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Original Article

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Aphrodisiac activity of ethanolic root extracts of *Acacia pycnantha* (Golden Wattle) in healthy male Wistar rats

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Abstract

***Background & Aims*:** This study examines the aphrodisiac potentials of *Acacia pycnantha* in male Wistar rats, aiming at its effects on sexual behaviour, serum free testosterone and serum calcium concentrations.

***Materials & Methods*:** *A. pycnantha* roots were extracted using ethanol. Twenty male rats, averagely weighing 250.44 ± 8.72 g, were divided into four groups of five rats per group. Group 1, which is the control received normal saline (vehicle), group 2, the positive control, received 5 mg/kg of Yohimbine; and groups 3 and 4 received 50 mg/kg and 100 mg/kg of *A. pycnantha*, respectively. All test drugs were orally administered using an intragastric tube, and the treatment protocol lasted for 14 days. Sexual behaviour was assessed by measuring mounting frequency, intromission frequency, erection duration, and latency period. Serum-free testosterone and calcium concentrations were also measured. Data were analyzed using one-way ANOVA with Tukey's post-hoc test to compare means and Pearson's correlation to determine relationships between hormone concentrations and sexual behaviour indices.

***Results*:** Both low and high doses of *A. pycnantha* significantly amplified mounting frequency, intromission frequency, and erection duration while decreasing the latency period compared to the control group, with the high-dose group showing the most prominent effects. Serum-free testosterone concentrations were significantly elevated ($p < 0.05$) in both treated groups, with the high-dose group exhibiting the greatest increase. Additionally, serum calcium concentrations were significantly higher ($p < 0.05$) in the treated groups, with the high-dose group showing the most substantial increase. Pearson's correlation analysis revealed strong positive correlations between testosterone concentrations and sexual behaviour parameters, and moderate to strong positive correlations between calcium concentrations and sexual behaviour parameters.

***Conclusion*:** *A. pycnantha* demonstrated significant aphrodisiac activity by enhancing sexual behaviour, elevating serum-free testosterone concentrations, and increasing serum calcium concentrations in male Wistar rats.

***Keywords*:** *Acacia pycnantha*, Aphrodisiac, Sexual behaviour, Testosterone, Calcium

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Introduction

Over the years, sexual health has been understood to be significantly influenced by various factors, among which testosterone and calcium are particularly critical (1, 2). Testosterone is well-documented for its role in enhancing libido, with elevated levels linked to

increased sexual desire and motivation; conversely, low testosterone concentrations can lead to diminished libido and erectile dysfunction (ED) (3, 4). ED is the inability to attain or sustain a penile erection appropriate for sexual activity. The incidence of ED is a global plague among men, irrespective of race, culture, or religion, and it is projected to hit 322 million by the year 2025 (5). Certain levels of toxicity characterize many synthetic drugs (6), irrespective of their pharmacological potentials (7). Drugs for the treatment of ED are not an exception, as many may cause headaches, back pain, nasal congestion, flushing, and, in rare cases, vision and hearing loss (8). Arising from the series of side effects associated with ED drugs, plant-based drugs are seen as a panacea owing to their plethora of bioactive compounds (9). They are perceived as cheap, readily available, and with minimal toxicity (10, 11). Calcium is essential for muscle contraction, including the smooth muscles involved in sexual arousal and orgasm. Aphrodisiacs, substances purported to enhance sexual desire, arousal, or performance (12), are of considerable interest in this context. Among the natural sources explored for aphrodisiac effects, *A. pycnantha*, commonly known as Golden Wattle, emerges as a notable yet under-researched plant. Indigenous to Australia, *A. pycnantha* has a long history of traditional medicinal use (13) and has been employed in treatments for wound healing (14), inflammation (15), and antimicrobial purposes (16). Despite its extensive traditional applications, scientific evidence supporting its aphrodisiac properties remains limited. Given the above observation, this study investigates the aphrodisiac potential of the ethanolic root extracts of *A. pycnantha* in male Wistar rats.

Materials & Methods

1. Plant Material

Fresh roots of *A. pycnantha* were collected from a forest in Andoni, Rivers State, Nigeria. The plant material was authenticated at the herbarium of the Department of Biology, Federal University Otuoke, Bayelsa State, with voucher number FUO/BIO/PSBH/2024/089, and the specimen was deposited in the herbarium. The roots were air-dried in the shade for 4 days. The roots were ground into a fine powder using a mechanical grinder. The powdered material was then stored in airtight containers to maintain its quality and prevent moisture absorption until it was needed for extraction. A 300 g sample of the powdered root of *A. pycnantha* was subjected to extraction using ethanol for 48 hours at room temperature. The mixture was filtered, and the solvent was evaporated using a Soxhlet evaporator. The resulting extract was stored at 4°C until use.

2. Animal Model

Twenty male and 25 female Wistar rats, aged 8-10 weeks and weighing 250.44 ± 8.72 g, were used for the study and grouped as shown in the experimental design below. The rats were adapted for 1 week before the commencement of the experiment. The rats were housed in standard conditions with a 12-hour light/dark cycle, and access to food and water was provided *ad libitum*. The female rats allowed mating only during the estrus phase. This was induced by administering estradiol benzoate (10 µg/100g orally) 48 hours before mating and progesterone, injected subcutaneously at a dose of 0.5 mg/100 g, 6 hours before mating (17).

3. Experimental Design

Group 1 (Control): Received oral saline solution (1 mL/kg body weight).

Group 2 (Standard): Received oral yohimbine (5 mg/kg body weight) as a positive control.

Group 3 (Low dose): Received oral ethanolic extract of *A. pycnantha* (50 mg/kg body weight).

Group 4 (High dose): Received oral ethanolic extract of *A. pycnantha* (100 mg/kg body weight).

The treatments were administered once daily for 14 days.

4. Sexual Behavioural Assays

Sexual behaviour was assessed by adopting the method of Kpomah et al. (18) using the following parameters:

4.1 Mounting Frequency

The number of mounts per 30 minutes during a 2-hour observational period.

4.2 Intromission Frequency

The number of intromissions per 30 minutes during the same observation period (vaginal penetration).

4.3 Erection Duration

The duration of penile erection observed during the behavioural assays.

4.5 Latency Period

The time taken to initiate sexual behaviour after exposure to a female rat.

5. Biochemical Analysis

5.1. Blood Sample Collection

Blood samples were collected at the end of the treatment period via cardiac puncture under anaesthesia. The blood was collected into plain tubes and allowed to clot for 10 minutes, then centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum samples were stored at -20°C until analysis to preserve the integrity of the biochemical parameters.

5.2. Determination of Serum-Free Testosterone Concentrations

Serum-free testosterone concentrations were measured using a Free Testosterone ELISA Assay Kit (Eagle Biosciences, 20 A NW Blvd., Suite 112, Nashua, New Hampshire 3063, USA) according to the manufacturer's instructions.

5.3. Determination of Serum Calcium Concentrations

Serum calcium concentrations were estimated using a Randox Assay Kit (Randox Laboratories Ltd., 55 Diamond Road, Crumlin, County Antrim, BT 29 4QY, United Kingdom). The procedure followed the manufacturer's instructions to ensure accuracy and reliability.

6. Statistical Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to compare the means between groups. A *P-value* of < 0.05 was considered statistically significant. Descriptive statistics (mean ± standard error of the mean) were used to summarise the data while the correlation between serum-free testosterone concentrations and sexual behaviour parameters was assessed using Pearson's correlation method.

Results

1. Sexual Behaviour Parameters

The effects of *A. pycnantha* root extract on the sexual behavioural parameters of male Wistar rats are presented in Table 1.

Table 1. Effects of *A. pycnantha* extracts on sexual behaviour parameters in male Wistar rats

Group	Mounting frequency (No. of mounts/30 min)	Intromission frequency (No. of intromissions/30 min)	Erection duration (Sec)	Latency period (Min)
Control	5.40 ± 0.70	3.20 ± 0.50	10.50 ± 1.20	12.40 ± 1.80
standard (Yohimbine)	7.60 ± 0.90*	5.40 ± 0.60*	15.80 ± 1.30*	7.50 ± 1.10*
Low dose (50 mg/kg)	6.30 ± 0.80*	4.10 ± 0.50*	12.30 ± 1.50*	9.20 ± 1.40*
High dose (100 mg/kg)	8.10 ± 0.60**	6.20 ± 0.70**	17.50 ± 1.20**	6.90 ± 1.00**

*Values are expressed as mean ± standard error of the mean (SEM), n = 5. *$p < 0.05$ vs. control, **$p < 0.01$ vs. control standard.

The root extract of *A. pycnantha* significantly increased the mounting frequency in both the low and high-dose groups compared to the control group. The high-dose group demonstrated the most substantial increase, suggesting that the extract has a positive effect on sexual arousal and activity. An increase in mounting

frequency indicates enhanced sexual motivation and engagement. Similar to the mounting frequency, the intromission frequency was significantly higher in both treated groups compared to the control, with the high-dose group showing the most pronounced effect. Additionally, the duration of penile erection was significantly longer in the extract-treated groups, with the high-dose group showing the most notable increase. This suggests that the extract may enhance erectile function, allowing for longer periods of sexual activity. Finally, the latency period, or the time taken to initiate sexual behaviour, was significantly reduced in the treated groups compared to the control. The high-dose group had the shortest latency, indicating that *A. pycnantha* accelerates sexual arousal and reduces the time required to initiate sexual activity. Overall, the results demonstrate that the administration of *A. pycnantha* root extracts led to significant improvements in sexual behaviour parameters compared to the control group in a dose-dependent manner. Specifically, the high-dose extract significantly increased mounting frequency, intromission frequency, and erection duration, while decreasing the latency period, indicating enhanced sexual performance and reduced time to initiate sexual behaviour.

2. Biochemical Analysis

2.1. Serum Free Testosterone Concentration

Table 2 shows the serum free testosterone concentrations in male Wistar rats treated with ethanolic root extracts of *A. pycnantha*.

Table 2. Serum free testosterone concentrations in male Wistar rats treated with ethanolic root extracts of *A. pycnantha*

Group	Serum free testosterone concentration (mg/dL)
Control	22.00 ± 1.80
Standard (Yohimbine)	29.00 ± 2.20*
Low dose (50 mg/kg)	25.00 ± 2.00*
High dose (100 mg/kg)	31.00 ± 2.50**

*Values are expressed as mean ± standard error of the mean (SEM), n = 5 *$p < 0.05$ vs. control, **$p < 0.01$ vs. control

The data presented in Table 2 reveal that serum free testosterone concentrations were significantly higher in both the low and high-dose groups compared to the control, with the high-dose group showing the most pronounced increase. This elevation in testosterone is consistent with the observed improvement in sexual behaviour parameters. The significant increase in serum free testosterone concentrations in the treated groups supports the notion that *A. pycnantha* enhances sexual performance by elevating testosterone concentrations.

2.2. Serum Calcium Concentration

The results from Table 3 reveal the effects of ethanolic root extracts of *A. pycnantha* on serum calcium concentrations in male Wistar rats. The control group had a serum calcium concentration of 8.50 ± 0.60 mg/dL, establishing a baseline for comparison. In the standard (Yohimbine) group, serum calcium concentrations increased to 9.20 ± 0.70 mg/dL, a statistically significant change ($p < 0.05$) compared to the control. This indicates that Yohimbine, known for its aphrodisiac properties, has a notable impact on calcium concentration. The low dose of *A. pycnantha* extract (50 mg/kg) resulted in a serum calcium concentration of 8.90 ± 0.50 mg/dL. This increase is significant ($p < 0.05$) compared to the control group, suggesting that even a lower dose of the extract positively affects calcium metabolism. The high dose (100 mg/kg) led to the highest serum calcium concentration of 9.60 ± 0.80 mg/dL, which was significantly higher than the control ($p < 0.01$). This dose-dependent increase highlights the potent effect of the high dose of *A. pycnantha* extract on calcium concentrations.

Table 3. Serum calcium concentrations in male Wistar rats treated with ethanolic root extracts of *A. pycnantha*

Group	Serum calcium concentration (mg/dL)
Control	8.50 ± 0.60
Standard (Yohimbine)	9.20 ± 0.80*
Low dose (50 mg/kg)	8.90 ± 0.50*
High dose (100 mg/kg)	9.60 ± 0.80**

*Values are expressed as mean ± standard error of the mean (SEM), n = 5. *$p < 0.05$ vs. Control, **$p < 0.01$ vs. Control

3. Correlation between Serum Free Testosterone Concentrations and Sexual Behaviour Parameters

Table 4 shows the correlation between serum-free testosterone concentration and sexual behaviour indices in male Wistar rats.

Table 4. Correlation between serum free testosterone concentration and sexual behaviour parameters in male Wistar rats

Parameter	Mounting frequency	Intromission frequency	Erection duration	Latency period
Serum Free Testosterone concentration (ng/dL)	0.85	0.88	0.90	-0.80

Values closer to 1 or -1 indicate a stronger correlation, with positive values indicating a positive correlation and negative values indicating a negative correlation.

The correlation coefficient of 0.85 indicates a strong positive relationship between serum free testosterone concentration and mounting frequency. Higher testosterone concentrations are associated with an increased number of mounts, suggesting that testosterone plays a significant role in enhancing sexual arousal and motivation.

A correlation coefficient of 0.88 demonstrates a very strong positive correlation between testosterone concentration and intromission frequency. This indicates that elevated testosterone concentration corresponds to a higher frequency of successful intromissions, further supporting the role of testosterone in improving sexual performance.

The coefficient of 0.90 reflects a very strong positive correlation between serum free testosterone concentrations and erection duration. Higher testosterone concentrations are associated with longer erection times, suggesting that testosterone positively influences erectile function. The negative correlation coefficient of -0.80 indicates a strong negative relationship between testosterone concentration and latency period. Higher testosterone concentrations are associated with a shorter latency period, meaning that increased testosterone facilitates quicker initiation of sexual activity. The correlation between increased testosterone and improved sexual behaviour parameters suggests that the aphrodisiac effects of the extract may be mediated through its impact on hormone levels.

4. Correlation between Serum Calcium Concentrations and Sexual Behaviour Parameters

Table 5 shows the correlation between serum calcium concentrations and sexual behaviour parameters in male Wistar rats.

Table 5. Correlation between serum calcium concentration and sexual behaviour parameters in male Wistar rats

Parameter	Mounting frequency	Intromission frequency	Erection duration	Latency period
Calcium concentration	0.65	0.60	0.70	-0.55

Values represent Pearson correlation coefficients. Values closer to 1 or -1 indicate a stronger correlation, with positive values indicating a positive correlation and negative values indicating a negative correlation.

The positive correlation coefficient of 0.65 indicates a moderate to strong positive relationship between serum calcium concentrations and mounting frequency. This suggests that as serum calcium concentrations increase, the frequency of mounting behaviour also tends to increase in male Wistar rats. The positive correlation coefficient of 0.60 shows a moderate positive relationship between serum calcium concentrations and intromission frequency. Higher serum calcium concentrations are associated with an increase in the number of intromissions. The correlation coefficient of 0.70 indicates a strong positive relationship between serum calcium concentrations and erection duration. Higher calcium concentrations are associated with longer durations of penile erection. The negative correlation coefficient of -0.55 indicates a moderate inverse relationship between serum calcium concentrations and latency period. As serum calcium concentrations increase, the time taken to initiate sexual behaviour decreases.

Discussion

The present study explored the aphrodisiac activity of ethanolic root extracts of *A. pycnantha* (Golden Wattle) using male Wistar rats. The findings indicate that *A. pycnantha* extracts significantly enhance sexual behaviour parameters and elevate serum free testosterone concentrations, corroborating the potential aphrodisiac properties of this plant.

Effects of *A. pycnantha* Root Extract on Sexual Behaviour Parameters

The sexual behaviour assays revealed that both low and high doses of *A. pycnantha* root extract significantly improved sexual behaviour parameters. The high-dose group exhibited the most pronounced effects. The increase in mounting and intromission frequencies aligns with studies on other aphrodisiac plants. For instance, *Ginseng* and *Tribulus terrestris* have demonstrated similar effects in enhancing sexual motivation and performance through increased mounting and intromission frequencies (19, 20). The significant increase in erection duration observed in the extract-treated groups is consistent with findings from studies on aphrodisiac herbs such as *Withania somnifera* (Ashwagandha), which has been shown to improve erectile function and sexual performance in animal models (21, 22). The reduction in latency period supports findings from studies involving Cnidium monnieri fruit extract, which has been reported to shorten the time to initiate sexual activity in male rats (23).

Effects of *A. pycnantha* Root Extract on Testosterone Concentrations

The increase in serum free testosterone concentrations in the treated groups suggests that the aphrodisiac effects of *A. pycnantha* may be mediated through its impact on testosterone. This is consistent with research on other plant extracts known to elevate testosterone concentrations. For example, Fenugreek extract has been shown to increase serum testosterone and improve sexual behaviour parameters (24). Similarly, Panax ginseng has been associated with increased testosterone concentrations and improved sexual function (25). Testosterone is a key male sex hormone produced primarily in the testes, and it plays a significant role in regulating libido and sexual function (4, 26). In both men and women, testosterone concentrations can influence sexual desire, arousal, and overall sexual satisfaction (27).

Effects of *A. pycnantha* Root Extract on Calcium Concentrations

The increase in serum calcium concentrations in the treated groups indicates that *A. pycnantha* extract highlight another mechanism through which the plant may exert its aphrodisiac effects. These findings indicate a dose-dependent effect of *A. pycnantha* on serum calcium concentrations, with both the low and high doses resulting in significant increases compared to the control. The high dose of *A. pycnantha* extract produced the most substantial effect, suggesting that the plant extract's impact on calcium metabolism might be more pronounced at higher doses. Overall, the increase in serum calcium concentrations with *A. pycnantha* treatment is consistent with its potential as an aphrodisiac. Calcium plays a critical role in physiological processes related to erectile function and muscle contraction. In the smooth muscle cells of the blood vessels in the penis, calcium ions are crucial for regulating muscle contraction and relaxation (28, 29). Calcium aids penile erection by promoting smooth muscle contraction (30), while phosphodiesterase type 5 (PDE-5) inhibitors enhance this process by preventing the breakdown of cyclic GMP, which also relies on calcium signaling for its effects on smooth muscle relaxation (31). The observed elevation in calcium concentrations could contribute to the improved sexual behaviour parameters noted in this study.

Correlation between Testosterone Concentration and Sexual Behaviour Parameters

The correlation analysis demonstrated a strong positive relationship between testosterone concentration and sexual behaviour parameters. The positive correlations observed with mounting frequency and intromission frequency align with studies showing that elevated testosterone concentrations are associated with increased sexual motivation and activity (32). The strong positive correlation with erection duration supports studies indicating that higher testosterone concentrations enhance erectile function (33). The negative correlation between testosterone concentrations and latency period aligns with findings that increased testosterone facilitates the quicker initiation of sexual activity (34).

Correlation between Calcium Concentrations and Sexual Behaviour Parameters

The results in Table 5 reveal that serum calcium concentrations are positively correlated with several key sexual behaviour parameters, including mounting frequency, intromission frequency, and erection duration. This suggests that calcium plays a significant role in sexual function and performance (28). The negative correlation with the latency period further supports the idea that higher calcium concentrations may facilitate quicker sexual responses. These findings align with existing knowledge of the role of calcium in muscle function and neurotransmission (35).

Conclusion

The results of this study confirm that ethanolic root extracts of *A. pycnantha* exhibit significant aphrodisiac activity, as evidenced by improvements in sexual behaviour parameters and elevated serum free testosterone and calcium concentrations. These findings are consistent with other research on aphrodisiac plants and testosterone and calcium modulation. The dose-dependent effects of *A. pycnantha* accentuate its potential as a natural aphrodisiac.

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None declared.

Ethical statement

Ethical approval for this study and the use of animals was granted by the Directorate of Research and Quality Assurance, Federal University Otuoke, Bayelsa State, via a letter referenced DRQA/FUO/0110/21/05/24.

Data availability

None declared.

Conflict of interest

The authors have no conflict of interest in this study.

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Author contributions

All the authors contributed equally to various aspects of the study, including the design, methodology, procurement of materials and assay kits, processing of results, discussion, and final submission.

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