

Effects of Aqueous Leaf Extracts of *Irvingia Wombolu* and *Irvingia Gabonensis* on Sperm Parameters in Male Wistar Rats

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Abstract

Male infertility is a prevalent global concern, underscoring the need for accessible alternative treatments. Medicinal plants like *Irvingia gabonensis* and the less-studied *Irvingia wombolu* are used in traditional medicine for reproductive health, but scientific validation of their efficacy is limited. This study investigated the effects of aqueous leaf extracts of *Irvingia wombolu* and *Irvingia gabonensis* on sperm parameters in male Wistar rats. Thirty rats were randomly divided into five groups (n=6). Group I (control) received distilled water, while Groups II and III received *Irvingia wombolu* extract at 200mg/kg and 400mg/kg, respectively. Groups IV and V received *Irvingia gabonensis* extract at 200mg/kg and 400mg/kg, respectively. All treatments were administered orally for 28 days. Sperm count, motility and morphology were analysed from the cauda epididymis. Data were expressed as mean $\pm$ SD and analysed using one-way ANOVA $p < 0.05$. Both extracts induced significant, dose-dependent improvements in all sperm parameters. The 400 mg/kg dose of *Irvingia gabonensis* produced the highest sperm count ($32.44 \pm 1.73 \times 10^6$) compared to the control ($26.21 \pm 2.12 \times 10^6$). Morphological abnormalities (head, neck, tail, cytoplasmic) were significantly reduced in all treated groups. Motility was markedly enhanced, with the percentage of rapid progressive sperm increasing to 84.86% in the *Irvingia gabonensis* 400mg/kg group, compared to 52.03% in the control. Histological examination revealed normal testicular architecture across all groups. Aqueous leaf extracts of *Irvingia wombolu* and *Irvingia gabonensis* significantly improved sperm quality in rats, supporting their traditional use and suggesting their potential as natural adjuvants for managing male infertility

Keywords: infertility, Sperm count, motility, morphology, *Irvingia wombolu*, *Irvingia gabonensis*

Introduction

Infertility is a major global health issue, impacting an estimated 15% of couples globally. Male factor infertility is implicated in close to 50% of these instances (Babakhanzadeh *et al.*, 2020). Crucial indicators of male fertility include sperm count, morphology and motility, which are essential metrics for evaluating the efficacy of spermatogenesis, the quality of sperm and their

capacity to achieve fertilization (Tanga *et al.*, 2021). Impairments in these critical sperm parameters can significantly diminish male reproductive potential (Salas-Huetos *et al.*, 2021). While assisted reproductive technologies (ART) offer solutions for many couples, their frequent high cost, invasive nature and limited availability in underserved regions underscore the need for more accessible and natural treatment options (Kalra, 2024). This has spurred significant research into affordable alternatives, particularly the therapeutic application of medicinal plants (Kalra, 2024).

The application of plant-based therapies from traditional medicine has a longstanding history in the treatment of fertility issues across diverse cultures (Singh, 2024). Within sub-Saharan Africa, numerous botanicals are employed in folk practices to improve male reproductive health, yet scientific evidence for their efficacy is often limited (Shai *et al.*, 2022). *Irvingia wombolu* and *Irvingia gabonensis*, two phylogenetically similar species from the Irvingiaceae family, are notable examples (Nguena-Dongue *et al.*, 2023). Indigenous to tropical Africa, these trees are commonly referred to as “bush mango” or “African wild mango” and are prized for their nutritional fruit and seeds, as well as their dense phytochemical profile and prospective health applications (Adeoye *et al.*, 2023).

Scientific research has focused more extensively on *Irvingia gabonensis* than on *Irvingia wombolu*, especially concerning its anti-diabetic, hypolipidemic, antioxidant and anti-inflammatory activities (Mgbemena *et al.*, 2019). The seeds of this species, known as “ogbono,” contain a high concentration of essential fatty acids, fiber, flavonoids and polyphenols (Adeoye *et al.*, 2023). These phytoconstituents are potentially responsible for offering protection against oxidative stress, a key pathogenic factor in male infertility (Uddandrao *et al.*, 2024).

Consequently, the antioxidant compounds present in *Irvingia* species could potentially aid in the restoration of testicular function and provide a defense against damage to sperm cells (Lopes-Ferreira *et al.*, 2025). Although *Irvingia gabonensis* has demonstrated positive impacts on blood glucose control and lipid metabolism, its specific effects on male fertility, particularly on semen quality metrics, have not been thoroughly investigated (Anake, 2023).

In contrast to *I. gabonensis*, *Irvingia wombolu* is less extensively researched and cultivated, despite their close botanical relationship (Olutumise *et al.*, 2023). It is characterized by possessing more bitter fruits and harder seeds, which are similarly employed in traditional medicine for diverse therapeutic applications (Okakpu *et al.*, 2025). Existing reports indicate it may have comparable pharmacological and phytochemical properties to *I. gabonensis*; however, there is a scarcity of scientific data validating its efficacy for reproductive health (Nguena-Dongue *et al.*, 2023). Furthermore, while the seeds of these species have been the focus of most studies, the leaves remain largely uninvestigated for their potential impacts on male reproductive parameters (Nguena-Dongue *et al.*, 2023).

Given the increasing prevalence of male infertility and the demand for alternative treatments, there is a pressing need for empirical studies that examine the potential reproductive health benefits of these indigenous plant species.

Materials and Methods

Collection and Identification of Plant Materials

A large quantity of fresh leaves of *Irvingia gabonensis* were collected from Aba in Osisioma L.G.A., Abia State, Nigeria. Leaves of *Irvingia wombolu* were collected from Amassoma town, Southern Ijaw L.G.A., Bayelsa state, Nigeria. The plants were identified by Prof. Kola Ajibesin of

the Department of Pharmacognosy, Niger Delta University. Voucher specimens were deposited for future reference.

Preparation of Plant Extracts

The collected leaves were thoroughly washed, air-dried under shade at room temperature for two weeks. Afterwards, they were ground into fine powder. 500g of the powder was soaked in 2 litres of distilled water and allowed to stand for 72 hours with intermittent stirring. The mixture was filtered with cheese cloth and the filtrate was evaporated in a water bath at 50°C to obtain the aqueous extracts.

Experimental animals and Ethical Consideration

This study utilized thirty adult male Wistar rats weighing 150-200g. The Research Ethical Committee of the College of Health Sciences at Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, granted ethical approval for all experimental procedures.

Experimental Design and Treatment

The rats were randomly divided into five groups as follows

Group I (Control): (distilled water)

Group II: Received 200 mg/kg of *Irvingia wombolu* extract

Group III: Received 400 mg/kg of *Irvingia wombolu* extract

Group IV: Received 200 mg/kg of *Irvingia gabonensis* extract

Group V: Received 400 mg/kg of *Irvingia gabonensis* extract

All treatments were administered orally using gavage over a period of 28 consecutive days. The dosage levels were chosen in accordance with prior research and preliminary toxicity evaluations, which confirmed their safety at the concentrations used.

Upon completion of the 28-day period, all animals were humanely euthanized under Isoflurane. Sperm samples were subsequently collected from the cauda epididymis for all conducted analyses.

Sperm Analysis

Determination of Sperm motility

The right cauda epididymis of each rat was incised to collect semen onto a pre-warmed glass slide. Two drops of warmed 2.9% sodium citrate solution were added to the semen sample, which was then covered with a coverslip. Sperm motility percentage was evaluated by visual examination at $\times 40$ magnification. Three distinct fields per sample were assessed for motility, and the mean value of these three readings was recorded as the final motility score, following the procedure outlined by Bearden and Fuquay (2000)

Four Grades of Sperm Motility

The percentage was calculated by assessing 100 randomly selected sperm cells.

Grade A: Motility Grade IV or Rapid Progressive Motility: This represents the strongest movement category, characterized by sperm cells swimming rapidly in a straight line.

Grade B: Motility Grade III or Slow/Sluggish Progressive Motility: Sperm in this category exhibit forward movement but at a slower pace and in a non-linear, curved, or crooked path.

Grade C: Motility Grade II or Non-Progressive Motility: Sperm in this category display tail movement but lack forward progression.

Grade D: Motility Grade I or Immotility: This category consists of sperm that are completely stationary and exhibit no movement whatsoever.

Determination of Sperm Count

An incision was made in the left cauda epididymis of each rat, and the exuding semen was immediately collected into a red blood pipette to the 0.5 mark. The semen was then diluted with warm normal saline to the 101 mark. Approximately 10 μ L of this mixture was transferred to a Neubauer hemocytometer chamber and examined under a microscope at $\times 40$ magnification.

Determination of Sperm Morphology

The eosin-nigrosin staining technique, as previously outlined, was employed to assess morphologically abnormal spermatozoa. Slide examination was conducted using a light microscope at 100x magnification. For each slide, 200 sperm cells were evaluated, and the percentage of abnormalities in the head, tail, and overall structure was calculated and expressed.

Sperm Morphology Defects

Four distinct grades of sperm defects were calculated by examining 50 randomly selected deformed cells

GROUP A: Head defect

GROUP B: Neck defect

GROUP C: Tail defect

GROUP D: Cytoplasmic defect

Statistical Analysis

The results were subjected to statistical analysis, and all values were presented as the mean $\pm$ standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). Statistical significance was considered at a p-value less than 0.05 ($p < 0.05$).

Results

Table 1. Mean sperm count of controls and normal rats administered with aqueous extracts of *Irvingia wombolu* and *Irvingia gabonensis* leaves for 28 days.

TREATMENT GROUP	CONTROL	Irvingia wombolu (200 mg/kg) (200 mg/kg))	Irvingia wombolu (400mg/kg) (400mg/kg)	Irvingia gabonensis (200mg/kg) (200mg/kg)	Irvingia gabonensis (400 mg/kg)
SPERM COUNT ($\times 10^6$)	26.21 $\pm$ 2.12 ^a	29.48 $\pm$ 1.98 ^b	31.89 $\pm$ 2.08 ^c	28.66 $\pm$ 1.53 ^b	32.44 $\pm$ 1.73 ^c

Data are expressed as the mean $\pm$ SD (n=6). Mean values within a row with different superscript letters are significantly different ($p < 0.05$)

Table 2. Mean sperm morphological abnormality count (%) of controls and normal rats administered with aqueous extracts of *Irvingia wombolu* and *Irvingia gabonensis* leaves for 28 days.

GRADE	CONTROL	<i>Irvingia wombolu</i> (200 mg/kg)	<i>Irvingia wombolu</i> (400 mg/kg)	<i>Irvingia gabonensis</i> (200 mg/kg)	<i>Irvingia gabonensis</i> (400 mg/kg)
Head defects	26.22 $\pm$ 3.12 ^a	21.56 $\pm$ 1.95 ^b	17.92 $\pm$ 1.99 ^c	22.45 $\pm$ 2.04 ^b	17.82 $\pm$ 2.01 ^c
Neck defects	23.93 $\pm$ 2.18 ^a	20.56 $\pm$ 6.01 ^b	16.72 $\pm$ 1.32 ^c	21.08 $\pm$ 3.18 ^b	18.12 $\pm$ 2.12 ^c
Tail defects	24.23 $\pm$ 2.31 ^a	20.94 $\pm$ 2.88 ^b	18.24 $\pm$ 1.96 ^c	20.87 $\pm$ 1.79 ^b	16.98 $\pm$ 2.67 ^c
Cytoplasmic defects	24.82 $\pm$ 2.07 ^a	22.18 $\pm$ 1.48 ^b	16.38 $\pm$ 1.88 ^c	20.26 $\pm$ 2.42 ^b	17.21 $\pm$ 2.03 ^c

Data are expressed as the mean $\pm$ SD (n=6). Mean values within a row with different superscript letters are significantly different ($p < 0.05$)

Table 3. Mean sperm motility assay (%) of controls and normal rats administered with aqueous extracts of *Irvingia wombolu* and *Irvingia gabonensis* leaves for 28 days.

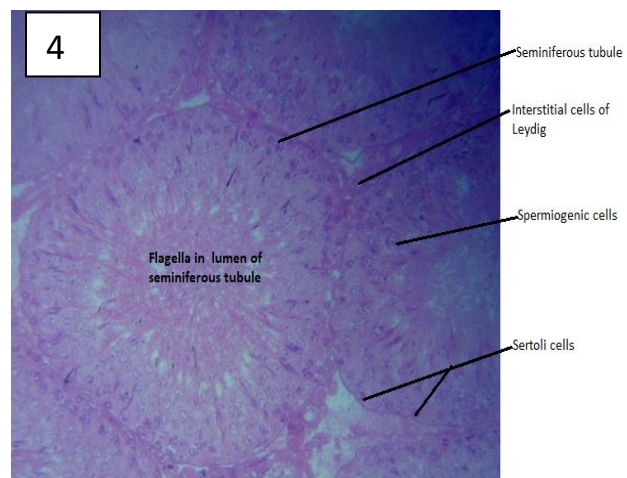
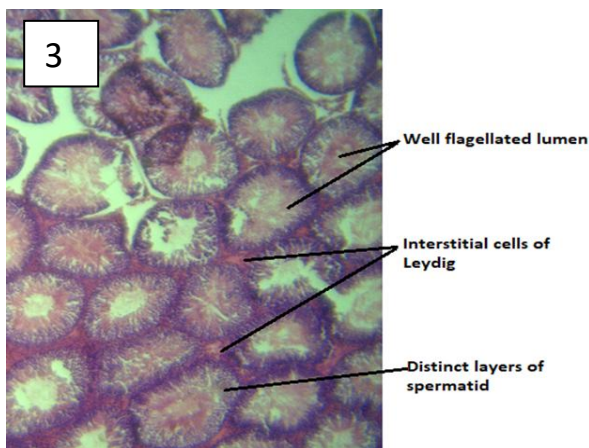
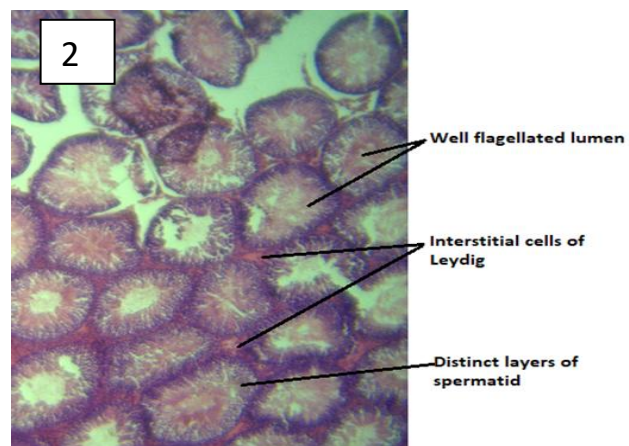
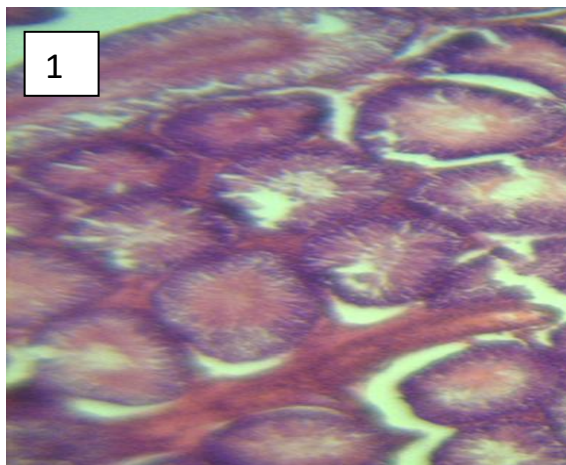
Type of Motility	CONTROL	<i>Irvingia wombolu</i> (200 mg/kg)	<i>Irvingia wombolu</i> (400 mg/kg)	<i>Irvingia gabonensis</i> (200 mg/kg)	<i>Irvingia gabonensis</i> (400 mg/kg)
Rapid progressive	52.03 $\pm$ 2.86 ^a	71.67 $\pm$ 4.89 ^b	84.34 $\pm$ 4.06 ^c	70.18 $\pm$ 3.12 ^b	84.86 $\pm$ 3.79 ^c
Slow/sluggish	21.96 $\pm$ 2.12 ^a	16.77 $\pm$ 1.86 ^b	13.85 $\pm$ 1.23 ^c	17.05 $\pm$ 1.88 ^b	13.18 $\pm$ 1.08 ^c
Non-progressive	22.54 $\pm$ 2.21 ^a	15.87 $\pm$ 2.14 ^b	11.87 $\pm$ 2.08 ^c	14.87 $\pm$ 1.03 ^b	12.05 $\pm$ 1.28 ^c
Immobile cells	10.45 $\pm$ 2.88 ^a	5.06 $\pm$ 1.22 ^b	4.82 $\pm$ 0.74 ^c	4.97 $\pm$ 1.45 ^b	4.55 $\pm$ 0.92 ^c

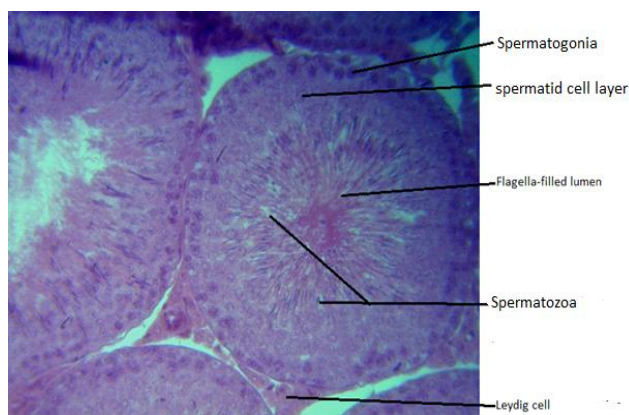
Data are expressed as the mean $\pm$ SD (n=6). Mean values within a row with different superscript letters are significantly different ($p < 0.05$)

Histology of Testes Tissue

Histological sections of testes tissue from control and experimental rats were stained with hematoxylin and eosin (H&E) and examined at 100x magnification administered with aqueous extracts of *Irvingia wombolu* and *Irvingia gabonensis* leaves for 28 days.

Plate 1 (Normal Control): Photomicrograph of testes tissue showing spermatid cell layers, flagellated lumen of seminiferous tubules and interstitial cells of Leydig. Tissue shows normal microstructure. **Plate 2 (Received 200 mg/kg of *Irvingia wombolu* extract):** Photomicrograph of testes tissue showing pockets normal spermatid cell layers and well flagellated lumens of seminiferous tubules. The interstitial cells of Leydig appear normal. Tissue shows normal microstructure. **Plate 3 (Received 400 mg/kg of *Irvingia wombolu* extract):** Photomicrograph of testes tissue showing spermatid cell layers, flagellated lumen of seminiferous tubules and interstitial cells of Leydig. Tissue shows normal microstructure. **Plate 4 (Received 200 mg/kg of *Irvingia gabonensis* extract):** Photomicrograph of testes tissue showing pockets normal spermatid cell layers and well flagellated lumens of seminiferous tubules. The interstitial cells of Leydig appear normal. Tissue shows normal microstructure. **Plate 5 (Received 400 mg/kg of *Irvingia gabonensis* extract):** Photomicrograph of testes tissue showing spermatid cell layers, flagellated lumen of seminiferous tubules and interstitial cells of Leydig. Tissue shows normal microstructure.





Discussion

The present study evaluated the effects of 28-day oral administration of aqueous leaf extracts of *Irvingia wombolu* and *Irvingia gabonensis* on sperm characteristics in male Wistar rats. The key parameters assessed included sperm count, sperm morphology and sperm motility. Across all parameters, both extracts produced significant, dose-dependent improvements compared to the control, with *Irvingia gabonensis* at 400mg/kg generally demonstrating the most pronounced effects.

Administration of both *Irvingia wombolu* and *Irvingia gabonensis* led to significant increases in sperm count relative to the control group. The increase was dose-dependent, with the highest sperm count ($32.44 \pm 1.73 \times 10^6$) observed in rats treated with *I. gabonensis* at 400 mg/kg. This suggests that the phytochemicals present in these plant extracts may stimulate spermatogenesis or enhance testicular function. The observed effects could be attributed to antioxidant and androgenic constituents such as flavonoids, alkaloids and phenolic compounds, which have been reported in both species (Nguena-Dongue *et al.*, 2023; Omale *et al.*, 2024). These compounds may protect testicular cells from oxidative damage and modulate hormonal pathways involved in spermatogenesis (Lopes-Ferreira *et al.*, 2025).

Sperm morphology is a critical determinant of male fertility, and morphological abnormalities often reflect underlying testicular or systemic dysfunction. The control group exhibited high levels of morphological abnormalities across all categories (head, neck, tail and cytoplasmic defects), whereas both plant extracts significantly reduced these abnormalities in a dose-dependent manner. The most notable reductions were seen with the 400 mg/kg doses of both extracts. For example, head defects decreased from 26.22% in the control to 17.92% and 17.82% for *I. wombolu* and *I. gabonensis*, respectively. This improvement in morphology is likely tied to the antioxidant properties of the extracts, which protect developing sperm from oxidative damage (Omale *et al.*, 2024).

Sperm motility is essential for successful fertilization and the current findings demonstrate that *Irvingia* extracts significantly enhance this parameter. Rapid progressive motility increased markedly in the treated groups, reaching up to 84.86% in the *I. gabonensis* 400 mg/kg group. Concurrently, slow, non-progressive, and immotile sperm counts declined significantly. These improvements may be due to enhanced mitochondrial function and energy availability within

sperm cells, facilitated by the bioactive constituents of the extracts. Compounds such as saponins and sterols, found in *Irvingia* species, have been linked to improved mitochondrial activity and sperm kinetics (Nguena-Dongue *et al.*, 2023; Shai *et al.*, 2022).

The reduction in immotile sperm (from 10.45% in controls to as low as 4.55%) further reinforces the potential of these extracts to counteract subfertility by preserving sperm vitality. Notably, the effects of both *Irvingia* species were comparable at equivalent doses, though *I. gabonensis* often showed slightly superior outcomes.

The dose-dependent nature of the effects observed suggests that the phytochemical constituents may act through mechanisms such as antioxidant activity, reducing reactive oxygen species (ROS) that damage sperm DNA and membranes, hormonal modulation, particularly enhancing testosterone levels, which directly support spermatogenesis and improved testicular histoarchitecture, promoting better Sertoli and Leydig cell function, as shown in related studies using other medicinal plants (Kaltsas, 2023).

Histological results indicate that neither extract of *Irvingia wombolu* nor *Irvingia gabonensis*, at the doses tested (200 mg/kg and 400 mg/kg), had any adverse (toxic) effect on the microstructure of the testes.

These findings are consistent with earlier reports on the fertility-enhancing potential of tropical medicinal plants, such as *Moringa oleifera* and *Garcinia kola*, which also exhibit antioxidant and androgenic activities (Shai *et al.*, 2022; Mbouché *et al.*, 2025). The current results not only align with those reports but also highlight the relative potency of *Irvingia* species, which are less commonly studied in this context.

Conclusion

The findings from this study provide strong evidence that aqueous extracts of *Irvingia wombolu* and *Irvingia gabonensis* significantly enhance male reproductive parameters in rats. The extracts improved sperm count, reduced morphological defects and increased sperm motility in a dose-dependent manner. These effects are likely mediated through antioxidative and androgenic mechanisms. The data support the traditional use of *Irvingia* species in fertility enhancement and suggest their potential as candidates for natural male fertility therapies.

Conflict of Interest

There is no conflict of interest.

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