Journal of Science and Practice of Pharmacy
December 2017; 4 (1): 182-188 Available at http://www.jsppharm.org
ISSN: 2449-0458 (print); 2449-0466 (electronic)

Original Research Article

Preliminary investigation of the aphrodisiac potential of the methanol extract and fractions of *Rhaphiostylis beninensis* Planch ex Benth (Icacinaceae) root on male rats

Josephine O Ofeimun *, Buniyamin A Ayinde
Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, University of Benin, PMB 1154, Benin City, 300001, Nigeria

* For correspondence: *Email: jofeimun@uniben.edu. Tel: +2348064942568*

Abstract

Purpose: The study investigated the claimed use of the root of *Rhaphiostylis beninensis* (Rb) as an aphrodisiac.
Methods: The methanol extract of the root of Rb as well as the chloroform and aqueous fractions were administered to male rats (100, 200, 400 mg/kg), which were mated with female animals that had been brought into oestrus by the sequential administration of 100 µg/animal of ethinyl oestradiol orally 48 h before and progesterone (1.0 mg/animal) subcutaneously, 6 h prior to mating. Tween 80 (5 %) and 50 mg/kg sildenafil served as the negative control and positive control respectively. Parameters determined were; mounting latency (ML), intromission latency (IL), ejaculation latency (EL), mounting frequency (MF), intromission frequency (IF), ejaculation frequency (EF), anogenital grooming (AG), genital sniffing (GS).
Results: The methanol extract and aqueous fraction were observed to show a dose dependent increase in MF, IF, EF, AG and GS, while a dose dependent decrease in ML, IL and EL was observed. The chloroform fraction showed the same pattern of dose dependent activity for all the parameters evaluated except for the IL, where the effect produced by 200 mg/kg was higher than that produced by 400 mg/kg dose.
Conclusion: This study provides evidence for theuse of the root of Rb in ethno-medicine as an aphrodisiac.

Keywords: Male sexual dysfunction, impotence, ethno-medicine, chloroform and aqueous fractions

Indexing: Index Copernicus, African Index Medicus

Introduction

Male sexual dysfunction (MSD) has been defined as any condition that prevents the attainment of sexual satisfaction in the male [1]. It has been identified as one of the common causes of infertility and it is known to manifest in various forms which includes; delayed or inhibited ejaculation, premature or retrograde ejaculation, low/inadequate libido and erectile dysfunction [2]. Erectile dysfunction, also sometimes referred as impotence is the consistent inability to achieve or maintain an erection sufficient for satisfactory sexual intercourse. While not considered a threatening health condition, it carries grave social and medical implication as it may be an indication of more worrisome health conditions such as diabetes, hypertension, hormonal imbalance and neurogenic diseases [3,4].

Aphrodisiacs are substances that arouse the sexual instincts, increase pleasure and performance and they have been used to treat some form of erectile dysfunction [5]. Several medicinal plants have been claimed to possess pro-sexual properties in ethno-medicine. One of such plant is *Rhaphiostylis beninensis* [6].

Rhaphiostylis beninensis Planch ex Benth (Rb) is a woody climber that is native to tropical Africa. In folkloric medicine, the leaf and root are used in the management of arthritis and rheumatism while the leaf decoction is used as a mouth wash and a wash for sores. The bark is used as a

laxative of choice for babies up to three months old with chronic constipation [6]. A decoction of the leaf is also taken for bronchial ailments while the leaf bark and root decoctions are used to expel roundworms. Goats forage the plant evoking the epithet in Igbo "Ike" meaning strong. The idea of impacting strength is also manifest in the root and bark which are prepared and sold to pregnant women to "strengthen the fetus" and also convey the idea of making the male organ strong, hence its use in ethno medicine as an aphrodisiac [7].

The plant is known locally in Nigeria by various names such as "Osumede" or "Usuende" (Bini), "Ikpokirikpo" or "Kpolokoto" (Igbo), "Atapara" or "Idiaapata" (Yoruba) [8]. Anti-inflammatory, analgesic, anti-bacterial, antiproliferative and growth inhibiting activities have been reported for the plant [8-10]. A thiourea derivative namely, N,N-di (4-methyoxybenzyl) thiourea with anti-inflammatory activity have been isolated from the root of the plant [11]. The present study was undertaken to evaluate the aphrodisiac potential of the plant with particular reference to the methanol extract, chloroform and aqueous fractions in male rats.

Methods

Plant collection and identification

Plant material was purchased at New Benin market in Oredo Local Government Area of Edo State in April 2014. It was identified at the Forestry Research Institute of Nigeria (FRIN) Ibadan by Dr Olufemi Shasanya, where a voucher specimen was deposited and the number (FHI-10068) allocated.

Preparation and extraction of the plant material

The plant was washed free of earthy material and the root bark was scrapped off and dried in the laboratory at room temperature for 1 week and thereafter in an oven maintained at 40 ⁰ C for 30 min. The dried plant material was milled and subjected to extraction (1.0 kg) in a Soxhlet extractor by defatting with petroleum ether (40-60 ⁰ C); (3.5 L), followed by extraction of the marc with methanol (2.5 L). The methanol extract was concentrated under reduced pressure using a rotary evaporator set at 40 ⁰ C and to obtain a dark brown sticky mass. The extract (40 g) was dissolved in methanol/water (1:10) and partitioned with chloroform exhaustively to yield the chloroform and aqueous fractions. These were concentrated by use of the rotary evaporator set at 40 ⁰ C. The aqueous fraction was further dried by placing it in an oven set at 40 ⁰ C.

Source and maintenance of animals

Adult albino rats of both sexes (180 - 220 g) were obtained from the Animal House of the School of Medicine, Ambrose Alli University Ekpoma, Edo State, and housed in the Animal House of The Department of Pharmacology, Faculty of Pharmacy University of Benin. They were allowed to acclimatize for two weeks, fed with pelletized animal feed (Livestock Feed, Lagos) and allowed access to water ad libitum. They were equally subjected to natural 12 h cycle of night and day. Handling was carried out according to the guidelines of the European convention for the protection of vertebrate animals [12] and ethical approval was obtained from the Ethical Committee of the Faculty of Pharmacy, University of Benin.

Experimentation

The method according to Yakubu *et al* [13] with a slight modification was adopted for this evaluation. The female animals were artificially brought into oestrus (heat) by the administration of ethinyl oestradiol orally (100 µg/ animal) 48 h before, and progesterone subcutaneously (1.0 mg/animal) 6 h prior to mating. The male animals were grouped into five groups of five animals each. Animals in group 1 received 10 ml/kg of 5 % Tween 80 and served as the negative control, while group 2, 3 and 4 received 100, 200 and 400 mg/kg of the methanol extract, chloroform or aqueous fraction. Group 5 animals received 50 mg/kg of sildenafil in 5 % Tween 80 and served as the positive control. Twenty minutes after administration of the negative control, extract/fraction or positive control, animals (one at a time) were placed in a plastic cage (30 cm × 18 cm × 30 cm) and allowed 10 min to acclimatize following which the female animals were introduced into the box (one male to one female animal at a time). The animals were observed for 30 min [14,15] with the aid of a video recording camera and an independent observer. Video camera recordings were transcribed and matched with that of the independent observer for the purpose of accuracy and elimination of bias. Similar

procedures were used to evaluate the effect of the chloroform and aqueous fractions.

The following male sexual parameters were observed, recorded and evaluated: Mounting frequency (MF), this is defined as the number of times the male animal mount the female from the time of introduction of the female without achieving intromission. A mount is operationally defined as the male assuming the copulatory position by lifting its fore body over the hind parts of the female by clasping her flanks with his forepaw [13]. Mounting latency; this is defined as the time interval between the introduction of the female and the first mount by the male [13].

Others parameters of sexual behavior observed include; intromission frequency, intromission latency, ejaculation frequency and ejaculation latency. Intromission refers to the introduction of one organ into another; operationally, the introduction of the penis into the vagina. Intromission frequency therefore refers to the number of intromissions from the time the female animal was introduced until ejaculation, while intromission latency is taken as the time interval between the introduction of the female animal into the cage and the first intromission by the male. This is usually characterizes by pelvic thrusting and springing dismounts [16]. Ejaculatory frequency refers to the number of times there was expulsion of semen by the male following vaginal penetration. It is characterized by longer, deeper pelvic thrusting and slow dismount, followed by a period of inactivity or reduced activity.

Ejaculation latency is defined as the time interval between the first intromission and ejaculation [16]. Also considered were anogenital grooming and genital sniffing. Anogenital grooming is the number of times the male assumes a sitting position on its hind part and scratches/rub its genital area. Genital sniffing refers to the number of times the male animal goes after the female and sniffs out its anal area without mounting. Animals were taken from the animal house to the laboratory for the experiment with dim red light in place and minimal disturbance daily for one week before the experiment to acclimatize them to the experimental environment. Equally the receptivity of the female animals was tested by exposing them to other male animals outside the test animals and only the most receptive females were selected for the experiment. All experiments were carried out between 2000 and 0300 h. Upon removal of each set of animals from the cages, they were cleaned out before the introduction of the next set.

Statistical analysis

Results were expressed as mean ± SEM of five animals per group. One way analysis of variance (ANOVA) was used to analyze the results, while the Duncan's multiple range test was used to establish level of significance at $p < 0.05$. GraphPad Instat software incorporated, California U.S.A was employed as the data analysis tool.

Results

The methanol extract (100, 200, 400 mg/kg) was observed to produce a dose dependent increase in MF, IF, EF, AG and GS, while a dose dependent decrease in ML, IL and EL was observed compared to the negative control. The highest activity was produced by the 400 mg/kg dose of the extract, whose effect on IL and AG were statistically significant ($p < 0.05$) (Table 1).

The chloroform fraction showed the least activity in all the parameters determined, however, activity equally followed a dose dependent pattern except in the activity of the fraction on IL, where it was observed that the 100 mg/kg dose of the chloroform extract produced a lower value (763.32 ± 16.59) compared to the 200 mg/kg dose which produced a latency time of 854.37 ± 11.21 sec (Table 2).

Similarly, the effect of the aqueous fraction was observed to follow a dose dependent pattern as the methanol extract and chloroform fraction. The effect produced by 200 mg/kg of aqueous fraction on mounting latency was statistically significant ($p < 0.05$) compared to the negative control (Table 3). Overall, the aqueous fraction produced the highest activity in all the parameters evaluated, with the chloroform fraction showing the least activity. The activity of the methanol extract was intermediate between the two fractions. However the effect produced by the aqueous fraction was less than that produced by the reference drug.

Table 1: Aphrodisiac activity of methanol extract of *Rhaphiostylis beninensis*

	Control	100 mg/kg	200 mg/kg	400 mg/kg	Sildenafil
MF	4.00 ± 0.12	6.50 ± 0.76	7.80 ± 0.22	9.00 ± 0.51	15.00 ± 3.17
ML	481.37 ± 9.30	316.27 ± 13.03	220.42 ± 6.41*	169.53 ± 6.29	69.20 ± 3.16
IF	2.60 ± 0.23	3.00 ± 0.29	3.20 ± 0.35	5.40 ± 0.76	8.00 ± 1.46
IL	748.34 ± 18.64	590.39 ± 17.30	437.21 ± 15.41	331.16 ± 13.30	183.29 ± 13.61*
EF	1.20 ± 0.11	1.60 ± 0.41	2.20 ± 0.19	2.60 ± 0.34	5.10 ± 0.44
EL	367.24 ± 11.24	328.23 ± 13.41	258.41 ± 11.09	208.24 ± 10.56	121.15 ± 10.36
AG	9.34 ± 1.19	11.12 ± 1.31	13.52 ± 1.26*	15.80 ± 1.57	21.95 ± 2.35
GS	5.50 ± 1.28	7.57 ± 1.15	8.23 ± 0142	9.31 ± 2.31	16.20 ± 0.81*

*Results are expressed as mean ± SEM, n = 5, p < 0.05**

Table 2: Aphrodisiac activity of chloroform fraction of methanol extract of *Rhaphiostylis beninensis*

	Control	100 mg/kg	200 mg/kg	400 mg/kg	Sildenafil
MF	4.00 ± 0.12	5.10 ± 0.32	5.80 ± 0.22	6.20 ± 0.35	15.30 ± 1.52
ML	402.11 ± 7.08	407.27 ± 11.03	342.15 ± 8.32	291 ± 7.11	81.53 ± 3.22*
IF	2.40 ± 0.51	2.80 ± 0.21	3.00 ± 0.38	3.60 ± 0.29	7.40 ± 0.35
IL	748.34 ± 18.64	763.32 ± 16.59	854.37 ± 11.21	663.17 ± 15.42	192.72 ± 11.23*
EF	1.20 ± 0.18	1.20 ± 0.13	1.40 ± 0.11	1.80 ± 0.21	6.60 ± 0.51
EL	392.85 ± 10.72	342.45 ± 10.53	307.38 ± 11.17	299.51 ± 9.27	121.15 ± 10.36
AG	10.02 ± 1.59	9.82 ± 1.09	11.20 ± 1.26*	11.75 ± 1.39	21.95 ± 2.35
GS	6.30 ± 1.28	6.30 ± 1.31	6.72 ± 1.42	7.46 ± 1.09	10.52 ± 2.22*

*Results are expressed as mean ± SEM, n = 5, p < 0.05**

Table 3: Aphrodisiac activity of the aqueous fraction of *Rhaphiostylis beninensis*

	Control	100 mg/kg	200 mg/kg	400 mg/kg	Sildenafil
MF	4.00 ± 0.12	7.20 ± 1.26	8.80 ± 1.32	10.00 ± 1.73	15.30 ± 1.52
ML	402.11 ± 7.08	235.33 ± 6.93	192.31 ± 4.38	156.54 ± 4.21	81.53 ± 3.22*
IF	2.40 ± 0.51	4.00.20 ± 0.49	4.60 ± 0.35	5.10 ± 0.38	7.40 ± 0.35
IL	748.34 ± 18.64	312.17 ± 17.12	263.21 ± 15.41	213.16 ± 18.30*	192.72 ± 11.23*
EF	1.20 ± 0.18	2.20 ± 0.13	2.60 ± 0.21	3.20 ± 0.19	6.60 ± 0.51
EL	392.85 ± 10.72	272.54 ± 10.32	251.41 ± 10.51	177.24 ± 11.69	121.15 ± 10.36
AG	10.02 ± 1.59	13.00 ± 0.93	15.50 ± 1.62*	18.20 ± 1.55	21.95 ± 2.35
GS	6.30 ± 1.28	8.40 ± 1.27	10.00 ± 1.68	10.60 ± 1.18*	10.52 ± 2.22*

*Results are expressed as mean ± SEM, n = 5, p < 0.05**

Discussion

Generally, medicinal plants with aphrodisiac activity have been shown to elicit their effect through various mechanisms. This involves a peripheral, hormonal, and neuronal component with a possible integration of all these mechanism [17,18]. The peripheral component has to do with the relaxation of the penile smooth muscle brought about by increased generation and release of nitric oxide, activation of cyclic adenosine monophosphate (cAMP), and or activation of the efflux of potassium ion with a resultant increase in the flow of blood to the penis and the production of an erection [19,20]. It has also been established that increased serum level of androgens particularly testosterone regulate the magnitude of penile erectile response by regulating the venous out flow from the cavernous spaces [21]. Equally involved is the neural system that depends on the stimulation and inhibition of dopamine receptors in the central nervous system to either enhance or impair sexual behavior [22].

Genital sniffing is a parameter used to measure pre-copulatory sexual behavior, the purpose being to stimulate sexual excitement. An increase in the number of times the male seeks out the female to sniff her odors is an indication of pre-copulatory sexual stimulation in the male [23]. In this study, the aqueous fraction of the methanol extract of Rb, was observed to produce an increase in GS that was statistically

significant compared to the control. This increase was similar to that produced by the reference drug, suggesting an increase in sexual stimulation and excitement by this fraction of the extract. Equally, increase in anogenital grooming is indicative of increased sexual stimulation in male rats. It plays a major role in the readiness of the adult male rat for reproduction [24]. It is also an important measure of the erectile status of the penis [25]. In this study, the methanol extract as well as the chloroform and aqueous fractions of RB at varying concentrations were observed to exhibit a dose dependent response in AG, with that produced by the aqueous fraction, at the highest dose (400 mg/kg) being higher than that seen with the extract and chloroform fraction. However, this was less than that seen with the reference drug.

Mounting and intromission frequencies are considered indices of libido and potency, while a decrease in mounting and intromission latencies are indicators of sexual arousability, motivation and potency [26]. The methanol extract, aqueous and chloroform fractions were observed to effect a dose dependent increase in mounting, intromission and ejaculatory frequencies, whereas a dose dependent decrease in mounting, intromission and ejaculation latencies was observed suggesting that these have ability to affect, arousal, motivation, potency and vigor positively. However, for each of the parameters considered, the aqueous fraction was seen to have a greater effect followed by the methanol extract and lastly the chloroform fraction.

The purpose of fractionating the methanol extract into the chloroform and aqueous phases is to partition the constituents present in the extract into non – polar (chloroform) and polar (aqueous) constituents. From the result of the study it can be deduced that the polar constituents present in the plant have a higher tendency to induce aphrodisiac effect in male rats compared to the non-polar constituents. Various phytochemicals have been reported to be present in the plant. These include alkaloids, tannins, flavonoids, saponins, steroids and reducing sugars (5). Except for reducing sugars, all the other constituents have been reported to enhance sexual stimulation by increasing endogenous testosterone level probably by raising the level of luteinizing hormone [27]. Equally, alkaloids present in some plants such as *Mucuna pruriens*have been credited with estrogenic properties that manifest as vasodilation of blood vessels of the penis with resultant erection, as well as increased spermatogenesis [28]. Saponins and particularly, those with a steroidal nucleus are known to have a nitric oxide like activity that bring about the relaxation of the smooth muscle of the corpus cavernosum. They are also known to produce increases in the levels of circulating testosterone with its resultant effect on male sexual behavior, which is enhanced sexual stimulation and excitement [29]. It is possible that the presence of any or all of these components in the extract and fractions are responsible for the observed activity.

Conclusion

This study provides evidence for the use of the root of *R. beninensis* in ethno medicine as an aphrodisiac. The methanol extract, chloroform and aqueous fractions of the plant revealed varying degree of activity with the aqueous fraction showing the highest activity. The effect observed could be due to the presence of constituents such as saponin, alkaloids, flavonoids, tannin andsteroidal triterpenoids reported to be present in the plant, and these may have exerted their effect through central and /or peripheral mechanisms. However, more work still needs to be done to identify, isolate and characterize constituent/s in the plant responsible for the observed activity and to identify the exact mechanism/s of action of extracts and such isolated constituents.

Acknowledgement

We wish to acknowledge Pharmacist Fabian Amaechina of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mr Gabriel O. Benjamin of the Department of Phytomedicine and Mr Benjamin O. Abhulimen of the Department of Science Laboratory Technology, Faculty of Life Sciences,

University of Benin for their assistance with this work.

Conflict of Interest

We declare that there is no conflict of interest associated with this work.

Contribution to authorship

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by us. The two authors jointly conceived and designed the work, while actual data collection and analysis was done by the first author. Both authors jointly contributed to the manuscript.

References

1. Lue TF, Male sexual dysfunction. In: McAnich JW, Lue TF, editors, Smith and Tanagho's General urology. 18th ed. New York McGraw Hill; 2013. pp. 463-482.
2. Dabhadkar DK, Zade V, Charjan A, Thakare G. Review of natural plants and its potential to treat sexual dysfunction in albino rats. Bio Bull. 2015;1(1):7-11.
3. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of theaqueous extract of *Fadogia agretis* (Schwanf Ex Hiem) stem in male albino rats. Asian J Androl 2005;7:399-404.
4. Kaplan HS. The sexual desire disorder: Dysfunctional regulation of sexual motivation.1st ed, New York, Brunner-Routledge; 1995.
5. Kotta S, Ansari SH, Ali J. Exploring scientifically proven herbal aphrodisiacs. Pharmacog Rev 2013;7:1-10.
6. Irvine FR. *Raphiostylis beninensis*: woody plants of Ghana with special references to their activity. Oxford University press London. 1961. pp. 547-549.
7. Burkill HM. Useful plants of West Tropical Africa. Vol 2: families E-I. Royal Botanical Gardens Kew, London.1985. p. 593-594.
8. Ofeimun JO, Onwukeame DN. Evaluation of phytochemical constituents and pharmacological activities of roots of *Raphiostylis beninnensis (*Icacinaceae*)*. J Pharm Sci Pharm Prac 2006;8:85-90.
9. Lasisi AA, Folarin OM, Dare EO, Akinloye OA, Fisuji MO. Phytochemical, antibacterial and cytotoxic evaluation of *Raphiostylis beninensis* (Hook F. Planch) stem bark extracts. Int J Pharm. Bio Sci 2011;2: 489-495.
10. Ofeimun JO, Mbionwu MI. Cytotoxic and growth inhibitory activity of aqueous extracts of root and leaf of *Raphiostylis beninensis* Planch Ex Benth and *Pyrenancantha staudtii* Engl (Icacinaceae). J Pharm Bioresour 2014;11:8-14.
11. Ofeimun JO, Ayinde BA, Igbe I, Aderogba M, Adhikari A, Amjad H, Iqbal MC. Anti-inflammatory constituent from the root of *Rhaphiostylis beninensis* (Icacinaceae). Res J Phytochem 2014: Doi 10.3923/1819- 3471.1-5.
12. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series, Strasbourg, ETS-123, 2005.
13. Yakubu MT, Akanji MA, Oladiji AT. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. Phcog Rev 2007;1: 49-56.
14. Gauthaman K, Adaikan PG, Prada RNV. Aphrodisiac properties of *Tribulus terrestis* extract (Protodioscin) in normal and castrated rats. Life Sci. 2002;71(12):1385-1396.
15. Agmo A. Male rat sexual behavior. Brain Res Protoc. 1997;1:203-209.
16. Dominguez JM, Hull EM. Dopamine, the medial pre-optic area and male sexual behavior. Physiol Behav 2005 86:356-368.
17. Abedi A, Parviz M, Karimian SM, Rodsari HZ. Aphrodisiac activity of aqueous extract of *phoenix dactylifera* pollen in male rats. Adv Sex Med. 2013;3:28-34.
18. Anderson KE, Wagner G. Physiology of penile erection. Physio.Rev. 1995; 75: 191-236.
19. Anderson KE. Pharmacology of penile erection. Pharmacol Rev 1997;53:417-450.
20. Burnett AL. Nitric oxide in the penis: Physiology and pathology. J.Urol. 1997; 157: 320- 324.
21. Klotz BW, Zimmerman J, Ruth P, Englemann U, Addicks K. Soluble guanylate cyclase and cGMP-dependent protein kinase I expression in the human corpus cavernosum. Int J Impot Res 2000; 12:157-164.
22. Lee SW, Wang HZ, Christ GJ. Characterization of ATP-sensitive potassium channels in human corporal smooth muscle cells. Int. J Impot Res 2006; 11:179-188.
23. Padashetty SA Mishra SH. Aphrodisiac studies of *Tricholepisglaberrima*with supportive action

from anti-oxidant enzymes. Pharmaceut. Biol. 2007; 45:580 -586.

24. Giuliano F, Rampin O, Schirar A, Jardin A, Rousseau JP. Autonomic control of penile erection: modulation by testosterone in the rat. J. Neuroendo. 1993; 5(6): 677-683.
25. Hernandez-Gonzalez M. Prepubertal genital grooming: Penile erection in relation to sexual behavior of rats. Physio Behavior 2000; 71: 51-56.
26. Rampin O, Jerome N, Suaudeau C. Proerectile effect of apomorphine in mice. Life Sci. 2003; 79(21):2329-2336.
27. Saksena S, Dixit VK. Role of total alkaloids of *Mucuna pruriens* Baker in spermatogenesis in Albino rats. Ind J Nat 1987; 3:3-7.
28. Hilnak Z. Pre-copulatory behavior in male rats: ethological analysis and functional consideration. Act. Nerv Super 1990;32(1): 12-34.
29. Yakubu MT, Akanji MA. Effect of aqueous extract of *Massularia acuminata* stem on sexual behavior of male rats. Evi. Based Comp. Alt. Med. 2011: http// dx. Doi.org 10.1155/2011/738103.