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Effects of Aqueous Extract of *Rafflesia cantleyi* Bud on Aphrodisiac Activity in Male Sprague Dawley Rats

^{1,2}Sumaia M.M. Bakoush, ³Wan A. Yaacob, ⁴Jumaat H. Adam and ¹Nazlina Ibrahim

¹School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia

²Department of Biology, Faculty of Science, Aljabel Algharbi University, Aljabel Algharbi, Libya

³School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia

⁴School of Environmental Sciences and Natural Resources, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia

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Corresponding Author:

Nazlina Ibrahim

School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan, Malaysia, Bangi, 43600, Selangor, Malaysia
Tel : +60389213815

ABSTRACT

Rafflesia cantleyi is locally known as Bunga Pakma has been used in Malaysian folk medicine by men as an energy drink or an aphrodisiac. However, there has been no evaluation of the effect of the repeated dosing of *R. cantleyi* bud. The current work was undertaken to evaluate the acclaimed aphrodisiac activity of *R. cantleyi* bud on male rats. The 40 adult male rats were divided into 4 groups (n = 10 per group/dose). Rats in group A (control) were administered with 1 mL of distilled water while those in groups B, C and D were given same volume containing 250, 500 and 1000 mg kg⁻¹ b.wt., of *R. cantleyi* bud water extract, respectively for 28 days. The effect of the extract on body weight, reproductive organ weight and serum testosterone concentration were determined. Sexual behavior parameters were monitored in male rats for weekly mounting test and on day 28 by pairing with a receptive female (1:1). The results revealed that the *R. cantleyi* bud water extract significantly increase body weight and serum testosterone content (p<0.05). Moreover, the bud extract markedly influence the orientation behavior of treated animals which showed more attraction towards female rats. Sexual behavior observations on the animals result revealed presence of precopulatory and copulatory behaviors (chasing, sniffing and mounting) by the tested male rats. The extract at doses 250, 500, 1000 mg kg⁻¹ b.wt. significantly increase the frequency of mount and intromission (p<0.05). In addition, the ejaculation latency was significantly prolonged. The mount and intromission latencies were reduced significantly whereas ejaculation frequency significantly increased (p<0.05). Computed percentage of mounted, intromitted, ejaculated, index of libido and copulatory efficiency were higher in treated group compared to control group. The present study demonstrates that water extract of *R. cantleyi* bud increases testosterone level and enhances sexual behavior in male rats. The dose taken must be cautiously monitored.

Key words: *Rafflesia cantleyi* bud, aphrodisiac, sexual behavior, sprague dawley male rats

INTRODUCTION

Malaysia has great potential to develop her abundant natural resources to increase the market based on herbal products. This is evident from a 1935 report that in Peninsular Malaysia alone there are about 550 genera of tropical plants, containing over 1,300 species possessing medicinal values (Ang *et al.*, 2000). Some of popular Malaysian plants undergoing extensive research are the male aphrodisiac *Eurycoma longifolia* Jack. (Tongkat Ali), *Labisia pumila* (Kacip Fatimah) *andrographis paniculata* (Hempedu Bumi), *Orthosiphon stamineus* (Misai Kucing), *Centella asiatica* (Pegaga), *Phyllanthus niruri* (Dukung Anak), *Momordica charantia* (Peria) and others (Nais, 2001).

Plants are claimed to possess aphrodisiac property by many traditional medicine systems including Ayurveda, Chinese, Malay and Unani medicines. Malaysia plants such as *Rafflesia* species has been acclaimed for aphrodisiac property and have been used in Malay traditional medicine (Ang *et al.*, 2000). In Peninsular Malaysia, *Rafflesia* buds or locally called as Bunga Pakma are used by men as an energy drink or as an aphrodisiac (Nais, 2001). An aphrodisiac is defined as substances that increases a sexual activity and improve sexual performance (Ang *et al.*, 2000). The bud was still sought after as a traditional medicine and biological studies on the genus *Rafflesia* are lacking. The effects of *R. cantleyi* buds on the male reproductive system has not been investigated yet. This study was undertaken to validate scientifically the aphrodisiac role of *R. cantleyi* bud as acclaimed by the traditional practitioners.

MATERIALS AND METHODS

Preparation of plant extract: *Rafflesia cantleyi* buds were collected from the state of Perak, Malaysia during months of July-August 2013. The buds were botanically identified by Dr. Jumaat Adam. A voucher specimen (AZIE02, AZIE03) was deposited in laboratory G104, Biology Building, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM).

Preparation of extracts: In this study, we were interested to determine the effect of the water extract commonly used by traditional herbal practitioners. The bud was washed with tap water to remove dusts and then rinsed with distilled water and cut into small pieces (~2 cm) and air dried at room temperature (~25°C) for 5-7 days. The dried bud was ground to a fine powder using grinder (Panasonic Model, Malaysia) and stored at -20°C until used. The dried bud powder (372.08 g) was extracted by boiling in distilled water (1.5 L) for 30 min. Resulting water extract was filtered through a tea strainer. Boiling of the powder in 1.5 L distilled water was repeated and pooled. Filtrate was centrifuged in 50 mL batches at 2000 rpm for 10 min in a centrifuge (Eppendorf-5810 R). The resulting

supernatant was filtered through filter paper Whatman (No. 1) placed on a funnel. Filtrate was dried in a freeze dryer and stored at 4°C in amber glass vials until use.

Animals: The 40 healthy adult male Sprague Dawley rats were assigned randomly into four main groups: A, B, C and D. Each comprised 10 adult male rats, weighing 250-300 g. They were obtained from the Animals House, UKM, Bangi and housed in plastic cages. The animals were acclimatized for a week to a 12 h light and dark cycle with free access to standard rodent pellet diet and water *ad libitum* before the experiment.

Experimental protocol: The experimental protocol and procedures used in this study has been approved by the UKM Animal Ethics committee (UKMAEC, No. 505). Group A rats received only distilled water and considered as the control group. The groups B, C and D were treated daily with 250, 500 and 1000 mg kg⁻¹ b.wt., of water extract of *R. cantleyi* bud, respectively. The doses were given orally using a gavage needle for 28 days. The animals were observed daily for clinical signs and mortality. Body weights and behavior were recorded every week during the study period and amounts of supplied food and water measured daily. On day 29, animals body weight were recorded before being anaesthetized. Blood samples were collected for serum testosterone analysis and the reproductive organs removed and weighed.

Mounting test: This test was performed weekly, 2 h prior to the experiment, the animals were weighed, transferred to a dark room, put in an individual cage and left for at least 1 h before the commencement of sexual behavior study. Mounting behavior of the male rats toward the receptive females were observed according to the method previously described by Meyerson *et al.* (1973) and Mahanem *et al.* (2004). Female rats used as mating stimuli were brought to heat with a single intramuscular injection of 0.125 mg kg⁻¹ estradiol benzoate and 1.25 mg kg⁻¹ of progesterone, 48 and 4 h before testing, respectively. Mounting behavioral test was conducted by placing a receptive female in the male cage. The male rat was expected to reach receptive female within 3 min. The time that the male started to mount the receptive female was considered as positive sexual behavior. Male which failed to respond within 3 min after being placed in the cage were considered as having negative sexual behavior. This test is useful to determine the willingness of male rats to sexual behavior towards the female.

Orientation activity: This activity was observed after the mounting test. Sexual behaviors of a male with female were observed from the cage side for prospective and precopulatory behaviors for 30 min. Orientation activity was analyzed in three segments as described by Islam *et al.* (1991), using the following scores:

- **Orientation towards female:** Sniffing and licking
- **Orientation towards self:** Non-genital grooming and genital grooming
- **Orientation towards environment:** Exploration, rearing and climbing

The cumulative score for each orientation behavior noted in during the observation period was later calculated.

Mating test: The time taken for the male rat's intromission activity was recorded. Male rats that couldn't have an intromission within the first 15 min fail the mating test. The following male sexual parameters were recorded or calculated during the observation period:

- **Mount Latency (ML):** Time from introduction of the female until the first mount
- **Intromission Latency (IL):** Time from introduction of the female until the first intromission
- **Ejaculation Latency (EL):** Time from the first intromission until ejaculation
- **Mount Frequency (MF):** The number of mounts in a series
- **Intromission Frequency (IF):** The number of intromissions in a series
- **Ejaculation Frequency (EF):** The number of times there was expulsion of semen by males after vaginal penetration-characterized by rhythmic contraction of the posterior abdomen

Other computed male sexual behaviour parameters include:

$$\text{Index of libido (\%)} = \frac{\text{No. mated}}{\text{No. paired}} \times 100$$

$$\text{Mounted (\%)} = \frac{\text{No. mounted}}{\text{No. paired}} \times 100$$

$$\text{Intromitted (\%)} = \frac{\text{No. of rats that intromitted}}{\text{No. paired}} \times 100$$

$$\text{Ejaculated (\%)} = \frac{\text{No. of rats that ejaculated}}{\text{No. paired}} \times 100$$

$$\text{Copulatory efficiency} = \frac{\text{No. of intromissions}}{\text{No. of mounts}} \times 100 \text{ (Agmo, 1997)}$$

Testosterone level: Serum testosterone was assayed using the enzyme immunoassay (EIA KIT; Cayman Chemical, USA, Catalog No. 582701). Samples were assayed in duplicate, according to the standard protocol provided in the assay kit.

Statistical analysis: Statistical analysis was performed with the Statistical Programs from the Social Science (SPSS) Version 17. Statistical significance of data was assessed by analysis of variance (ANOVA) and differences were considered significant at (p<0.05). Significant difference between control and experimental groups were assessed by Tukey test and Least Significant Difference (LSD). All data was expressed as Mean±Standard Deviations (SD).

RESULTS

Effect of water extract of *R. cantleyi* bud on body weight: During the 28 days of observation, there are significant change in the body weights of treated rats compared to control rats (p<0.05) as result shown in Table 1.

Relative organ weights: No significant change (p>0.05) in the epididymis weight and relative organ to body weight in animals treated with the water extract. An increase in testes and relative organ to body weight was significantly (p<0.05) in animals receiving 500 and 1000 mg kg⁻¹ doses as shown in Table 2.

Effect of *R. cantleyi* bud extract on testosterone serum levels: Figure 1 showed a dose dependent increase in the level of serum testosterone produced by male rats treated with water

Table 1: Weekly body weight (g) of rats treated with different doses of *R. cantleyi* bud water extract

Groups	Dose (mg kg ⁻¹ day ⁻¹)	Body weight (g days ⁻¹)				
		0	7	14	21	29
A	Control	227.84±14.82	235.85±13.1	249.39±16.59	258.83±15.11	267.31±8.92
B	250	227.26±7.60	237.00±8.04	253.25±8.61	262.64±9.20*	274.49±18.6*
C	500	229.42±8.65	241.26±8.28	259.66±9.22*	267.85±8.55*	283.99±10.31*
D	1000	236.12±15.86	248.91±15.86*	267.51±18.18*	278.56±17.23*	290.15±9.78*

Values are represented as Mean±SD of 10 replicates, *Significant difference in body weight compared to control at (p<0.05)

Table 2: Relative organ weights of sprague dawley rats treated with water extract of *R. cantleyi* bud

Groups	Dose (mg kg ⁻¹ day ⁻¹)	Epididymis (g)	Relative epididymis to body weight	Testes (g)	Relative testes to body weight
A	dH ₂ O	0.31±0.01	0.10±0.00	1.95±0.10	0.67±0.00
B	250	0.33±0.01	0.12±0.0	2.13±0.02	0.78±0.01
C	500	0.35±0.01	0.12±0.01	2.41±0.06*	0.82±0.00*
D	1000	0.35±0.01	0.12±0.01	2.68±0.12*	0.86±0.09*

Values are represented as Mean±SD, *Significant difference (p<0.05) compared to control

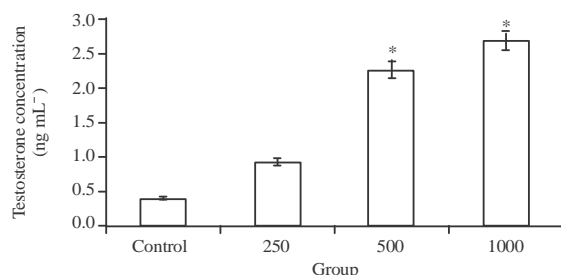


Fig. 1: Levels of serum testosterone following oral administration of *R. cantleyi* water extract compared to control group. *significant difference of serum testosterone in treatment compared to control ($p < 0.05$)

Table 3: Mean of time for males (n = 10) to reach and subsequently mount the receptive female after being treated with *R. cantleyi* extract at different doses compared to control

Dose (mg kg ⁻¹)	Mean time \pm SE (sec) (week)			
	1	2	3	4
Control	NR	NR	354.5 \pm 4.9	348.30 \pm 6.29
250	NR	294 \pm 14.0*	242.8 \pm 5.41*	238.00 \pm 4.83*
500	228.2 \pm 6.64*	165 \pm 9.43*	125.1 \pm 3.9*	101.69 \pm 7.18*
1000	207.0 \pm 7.02*	141 \pm 3.36*	101.0 \pm 5.9*	76.920 \pm 8.81*

NR: No. response even at 360 sec, *Significant difference ($p < 0.05$) compared to control

extract of *R. cantleyi* bud. The result revealed significant increase in serum testosterone level was observed in treated groups (500 and 1000 mg kg⁻¹) when compared to control group ($p < 0.05$). There was no significant difference between control and 250 mg kg⁻¹ treated group ($p > 0.05$).

Effect of *R. cantleyi* bud extract in mounting test: The administration of water extract of *R. cantleyi* for 28 days to male rats resulted in remarkable increase in mounting of the male rats after consuming of water extract of *R. cantleyi* bud (for treated group) compared (distilled water) control group as shown in Table 3. The extract at doses 500 and 1000 mg kg⁻¹ b.wt., treated male rats displayed higher sexual activity from the first week of treatment compared to treated at 250 mg kg⁻¹ and control group. The period of time to reach the female decrease significantly ($p < 0.05$) with prolonged treatment with the extract. There was no significant difference between animals treated with 500 and 1000 mg kg⁻¹ ($p > 0.05$) but significant difference was noted when compared to control ($p < 0.05$).

Effect of *R. cantleyi* bud extract on orientation behavior: Water extract at the doses of 250, 500 and 1000 mg kg⁻¹ b.wt., markedly influenced the orientation behavior of the treated animals, which showed more attraction towards female rats. The result revealed significant increase in number of licking ($p < 0.05$) and the sniffing ($p < 0.05$) of treated males towards receptive female compared to control group. The behavioral

assessment of rats towards environment (exploration and climbing) decreases in experimental animals ($p > 0.05$), while rearing was significantly increase on treated animals at the dose level of 500 and 1000 mg kg⁻¹ b.wt. The result showed also significant increase ($p < 0.05$) on the genital grooming of treated animals while decrease on nongenital grooming as compared to control group Table 4.

Effect of *R. cantleyi* bud extract on mating behavior: Water extract of *R. cantleyi* bud at doses 250, 500 and 1000 mg kg⁻¹ b.wt., showed significant increase ($p < 0.05$) on Mount Frequency (MF), Intromission Frequency (IF) and Ejaculation Latency (EL) compare to distilled water control group. Moreover, it causes significant decrease ($p < 0.05$) in the Mount Latency (ML) and Intromission Latency (IL) compared to control group as shown in Table 5.

The computed male sexual behaviour parameters which included percentage of mounted, intromitted, ejaculated, index of libido and copulatory efficiency were higher in the extract treated rats compared to the control group (Table 6). In contrast, the extract reduced the inter-copulatory interval of the treated animals in compared to control group.

DISCUSSION

Rafflesia has been used traditionally in medicinal practitioners as aphrodisiac. The preliminary phytochemical screening of aqueous *R. cantleyi* bud extract revealed the presence of alkaloids, flavonoids, tannin and steroids. The result indicates that the aqueous extract of *R. cantleyi* causes increase in animal body weight as well as in sexual organs such as testes and epididymis. Genesis of steroids is one of the causes of increased body and sexual organ weight and an increase in these parameters could be regarded as a biological indicator for effectiveness of the plant extract in improving the genesis of steroidal hormones. It has been reported that steroids constituents found in many plants possess fertility potentiating properties (Thakur and Dixit, 2007). Also, the study revealed the presence of flavonoids in *R. cantleyi* bud extract which has been implicated to have a role in altering androgen levels (Padashetty and Mishra, 2007). Since androgenic effect is attributable to testosterone levels in blood (Amini and Kamkar, 2005). The weight, size, secretary function of testes, epididymis and seminal vesicle are closely regulated by androgens. It is well established that androgens are the major regulators of growth, structure and functions of accessory sex organs (Agrawal *et al.*, 1986). Testosterone is the most important androgen secreted from the interstitial leydig cells. It has many important roles in the male reproductive functions such as inhibition of Gonadotropin Releasing Hormone (GnRH) and Luteinizing Hormone (LH) secretion, induction and maintenance of the differentiation of male accessory reproductive organs (Dohle *et al.*, 2003).

Sexual behavior in male is a complicated phenomenon under the control of endocrine, central and peripheral nervous

Table 4: Effect of water extract of *R. cantleyi* bud on orientation activity in male rats

Treatment group (mg kg ⁻¹ b.wt.)	Mean activity score towards female		Mean activity score towards environment			Mean activity score towards self	
	Licking	Sniffing	Exploration	Rearing	Climbing	Nongenital grooming	Genital grooming
Control	17.4±3.38	9.0±4	28.6±3.04	22.8±2.48	4.2±0.97	36.8±3.56	30.8±4.01
250	21.8±1.30*	18.0±4	28.2±4.08	23.8±2.16	3.6±3.04	33.8±2.38	32.8±1.92
500	28.0±2.54*	25.4±3.04*	24.4±6.46	25.8±1.48	4.4±2.07	31.8±3.16	37.4±1.81*
1000	31.4±4.87*	29.0±3.87*	23.8±3.42	27.2±1.87	4.0±0.89	28.8±1.16	40.2±2.94*

Value are given as Mean±SD, N = 10, *Significant difference (p<0.05) compared to control

Table 5: Effect of water extract of *R. cantleyi* bud on mating behaviour in male rats

Dose (mg kg ⁻¹ b.wt.)	Mount frequency	Mount latency (sec)	Intromission frequency	Intromission latency (sec)	Ejaculation frequency	Ejaculation latency (sec)
Control	3.8±1.135	377.9±16.01	3.3±0.67	395.8±10.79	0.5±0.52	345.4±12.066
250	9.6±1.56*	245.5±8.40*	8.9±1.57*	246.1±9.80*	0.9±0.7	430.6±12.74*
500	14.4±1.62*	110.0±9.36*	14.6±1.68*	129.7±20.52*	1.1±0.7	501.5±8.05*
1000	16.9±1.44*	97.9±7.62*	17.6±1.49*	114.8±10.48*	1.7±0.45*	519.4±14.31*

Value are given as Mean±SD, N = 10, *Significant difference (p<0.05) compared to control

Table 6: Effect of water extract of *R. cantleyi* bud on computed male rat sexual behaviour parameters

Doses (mg kg ⁻¹)	Mounted (%)	Intermitted (%)	Intromission ratio	Ejaculated (%)	Index of libido (%)	Copulatory efficiency	Intercopulatory interval (sec)
Control	60	40	0.46	40	50	86.84	684.15±7.77
250	100	60	0.48	60	70	92.7	277.81±7.97*
500	100	80	0.5	80	100	100	176.80±15.58*
1000	100	100	0.51	100	100	100	146.30±16.94*

Value are given as Mean±SD, N = 10, *Significant difference (p<0.05) compared to control

systems. Aphrodisiac activity in *Eurycoma longifolia* has been suggested to be associated with a 4.3 kDa bioactive peptide that was also detected in *Rafflesia* sp. (Asiah *et al.*, 2007). This potential phytoandrogen has been reported to increase testosterone levels in rat leydig cell (Sambandan *et al.*, 2004). The presence of similar peptide should therefore be confirmed if available in *R. cantleyi*.

Sexual behavior is enhanced by elevated testosterone levels. Another logical candidate that might attribute to the sexual behavior is the flavonoids constituent of the *R. cantleyi* bud extract (Bakoush *et al.*, 2015). It has been reported that flavonoids alter the androgen levels (Gauthaman *et al.*, 2002). The alteration in the androgen level was supported by the increase in testosterone content of the animals in the present study. In addition, the bud extract also has alkaloids that may have ergogenic properties by inducing vasodilation of blood vessels which consequently result in erection (Agmo, 1997). Alkaloids have also been reported to facilitate of sexual behavior (Adimoelja, 2000).

Sexual behavior in animal models is considered useful in predicting the potential similar effects of chemicals in human (Hull and Dominguez, 2007). The present investigation reveals that the aqueous extract of *R. cantleyi* can enhance male sexual behavior. In male rats, latency for mount and intromission are considered as indicators of the sexual motivation, whereas intromission and ejaculation frequencies are considered as behavioral indication of sexual performance and facilitation (Neill *et al.*, 1990). The result showed significantly increase in Mount Frequency (MF) and Intromission Frequency (IF) as compared to control. The MF and IF are considered the indices of both libido and potency. The *R. cantleyi* bud extract

significantly decreases the mount and intromission latencies indicating enhancement of sexual motivation. The significant increase in computed male sexual behavior parameters in this study are indications of sustained increase in sexual activity and aphrodisiac property inherent in the *R. cantleyi* bud extract. Nevertheless, caution must be taken on the dose consumed due to the effect on the organs especially liver and kidney as indicated by this study.

CONCLUSION

The results of this present study demonstrate that water extract of *R. cantleyi* bud enhance sexual behavior in male rats that may be attributed by the presence of alkaloids, steroid and flavonoids. This study supports the traditional medicinal use of *R. cantleyi* bud as an aphrodisiac from nature. Caution must be taken on the suitable dose. Another important point is that this plant is facing extinction. Results from this study should not be used as a reason for hunting the plant down. The unique property of the important constituent from *R. cantleyi* needs to be explored for synthetic production.

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