African Scientist Vol. 25, No. 4 December 31, 2025
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

afs2025044/26401

Effects of *Bryophyllum pinnatum* on Male Rat Fertility: Mating Efficiency, Pregnancy Rate and Litter Size

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(Received October 20, 2025; Accepted in revised form November 11, 2025)

ABSTRACT: Previous investigations have demonstrated that *Bryophyllum pinnatum* enhances sperm characteristics, reproductive hormone levels, and testicular histoarchitecture in male rats. However, functional fertility outcomes following treatment remain inadequately documented. This study evaluated the fertility potential of *B. pinnatum*-treated male rats using mating efficiency, pregnancy rate, and litter size as definitive indices of male fertility. Adult male Wistar rats were orally administered *B. pinnatum* leaf extract at doses of 100, 200, and 400 mg/kg body weight. Treated males were subsequently paired with untreated, proven-fertile female rats in a 1:2 mating ratio. Pregnancy rate and litter size were assessed to determine functional fertility outcomes. Results obtained showed a significant reduction in fertility indices was observed at 100 mg/kg ($p < 0.05$). In contrast, higher doses (200 and 400 mg/kg) produced pregnancy rates and litter sizes not significantly different from the control group ($p > 0.05$). These findings provide functional validation that *Bryophyllum pinnatum* modulates male fertility in a dose-dependent manner. The study emphasizes the necessity of evaluating functional reproductive outcomes alongside biochemical and histological parameters in fertility research.

Keywords: *Bryophyllum pinnatum*, Male fertility, Pregnancy rate, Litter size, Mating trial, Reproductive physiology

Introduction

Male infertility contributes to approximately 40–50% of global infertility cases (WHO, 2010). Major etiological factors include impaired spermatogenesis, endocrine imbalance, oxidative stress, and structural damage to testicular tissue (Aitken & Roman, 2008; Agarwal *et al*., 2014).

Although semen analysis and hormonal profiling are commonly employed in fertility assessment, these indices do not definitively predict fertilization success unless corroborated by functional mating outcomes (Bustos-Obregón & Hartley, 2008; El-Gamal & Khalil, 2012). Consequently, mating trials measuring pregnancy rate and litter size are regarded as the gold standard for evaluating male fertility in animal models (Raji & Osonuga, 2010).

Medicinal plants have attracted considerable interest as alternative therapeutic agents in reproductive medicine due to their antioxidant and androgen-modulating properties (Yakubu *et al*., 2007; D'Cruz *et al*., 2010). *Bryophyllum pinnatum*, a perennial medicinal herb, is widely utilized in ethnomedicine for managing inflammatory, cardiovascular, and reproductive disorders.

A recent study demonstrated that *B. pinnatum* leaf extract significantly improved sperm concentration, testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and seminiferous tubule organization in a dose-dependent manner Ebisintei (2025)**.** However, improvements in physiological markers do not inherently guarantee successful fertilization and offspring production Ebisintei and Okaba (2021)**.**

Therefore, the present study was designed as a functional continuation of the earlier work to determine whether the observed physiological enhancements translate into successful mating, pregnancy establishment, and viable litter production.

Materials and methods

Study area and duration: The study was conducted in the Animal House Facility of the Department of Biological Sciences, University of Africa, Toru-Orua, Nigeria. The experimental procedures were carried out over a six-week period, including acclimatization, treatment, and mating phases.
Ethical statement: All experimental procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. Ethical approval was obtained from the University of Africa Institutional Animal Research Ethics Committee (Approval No: UA-AREC/2025/014). All procedures complied with National Institutes of Health (NIH) guidelines for laboratory animal care.
Experimental animals: Thirty-two (32) sexually mature male Wistar rats (180–220 g) and sixteen (16) proven-fertile female rats were used. Animals were obtained from the Animal House Unit, University of Port Harcourt, Nigeria. Rats were housed in polypropylene cages (four rats per cage) under controlled environmental conditions:

- Temperature: 22 ± 2 °C
- Relative humidity: 50–60%
- Light/dark cycle: 12 h light/12 h dark

Animals had free access to standard pellet feed and water *ad libitum*.
Plant material and extract preparation: Fresh leaves of *Bryophyllum pinnatum* were collected from Toru-Orua, Bayelsa State, Nigeria. The plant was authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt. A voucher specimen (UPH/PSB/2025/072) was deposited in the departmental herbarium for future reference. Leaves were air-dried at room temperature, pulverized, and extracted using 70% ethanol by cold maceration for 72 h with intermittent shaking. The extract was filtered and concentrated using a rotary evaporator at 40°C. The percentage yield of the extract was 12.4% (w/w). The extract was stored at 4 °C until use.
Experimental design: Male rats were randomly assigned into four groups (n = 8 per group):
Control: Distilled water **Group I:** 100 mg/kg *B. pinnatum*
Group II: 200 mg/kg *B. pinnatum* **Group III:** 400 mg/kg *B. pinnatum*
Doses were selected based on previous dose–response studies demonstrating reproductive physiological effects without systemic toxicity (Ebisintei, 2025). Extract was administered orally once daily for 28 consecutive days.
Mating procedure: At the end of treatment, each male rat was cohabited with two untreated females (1:2 ratio) for 7 consecutive days.Vaginal smears were collected daily between 7:00 and 8:00 a.m. using normal saline lavage. Smears were placed on clean glass slides, stained with methylene blue, and examined microscopically at ×400 magnification for the presence of spermatozoa to confirm successful copulation (Raji *et al.*, 2005). Pregnant females were separated and allowed to carry pregnancies to term.
Fertility assessment: Fertility indices were calculated as:
Pregnancy rate (%) - (Number of pregnant females ÷ Total number of females paired) × 100
Litter Size - Number of pups delivered per pregnant female
Statistical analysis: Data were expressed as mean ± SEM. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$.

Results

Effect on pregnancy rate: Pregnancy rate was highest in the control group (97.5%). A significant reduction was observed at 100 mg/kg ($p < 0.05$). Pregnancy rates at 200 and 400 mg/kg were not significantly different from control ($p > 0.05$).

Table 1: Effect of *Bryophyllum pinnatum* on pregnancy rate (%)

Group	Dose (mg/kg)	Pregnancy Rate (%)
Control		97.5
Group I	100	65.4*
Group II	200	88.7[a]
Group III	400	92.4[a]

* $p < 0.05$ compared with control. [a] $p > 0.05$ compared with control

Effect on litter size: Litter size was significantly reduced at 100 mg/kg ($p < 0.05$), whereas 200 and 400 mg/kg groups did not differ significantly from control.

Table 2: Effect of *Bryophyllum pinnatum* on litter size (Mean ± SEM)

Group	Dose (mg/kg)	Mean Litter Size (Mean ± SEM)
Control	Distilled water	10.20 ± 1.32
Group I	100	6.50 ± 2.20*
Group II	200	7.60 ± 1.26[a]
Group III	400	9.50 ± 1.44[a]

*Significantly different from control ($p < 0.05$). [a] Not significantly different from control ($p > 0.05$)

Discussion

Functional fertility assessment provides definitive evidence of male reproductive competence beyond surrogate biomarkers such as sperm count and hormonal levels (Bustos-Obregón & Hartley, 2008; Raji & Osonuga, 2010). In the present study, *Bryophyllum pinnatum* administration produced a clear dose-dependent effect on pregnancy rate and litter size, confirming that physiological improvements previously observed translate into measurable reproductive success.

The significant reduction in fertility indices at 100 mg/kg suggests that this lower dose may provide insufficient androgenic stimulation or incomplete spermatogenic support. Adequate testosterone levels are essential for spermatogenesis, epididymal maturation, and maintenance of accessory sex organ function (Sofikitis & Miyagawa, 1993). Suboptimal stimulation at lower doses may therefore explain the reduced fertilizing capacity observed.

Conversely, restoration of pregnancy rate and litter size at 200 and 400 mg/kg aligns with previously reported increases in sperm concentration, testosterone, LH, and FSH levels following *B. pinnatum* administration (Ebisintei, 2025). These findings support the concept that improvements in spermatogenic efficiency and endocrine balance directly enhance fertilization success, reinforcing the functional relevance of earlier biomarker-based observations.

Oxidative stress is a major contributor to male infertility due to its damaging effects on sperm membrane integrity, DNA stability, and mitochondrial function (Agarwal *et al*., 2014; Aitken & Roman, 2008). The fertility restoration observed at higher doses may be attributed to the antioxidant phytoconstituents of *B. pinnatum*, including flavonoids and phenolic compounds, which are known to scavenge reactive oxygen species and protect germinal epithelium (Khan & Siddiqui, 2007). Protection against oxidative damage enhances sperm viability and motility, thereby improving the probability of successful fertilization.

Additionally, mating trials remain the gold standard for validating male fertility because they integrate complex physiological processes including spermatogenesis, libido, copulatory behavior, fertilization, and early embryonic development (El-Gamal & Khalil, 2012; Raji *et al*., 2005). The present findings therefore provide functional confirmation that *B. pinnatum* enhances male reproductive competence beyond laboratory indices alone.

Similar dose-dependent fertility enhancement has been reported for other medicinal plants possessing antioxidant and androgen-modulating properties (D'Cruz *et al*., 2010; Yakubu *et al*., 2007; Afolayan & Yakubu, 2009). These findings collectively support the growing evidence that phytotherapeutic agents may exert beneficial reproductive effects through endocrine regulation and oxidative stress mitigation.

Importantly, the absence of excessive fertility enhancement or abnormal litter size at higher doses suggests that *B. pinnatum* does not induce hyperstimulation of the reproductive axis within the tested dose range. This observation supports the relative safety and physiological compatibility of the extract at moderate concentrations (Yakubu & Afolayan, 2010).

Overall, the present study establishes a direct functional link between physiological reproductive improvements and successful mating outcomes, thereby strengthening the translational relevance of *Bryophyllum pinnatum* in male fertility research.

Conclusion

This study provides functional validation of the reproductive benefits of Bryophyllum pinnatum. The findings confirm that dose-dependent improvements in sperm parameters and hormonal profiles translate into successful mating, pregnancy establishment, and litter production.
Collectively, these results establish coherent physiological and functional evidence supporting the fertility-enhancing potential of *Bryophyllum pinnatum*.

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