

Full Length Research Paper

Evaluation of aphrodisiac properties of the aqueous extract of the trunk barks of *Spathodea campanulata* P. Beauv. (Bignoniaceae) on albino rats (*Rattus norvegicus*)

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***Spathodea campanulata* P. Beauvois (Bignoniaceae)** is a tree from the tropical and subtropical forests of Africa, used in folk medicine for the treatment of several diseases such as gastric pain, rheumatism, lumbago, cataracts and some intestinal parasites. In West Cameroon, traditional healers use a decoction of the bark of the trunk as an aphrodisiac in males. The objective of the present study was to evaluate the aphrodisiac activity of the aqueous extract of the trunk barks of *S. campanulata* in male rats. The male rats were divided into five lots: A, B, C, D and E of six animals each. Lot A received 5 ml/kg of distilled water daily for 8 days (negative control). Lot B received 5 mg/kg of Sildenafil Citrate (Viagra®) daily for 8 days (positive control). Lots C, D and E received 200, 400 and 800 mg/kg, respectively of the aqueous extract of the trunk barks of *S. campanulata* daily for 8 days. On the first, fourth and eighth day of administration, the copulatory parameters were observed and recorded. The extract induced an increase in erectile function stimulation through the significant increase ($p < 0.001$) in the number of erections, the frequency of mount and a decrease in mount latency, reflecting an increase in sexual stimulation; an increase in the frequency of intromission ($p < 0.001$) and a decrease in intromission latency, reflecting a stimulation of sexual performance. There was also an increase in ejaculation frequency and ejaculation latency ($p < 0.001$). These results indicate a pro-ejaculatory aphrodisiac potential of the aqueous extract of the trunk barks of *S. campanulata* in male rats and would justify the empirical use of this plant in the treatment of erectile dysfunction in humans, in traditional medicine.

Key words: *Spathodea campanulata*, aqueous extract, erectile dysfunction, aphrodisiac, male rat.

INTRODUCTION

Erectile dysfunction (ED) commonly known as sexual impotence is an inability to achieve or maintain sufficient

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penile erection to maintain full and satisfying sexual intercourse for at least six months (NIH Consensus Development Panel on Impotence, 1993). In 1995, about 152 million people worldwide were affected by ED and these numbers could reach 325 million by 2025 (Meuleman, 2003; Oladiji et al., 2013). In 2012, the global prevalence of ED was estimated at 20%, with a proportion increasing sharply with age (Andersson and Wagner, 1995). In the population aged 40 to 70, the incidence of erectile dysfunction is approximately 50%. Among men aged 18 to 69 who are sexually active, 47% reported at least an occasional erectile dysfunction and 7% persistent erectile dysfunction (VIDAL-Erectile Dysfunction-The disease, 2018). It appears that erectile dysfunction (ED) represents a real public health problem that profoundly affects the quality of life of patients and their partners (NIH Consensus Development Panel on Impotence, 1993; Elbendary et al., 2009). The causes of ED are organic, metabolic and/or psychological (ignorance of sexuality, anxiety, performance anxiety, family background and childhood, extramarital relationships or breakdown) (NIH Consensus Development Panel on Impotence, 1993). It is therefore crucial to improve the quality of sexual life of men who have it, as well as that of their partners, by providing adequate and effective treatment.

Thus, treatment options such as penile prostheses, psychosexual and oral therapies are implemented to remedy this problem (Porst, 2002; Meuleman, 2003). There has been considerable progress in research over the last few decades in the medical management of ED, and oral treatments are nowadays the most widely used, particularly in addition to the discovery of phosphodiesterase type 5 (PDE5) inhibitors such as Sildenafil, Tadalafil or Vardenafil, the use of α 2-adrenergic receptor antagonists such as Yohimbine or dopamine receptor agonists such as Apomorphine (Porst, 2002; Pryor, 2002; Fink et al., 2002; Drewes et al., 2003). The high cost of these conventional treatments as well as the poverty of the populations of African countries have aroused in recent years a growing interest and a strong demand for traditional herbal medicines. Several plants are then used by African populations as having aphrodisiac properties that is to say that they are known to be able to create or stimulate sexual desire (Tajuddin et al., 2004; Singh et al., 2013a). Some of these plants have been the subject of scientific research (Tajuddin et al., 2003; Watcho et al., 2006; El-Tantawy et al., 2007; Sanda et al., 2012) and others not researched on. This is the case of *Spathodea campanulata* P. Beauv. (Bignoniaceae) locally called "foukfouk" or "foufougue" which is used by traditional healers in the region of West-Cameroon (Roger et al., 2015) and West African regions for the treatment of ED (KondakuMbuta and Institute for Health Sciences Research, Forest Genetic Resources Program in Sub-Saharan Africa, 2012). Thus, it is essential to make a scientific contribution to their use.

This study was undertaken to evaluate the aphrodisiac

properties of aqueous extracts of the trunk bark of *S. campanulata* (Bignoniaceae) in albino rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Plant material

Harvest and identification

The bark of the trunk of *S. campanulata*, was harvested in November 2016 in the village of Foto district of Dschang, Department of Menoua in the region of West Cameroon. The identification of the plant was carried out at the national herbarium of Cameroon in comparison with the sample of *S. campanulata* collected by D. W. Thomas at the national herbarium under the number 55489 HNC/YA of the family Bignoniaceae. A certificate of identification has been issued for this purpose.

Preparation of extracts

Preparation of the aqueous extract of *S. campanulata*: The bark of the trunk of *S. campanulata* was sorted, cleaned and dried at room temperature away from the sun for ten days. They were then milled with an electric grinder and the resulting powder was subjected to aqueous extraction on one hand and to organic solvents by increasing polarity on the other hand: 3750 g of powder was macerated for 72 h in 5 L of water. The mixture was stirred every 24 h. After filtration with Whatman No. 3 paper, the macerate obtained was concentrated in an oven at 55°C. The aqueous extract obtained weighed 69.2 g (1.86%). The stock solutions of respective concentrations 11.5, 27 and 48 mg/ml were prepared by diluting the extract in distilled water and stored at 4°C for later use.

Phytochemical screening

The phytochemical screening of the aqueous extract of the trunk barks of *S. campanulata* was carried out in the phytochemistry laboratory of the Faculty of Medicine and Pharmaceutical Sciences (FMSP), according to the protocol described by Harborne (1980) slightly modified.

Animal material and ethical considerations

The animal species on which the present studies were conducted were rats (*Rattus norvegicus*) of the Wistar albino strain. The rats were raised in the pet shop of the Faculty of Medicine and Pharmaceutical Sciences (FMSP) at room temperature following the circadian rhythm. These animals received daily standard food and drinking water *ad libitum*. Rats were bred in cages with shavings that were changed every 2 days. For this study, we selected male and female sexually experienced Wistar albino rats, aged 12 to 16 weeks, weighing between 144 and 212 g. Ovariectomized females were induced artificially into estrus for our different experiments.

Induction of estrus

Females were induced into estrus by successive administration of estradiol benzoate (15 μ g/kg) and progesterone (60 μ g/kg), respectively for 48 and 6 h before the start of the experiment. This protocol was approved by the Institutional Ethics Committee of the University of Douala.

Table 1. Phytochemical screening of the bark of the trunk of *Spathodea campanulata*.

Test	Secondary metabolites	Solvent extracts			
		Hexane	Ethylacetate	Methanol	Aqueous
Dragendorff	Alkaloids	+	+	+	+
Liebermann Bushard	Terpenes and sterols	-	-	-	-
Saponins	Saponosides	-	+	+	+
Acetate	Coumarin	-	-	-	-
Ferric Chlorure	Phenols	-	-	+	+
Shinoda	Flavonoids	-	-	+	+
	Tanins	-	-	+	+
Fehling Liquor	Reducing sugars	-	-	+	+
	Anthocyanins	-	-	-	-

+: Present; -: absent.

Treatments of experimental animals

Thirty vigorous males submitted successfully to the grip test were divided into 5 groups of 6 rats each and treated orally as follows: Lot A (negative control) received 5 mL/kg of distilled water daily for 8 days, Lot B (positive control) received 5 mg/kg of Sildenafil Citrate (Viagra®) daily for 8 days, Lots C, D and E received 200, 400 and 800 mg/kg aqueous extract of the trunk bark of *S. campanulata*, daily for 8 days. These substances were administered using an orogastric feeding tube.

Study protocol for male sexual behaviour

The study was conducted between 13:00 and 17:00 to avoid any potential interference with the increase in natural libido observed in the mornings. The observations were then made on the 1st, 4th and 8th day of treatments administration. Thus, each rat was placed in a cage for 1 h for acclimation, and then a receptive female was introduced into the cage. The pair of animals was observed closely for 30 min. During this time, the following copulation parameters were counted and latency times measured:

- (1) The frequency of the mounts which corresponded to the number of mounts, with or without intromissions preceding an ejaculation;
- (2) The number of erections;
- (3) The frequency of intromissions which corresponded to the number of intromissions preceding an ejaculation;
- (4) The frequency of ejaculations which is the number of ejaculations recorded during the observation time;
- (5) The latency of the ride which is the time between the introduction of a female in the cage and the first mount;
- (6) The intromission latency time which is the time between the introduction of the female and the first intromission;
- (7) The latency time of ejaculation which is the time that separates the first intromission from the first ejaculation (Pfaus et al., 2016).

Statistical analysis

Data were entered into an Excel spreadsheet (Microsoft Office 2007, USA) and analyzed with Statview software version 5.0 (SAS Institute, Inc., USA). Quantitative data were presented as mean \pm standard deviation (SD) in graphs and tables. One-way order of variance analysis was used to compare the averages between two

and more than two groups, respectively. The Newman-Keuls post hoc test was used to make the multiple pair comparisons. The materiality threshold was set at p-value 0.05.

RESULTS

Phytochemical screening

The phytochemical screening of the aqueous extract of the trunk bark of *S. campanulata* revealed the presence of the secondary metabolites shown in Table 1.

Effects of the aqueous extract of *S. campanulata* on the mount frequency

The effects of the aqueous extract of *S. campanulata* on the frequency of mounts on days 1, 4 and 8 are shown in Table 1. On day 1, we observed a significant increase ($p < 0.05$) in the frequency of mounting rise for the batches treated at 200 and 400 mg/kg compared to the negative control. This increase was more marked for the lot at 400 mg/kg. On day 4 of experimentation, a significant increase ($p < 0.001$) of the riding frequency was observed in all the animals treated with the extract compared to those treated with distilled water. At day 8, the riding frequency was significantly ($p < 0.05$) increased for lots (or batches) treated at 400 and 800 mg/kg of extract, compared to the control receiving distilled water (Table 2).

Effects of the aqueous extract of *S. campanulata* on the frequency of intromission

Table 2 shows the effects of the aqueous extract of *S. campanulata* on the frequency of intromission in the different groups of animals on days 1, 4 and 8 of the experimental period. On day 1, we observed a significant

Table 2. Effects of aqueous extract of *S. campanulata* on the mount frequency, on the intromission frequency, the number of erections the ejaculation frequency. All values were expressed as average + SD

Lots	Observed parameters (per 30 min)											
	FM			FI			NE			FE		
	J1	J4	J8	J1	J4	J8	J1	J4	J8	J1	J4	J8
Eau distillée	15 ± 0	13.00 ± 0	21 ± 0	18.83 ± 5.44	13.03 ± 3.63	5.32 ± 1.56	22.33 ± 3.64	19.83 ± 3.47	24.33 ± 3.04	0.83 ± 0.3	1.33 ± 0.42	1.33 ± 0.3
Citrate de Sildenafil	27.67 ± 5.95	31.50 ± 2.63	34.33 ± 1.99	25.00 ± 5.32	13.34 ± 2.17	5.45 ± 2.33	24.00 ± 6.11	25.17 ± 2.65	27.83 ± 2.69	1.17 ± 0.4	1.67 ± 0.33	2.17 ± 0.33
<i>S. campanulata</i> 200 mg/kg	23.33 ± 2.87	31.60 ± 1.99	31.17 ± 5.30	20.50 ± 2.51	6.16 ± 1.74	2.51 ± 5.00	22.50 ± 2.77	29.80 ± 2.15	27.50 ± 5.50	0.83 ± 0.17	1.6 ± 0.24a	1.33 ± 0.33
<i>S. campanulata</i> 400 mg/kg	29.00 ± 4.66	29.33 ± 1.36	36.33 ± 4.26	23.17 ± 4.22	10.34 ± 1.68	4.22 ± 3.95	27.50 ± 5.05	31.00 ± 2.58	36.17 ± 3.91	1.33 ± 0.33	2.67 ± 0.21	2.17 ± 0.17
<i>S. campanulata</i> 800 mg/kg	21.67 ± 2.64	32.67 ± 3.95	33.50 ± 4.98	18.83 ± 2.18	5.35 ± 3.61	2.18 ± 4.43	16.33 ± 1.41	32.67 ± 3.58	27.83 ± 5.13	0.67 ± 0.2	1.83 ± 0.3b	1.33 ± 0.42

FM: Mount frequency, FI: intromission frequency, NE: number of erections, FE: ejaculation frequency.

($p < 0.05$) increase in the frequency of intromission in the animals receiving 200 and 400 mg/kg extract compared to the negative control group. When we compared with the positive control, we observed that only the difference with the animals receiving 800 mg/kg of extract was significant ($p < 0.001$). On day 4, we observed a significant decrease ($p < 0.001$) of the intromission frequency for the lots treated with the extract at different doses compared to the negative control.

Effects of aqueous extract of *S. campanulata* on the number of erections

On day 1, we observed that the number of erections significantly increased ($p < 0.05$) in animals receiving different doses of 200 and 400 mg/kg of the extract compared to the negative control. On day 4 of administration, we observed a higher significant increase ($p < 0.001$) in the number of erections for the different lots treated with the extract compared to the negative control. The erection number for the positive control lot increased significantly every 3 days compared to the negative control (Table 2).

Effects of the aqueous extract of *S. campanulata* on the ejaculation frequency

On day 1, the frequency of ejaculations in animals receiving 200 and 400 mg/kg of extract was significantly increased ($p < 0.05$), compared to that of the negative control group. The difference with the positive control is not significant. At day 4, we observed a significant ($p < 0.001$) increase in ejaculation frequency for the 400 and 800 mg/kg treated groups compared to the negative control. The batch at 200 mg/kg showed a significant difference ($p < 0.05$) compared to the same negative control. Also, we observed a significant increase ($p < 0.05$), only for the batch treated with 400 mg/kg of extract compared to the positive control. On day 8, among the lots that received the extract, the lot receiving 400 mg/kg of extract was the only one that showed a significant difference ($p < 0.05$) compared to the negative control group (Table 2).

Effects of aqueous extract of *S. campanulata* on mount latency

On day 1, we observed a significant decrease

($p < 0.001$ and $p < 0.05$) of latency in all animals receiving doses of 200, 400 and 800 mg/kg compared to the negative control group that received distilled water. On day 4, we observed a significant decrease ($p < 0.001$) for the batches receiving 400 and 800 mg/kg of extract compared to the negative control. Compared to the positive control, we observed a significant increase in the latency of rats in rats receiving with 200 mg/kg of extract. On day 8, we observed a significant decrease in the latency of the batch treated at 200 and 400 mg/kg (Table 3).

Effects of the aqueous extract of *S. campanulata* on intromission latency

On day 1, we observed a significant decrease ($p < 0.05$) in intromission latency for the batches receiving 200 and 800 mg/kg/day and a significant decrease ($p < 0.001$) for the lot treated at 400 mg/kg compared to the negative control group receiving distilled water. Compared with the positive control lot, there was a significant increase ($p < 0.001$) of the intromission latency time in rats given the 800 mg/kg extract. On day 4, we

Table 3. Effects of aqueous extract of *S. campanulata* on mounting latency, intromission latency, ejaculation latency. All values were expressed as average + SD.

Lots	Observed parameters (s)								
	TLM			TLI			TLE		
	J1	J4	J8	J1	J4	J8	J1	J4	J8
Eau distillée	71.33 ± 15.05	66.5 ± 2.06	91.5 ± 2.26	85.33 ± 5.11	75.50 ± 2.42	99.17 ± 9.74	725.67 ± 25.94	724.83 ± 26.94	602.00 ± 5.96
Citrate de Sildenafil	59.17 ± 22.82	58.83 ± 2.24	73.33 ± 3.56	59.67 ± 4.49	63.50 ± 3.2	81.33 ± 3.09	353.0 ± 57.7	304.50 ± 10.42	255.33 ± 7.54
<i>S. campanulata</i> 200 mg/kg	62 ± 11.07	75.17 ± 7.18	31.83 ± 3.61	82.00 ± 5.23	77.50 ± 1.77	34.83 ± 3.04	659.33 ± 33.51	639.83 ± 33.52	578.17 ± 2.81
<i>S. campanulata</i> 400 mg/kg	41.83 ± 9.42	18.17 ± 4.7	28.67 ± 1.18	50.50 ± 8.49	19.83 ± 4.6	28.67 ± 1.28	371.67 ± 45.87	351.50 ± 74.33	301.83 ± 7.3
<i>S. campanulata</i> 800 mg/kg	85.5 ± 19.05	32.67 ± 7.22	104.17 ± 4.48	87.00 ± 9.39	35.67 ± 7.99	106.00 ± 1.94	507.50 ± 15.49	496.50 ± 11.04	471.67 ± 10.01

TLM: Mounting latency, TLI: intromission latency, TLE: ejaculation latency.

observed a significant decrease in intromission latency time ($p < 0.001$) for the lot receiving 800 mg/kg extract compared to the negative control. We also observed a significant decrease ($p < 0.05$) of this same parameter for the batch receiving the extract at 400 mg/kg. On day 8, a significant decrease ($p < 0.001$) in intromission latency was observed for lots (batches) receiving 200 and 400 mg/kg compared to the negative control (Table 3).

Effects of aqueous *S. campanulata* extract on ejaculation latency

The first day of the experiment showed a significant ($p < 0.05$) increase in ejaculation latency in rats having received the dose of 200 mg/kg of the extract compared to those receiving distilled water. On day 8 of the experiment, the comparison with the negative control receiving distilled water showed a significant ($p < 0.05$) increase in ejaculation latency in the animals treated with the extract at doses of 200 and 800 mg/kg (Table 3).

DISCUSSION

The overall objective of this study was to evaluate

the aphrodisiac properties of the aqueous extract of the trunk barks of *S. campanulata* P. Beauv, a plant used in parts of sub-Saharan Africa and particularly in the West Region of Cameroon to treat erectile dysfunction. The findings of this study shows that high dose administration with aqueous extract of *S. campanulata* trunk bark stimulated the copulatory activity of treated rat lots, compared to the negative control group. Specifically, oral administration of the aqueous extract of *S. campanulata* to sexually experienced rats significantly increased the number of erections ($p < 0.05$), frequency of mounts, intromissions and ejaculations for the lot received 400 mg/kg of aqueous extract of *S. campanulata* trunk bark. Latency mount and intromission time were reduced for the same batch, while ejaculation latency time significantly increased ($p < 0.05$). Similar effects have been reported by Abedi et al. (2012) who evaluated the effects of the aqueous extract of pollen grains of *Phoenix dactylifera* on the sexual behavior of male rats. Thus, the extract would contain molecules that maintain erection and increase sexual motivation (Singh et al., 2013a). This is confirmed by the significant increase in the frequency of erections and ascents as well as the decrease in the latency of rats in the extract-treated rats compared to the

negative control rats. On the other hand, the decrease in the latency period of ascending and intromission is an indicator of an aphrodisiac action (Yakubu et al., 2007). Indeed, the latency of riding and intromission are inversely proportional to the sexual motivation. Therefore, the significant decrease in elevation and intromission latency observed in rats receiving *S. campanulata* extract at 400 mg/kg on day 4 may suggest a stimulation of motivation and sexual arousal. These results go in the same direction as those obtained by Sanda et al. (2012) who also observed a marked increase in the frequency of erections, ascites and a decrease in the latency of mount in normal adult rats treated with aqueous extracts of *Allanblackia floribunda* and *Glycyrrhiza glabra*.

From these results, we can suggest an increase in motivation and sexual desire induced by the aqueous extract of *S. campanulata* trunk bark. This pro-sexual effect could be attributed to the existence of saponins (Drewes et al., 2003), flavonoids and alkaloids revealed in phytochemical studies of this plant. Indeed, the steroidal nature of saponins could act as an intermediary in the androgen production pathway. Saponins could also bind to steroid hormone receptors, which could lead to conformational changes and

contribute to an increase in the function of these hormones; or they could bind to the enzymes involved in testosterone synthesis and increase its production (Gauthaman and Ganesan, 2008). Saponins also have a peripheral action by stimulating the release of nitric oxide (NO) in vascular smooth muscle (Abedi et al., 2012). The NO being a mediator involved in the relaxation of vascular smooth muscle tissue, will induce in erectile bodies of the penis, this increased vaso-relaxation is at the origin of an increase in the number of erections, hence the result that we got.

In addition, the reported antioxidant properties of *S. campanulata* (Mangambu et al., 2014) and well-known flavonoids (reported as an elevator of androgen levels in animals) (Pelissero et al., 1996) also contribute to the observed aphrodisiac effect. Similarly, the presence of alkaloids, known for their ergogenic properties, can act either by inducing vasodilatation of the blood vessels through NO production and ultimately leading to erection or by stimulating steroidogenesis in animal testes. Therefore, it is possible that the active ingredient contained in *S. campanulata* extract may have crossed the animal's blood-brain barrier to exert its aphrodisiac effect on the hypothalamic-hypophyso-testicular axis. It is well documented that in erectile function, androgens stimulate the expression of the neuronal isoform of nitric oxide synthase (nNOS) and modulate the activity of phosphodiesterase type 5 (Mills et al., 1994). Alkaloids can also act by relaxing the smooth muscles of cavernous bodies in the copulatory organ of male rats. This aphrodisiac effect would be completely different from the action of Viagra® (sildenafil citrate), the reference molecule used in this study. Indeed, sildenafil citrate is a non-androgenic aphrodisiac that acts directly on penile erectile tissue inhibiting the activity of phosphodiesterase type 5, the enzyme involved in the specific degradation of cGMP, the second messenger involved in the mechanism of erection. The prolonged action of cGMP is at the base of the aphrodisiac effect of sildenafil citrate.

Conclusion

This study focused on the evaluation of the aphrodisiac activity of the aqueous extract of *S. campanulata* P. Beauv. We obtained the different extracts and characterized the large groups of secondary metabolites present in the extract. In addition, we tested the aphrodisiac activity compared to distilled water as a negative control on one hand and sildenafil citrate (Viagra®) on the other hand, which is a reference aphrodisiac sold in pharmacies as a positive control. The qualitative analysis of extracts of *S. campanulata* P. Beauv shows the presence of flavonoids, alkaloids, phenolic compounds, tannins, reducing compounds, saponins and coumarins. The study of the aphrodisiac activity shows that *S. campanulata* P. Beauv increases sexual desire in rats significantly compared to the

negative control. Oral administration of the aqueous extract of the trunk barks of *S. campanulata* P. Beauv at doses of 200, 400 and 800 mg/kg body weight over a period of 8 days influenced the copulatory activity of normal and sexually experienced male Wistar albino rats. Compared with the animals in the negative control groups, the effects of the extract on the sexual behavior of the treated rats were materialized by stimulation of sexual motivation, effect confirmed by the increase in the frequencies of erections and mounts as well as the decrease in the latency of mount; stimulation of sexual performance, effect confirmed by the increase in the frequency of intromissions as well as the decrease of the latency of intromission; stimulation of sexual pleasure by an increase in the frequency of ejaculation and the latency of ejaculation, effect confirmed by the increase of the frequency of ejaculations and the increase of the latency of ejaculation (Singh et al., 2013b). It can be concluded that the aqueous extract of the trunk barks of *S. campanulata* P. Beauv have a pro-ejaculatory aphrodisiac effect. These results would justify the empirical use of this plant to fight against erectile dysfunction.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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