundoum PC, Ngaha MN, *et al.* Fertility enhancing effects of aqueous extract of *Rauvolfia vomitoria* on reproductive functions of male rats. *J Exp Integr Med* 2014; 4(1):43-49.
19. Kenmogne H, Bend E, Oundoum OCP, *et al.* Effects of aqueous root extract of *Carpolobia alba* G. Don on sexual behaviour in adult male rats. *Andrologia*, 2016 ; 48(8):908-914.
20. Carro-Juarez M, Rodriguez-Santiago MG, Franco MA, Hueletl-Soto ME. Aphrodisiac activity of the aqueous crude extract of Purple Corn (*Zea mays*) in male rats. *J Evid Based Complementary Altern Med* 2017; 22(4):637-645.
21. Govaerts R, Frodin DG, Radcliffe-Smith A. World Checklist and Bibliography of Euphorbiaceae (and Pandaceae) 1-4: 1-1622. The Board of Trustees of the Royal Botanic Gardens, Kew. 2000.
22. Leonard J. Revision of Zairian species of the genus *Pycnocomma* Benth. (Euphorbiaceae). *Bull Jard Bot Nat* 1995; 65:37-72.
23. Hawthorne, Jongking. Plant resources of tropical Africa (PROTA), 2006; p. 537-538.
24. The Council of the European Communities. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *OJEC* 1986; 358:1-29.
25. Cariton AE. Experimental surgery of the genital system. In: William IG, James EH, editors. *Methods of animal experimentation: Research surgery and care of the research animal; Part B Surgical approaches to organ systems*. Orlando: Academic Press Inc, Florida: 1986.
26. Wankeu-Nya M, Watcho P, Nguenefack TB, Carro-Juarez M, Tapondjou L, Kamanyi A. Effects of *Dracaena arborea* (Dracaenaceae) on sexual dysfunction in 4 weeks hyperglycemic male rats. *Asian Pac J Trop Med* 2014; 7:609-619.
27. Hull EM and Dominguez JM. Sexual behavior in male rodents. *Horm Behav* 2007; 52(1):45-55.
28. Dewsbury DA. A quantitative description of the behaviour of rats during copulation. *Behaviour* 1967; 29:154-178.
29. Beach FA. Characteristics of masculine "sex drive" Nebraska Symposium on Motivation. *University of Nebraska Press* 1956, Lincoln. 1- 31.
30. Ang HH, Ngai TH, Tan TH. Effects of *Eurycoma longifolia* Jack on sexual qualities in middle aged male rats. *Phytomedicine* 2003; 10:590-593.
31. Carro-Juarez M, Lobaton I, Benitez O, Espiritu A. Pro-ejaculatory effect of the aqueous crude extract of *cihuapatli* (*Montanoa tomentosa*) in spinal male rats. *J Ethnopharmacol* 2006; 106:111-116.
32. Barfield RJ and Geyer LA. The ultrasonic post ejaculatory refractory period of the male rat. *J Comp Physiol Psychol* 1975; 88:723-734.
33. Cartledge J, Minhas S, Earsdley I. The role of nitric oxide in penile erection. *Expert Opin Pharmacother* 2001; 2:1-13.
34. Giuliano F. Control of penile erection by the melanocortinergic system: experimental evidences and therapeutic perspectives. *J Androl* 2004; 25:683-694.
35. Dominguez J, Riolo JV, Xu Z, Hull EM. Regulation by the medial amygdala of copulation and medial preoptic dopamine release. *J Neurosci* 2001; 21:349-355.
36. Morelli A, Filippi S, Mancina R. Androgens regulate phosphodiesterase type 5 expressions and functional activity in corpora cavernosa. *Endocrinology* 2004; 145:2253-2263.
37. Putnam SK, Du J, Sato S, Hull EM. Testosterone restoration of copulatory behavior correlates with medial preoptic dopamine release in castrated male rats. *Hormone of Behavior*, 2005; 39:216-224.
38. Everitt BJ. Sexual motivation: A neural and behavioral analysis of the mechanisms underlying appetitive and copulatory responses of male rats. *Neurosci Biobehav Rev* 1990; 14:217-232.
39. Ushie OA, Onen AI, Ugbogu OC, Neji PA, Olumide VB. *Phytochemical Screening and Antimicrobial Activities of Leaf Extracts of Swietenia macrophylla*. *Chem Search Journal* 2016; 7(2):64-69.
40. Yin C, Xie L, Guo Y. Phytochemical analysis and antibacterial activity of *Gentiana macrophylla* extract against bacteria isolated from burn wound infections. *Microb Pathog* 2018; 114:25-28.
41. Hnatyszyn O, Moxatelli V, Rondina R, Costa M, Arranz C, Balaszczuk A, *et al.* Flavonoids from *Achyrocline satureioides* with relaxant effects on the smooth muscle of Guinea pig corpus cavernosum. *Phytomedicine* 2004; 11:366-369.

42. Gauthaman K, Ganesan AP. The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction: an evaluation using primates, rabbit and rat. *Phytomedicine* 2008; 15:44-55.
43. Chaiyasut C, Kusirisin W, Lailerd N, Lertrakarnnon P, Suttajit M, Srichairatanakool S. Effects of phenolic compounds of fermented Thai indigenous plants on oxidative stress in streptozotocin-induced diabetic rats. *Evid Based Complementary Altern Med* 2011; 1-10.
44. Yoshitake, T., Yoshitake, S., & Kehr, J. The *Ginkgo biloba* extract EGb 761(R) and its main constituent flavonoids and ginkgolides increase extracellular dopamine levels in the rat prefrontal cortex. *Br J Pharmacol* 2010; 159(3):659-668.
45. Chauhan, NS, Sharma V, Dixit VK, Thaku M. A review on plants used for improvement of sexual performance and virility. *BioMed Research International* 2014; 1-19.
46. Kpomah ED, Uwakwe AA, Abbey BW. Aphrodisiac studies of diherbal mixture of *Zanthoxylum leprieurii* Guill. & Perr. and *Piper guineense* Schumach. & Thonn. on Male wistar rats, *Global J Res. Med. Plants Ind Med* 2012; 1(9):381-390.
47. Sandroni P. Aphrodisiacs past and present: a historical review. *Clin Auton Res* 2001; 11:303-307.
48. Alok S, Ratendra K, Ramandeep S. Nature's Aphrodisiacs - A Review of Current Scientific Literature. *Int J R Adv Pharm Res* 2013; 3(2):1-20.

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