

The Therapeutic Effect of *Picralima nitida* Aqueous Fruit Extract on Hormonal Markers of Infertility in Adult Female Albino Rats

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Abstract

Hormonal imbalance remains a leading cause of infertility, especially in low-income countries. Reproductive health is a comprehensive concept that encompasses the entire well-being of individuals in relation to all aspects of the reproductive system at all stages of life. This study evaluated the phytochemical profile and effect of *P. nitida* aqueous fruit extract on reproductive hormones in female albino rats and compared it with the contraceptive Levonorgestrel/Ethinylestradiol (Levofem®). Twenty (20) adult female albino rats were divided into five (5) groups: Control, *P. nitida* extract at 100, 200, and 300 mg/kg and LNG/EE 0.005 mg/kg, for twenty-eight (28) days. Phytochemicals were quantified spectrophotometrically and via HPLC. Serum estradiol, progesterone, and prolactin were assayed by Fluorescence Immunoassay. Data were analyzed using ANOVA at $p < 0.05$. Saponins (0.695 mg/g) and glycosides (0.509 mg/g) were the most abundant phytochemicals. HPLC revealed ferulic acid (146.43 mg/L), salicylic acid (84.16 mg/L), and saponins (75.13 mg/L) as the most abundant bioactive compounds. The extract caused significant, dose-dependent changes in hormone levels. At 300 mg/kg, prolactin decreased significantly compared to control, while progesterone and estradiol increased, showing effects distinct from LNG/EE which suppressed progesterone. *P. nitida* aqueous fruit extract exhibited a fertility-supportive hormonal profile by reducing prolactin and enhancing progesterone and estradiol levels. This suggests the plant's extract has potential for managing infertility-related hormonal imbalance.

Keywords: *Picralima nitida*, Infertility, Progesterone, Estradiol, Prolactin, Phytochemical.

Introduction

Picralima nitida, popularly known as "Akuamma," is a prominent West African medicinal plant belonging to the *Apocynaceae* family. Indigenous to regions spanning from Nigeria to Uganda, the plant has long been a cornerstone of ethnomedicine, utilized for its analgesic, antimalarial, and antimicrobial properties (Adeyemi *et al.*, 2021). While the seeds are most recognized for their high indole alkaloid content, specifically akuammine and pseudoakuammigine, recent pharmacological interest has shifted toward exploring the

plant's broader systemic effects, including its role in reproductive health (De Witte *et al.*, 2016; Olajide *et al.*, 2022).

Reproductive health remains a global priority, particularly in low and middle-income countries where access to safe and effective fertility management is often hindered by socio-economic and cultural barriers (WHO, 2025; Pillai & Maleku, 2023). While synthetic hormonal contraceptives and fertility drugs are widely available, they are frequently associated with adverse effects ranging from pelvic inflammatory diseases to systemic hormonal imbalances (Pillai & Maleku, 2023; Teal & Edelman, 2021). Consequently, there has been a growing demand for plant-based alternatives that offer therapeutic efficacy with fewer to no side effects. Therefore, comparing the plant's effects with standard contraceptive would provide a clearer basis for its potential therapeutic use.

The endocrine regulation of the female reproductive system depends on a delicate balance within the Hypothalamic-Pituitary-Ovarian (HPO) axis, where hormones such as Estrogen, Progesterone, and Prolactin dictate fertility outcomes. However, there is a significant gap in the scientific literature regarding the specific impact of the aqueous fruit extract on reproductive markers, hence, the need for this research.

Statement of the Problem

Despite the widespread traditional use of *P. nitida*, its specific effects on the hormonal markers of infertility remain largely unvalidated. Current birth control methods and fertility treatments often carry risks of uterine perforation, allergic reactions, and long-term endocrine disruption (Woods, 2016). This calls for an urgent need to investigate whether *P. nitida* could serve as a potential natural fertility enhancer or a botanical contraceptive agent by evaluating its impact on key reproductive hormones.

Conceptual Framework

The fertility-supportive potential of *P. nitida* aqueous fruit extract is proposed through modulation of the Hypothalamic-Pituitary-Ovarian (HPO) axis. Phytochemicals identified in the extract, particularly saponins, phenolic acids including ferulic acid and salicylic acid, and flavonoids, are reported to interact with hypothalamic gonadotropin-releasing hormone (GnRH) neurons and receptors (Balkrishna *et al.*, 2025). This interaction is hypothesized to up-regulate ovarian synthesis of estradiol and progesterone while suppressing prolactin secretion from lactotroph cells. Consequently, the extract is expected to produce a hormonal profile that supports follicular development and luteal function, contrasting with the suppressive effect of LNG/EE on endogenous progesterone.

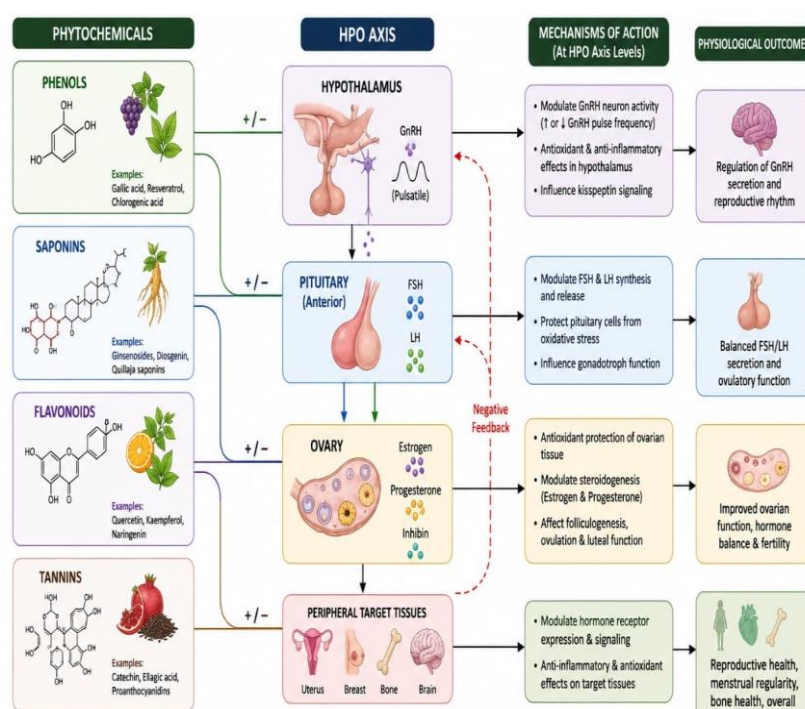


Figure 1: Illustration of the proposed mechanistic pathway linking phytochemical classes to HPO axis targets and downstream hormonal outcomes.

Hypothesis

We hypothesized that the aqueous fruit extract of *P. nitida* would modulate HPO axis activity by producing hormonal effects either comparable or contrasting with LNG/EE contraceptive action.

Aim and Objectives

The primary aim of this study is to investigate the therapeutic effects of *P. nitida* aqueous fruit extract on hormonal markers of infertility in adult female albino rats. The specific objectives are to:

1. Quantify the phytochemical and bioactive compounds in the extract using Spectrophotometer and HPLC.
2. Assess the impact of the extract on serum levels of Prolactin, Progesterone, and Estrogen.
3. Compare the efficacy of the extract against a standard hormonal birth control (LNG/EE).

Materials and Methods

Materials

Chemicals and Equipment

Reagents/chemicals

All reagents used for this research were of analytical grade. These reagents include; Ethanol, Hydrochloric acid (HCl), Dragendorff's reagent, Lead ethanoate solution, Acetic anhydride, concentrated Tetraoxosulphate (vi) acid (H_2SO_4), Ferric acid solution, Fehling's solution A & B, Acetic acid, Formaldehyde, Ethyl acetate, Dilute Ammonia (NH_3), Potassium ferricyanide, Petroleum ether, Chromogen solution, Folin-ciocalteu reagent, Sodium carbonate (iv), Lead acetate, Chloroform, Glacial acetic acid.

Apparatus/Equipment

The following materials were used for this research; Manual Weighing Balance, Batch Bottles, Distilled Water, Whatman Filter Papers, Rotary Evaporator, Spectrophotometer, HPLC, LNG/EE, Hormonal Assay Kit, Surgical Knife, Plain Bottles, Food Dehydrator, Blender, Beaker, Refrigerator.

Experimental Animal

Twenty (20) Adult female and five (5) male albino rats were obtained from B.S.U, Makurdi and bred in the animal house of the Department of Biochemistry, Nasarawa State University and were used for the experiment. They were maintained under standard conditions where they were fed with rat pellets and were allowed unrestricted access to water. The animals were acclimatized for two (2) weeks before the experiment and were subjected to hormonal assay to affirm fertility before treatment. All experimental procedures involving the animals were conducted in accordance with established institutional ethical guidelines for the care and use of laboratory animals.

Plant Material

The fruit of the plant was bought in late May from the Ultra-Modern New Market, Borikiri, Port Harcourt Local Government Area of Rivers State. It was identified by Josiah C. Onovo MACSS, MGSN (Professor of Cytogenetics and Plant Breeding), Nasarawa State University, Keffi, Nasarawa State.



Figure 2: Image showing the *Picralima nitida* fruits, developing on its tree.

Source: Field Survey.



Figure 3: Image showing mature and ripe *Picralima nitida* fruits.

Source: Field Survey.

Methods

Ethical Clearance/Consent

Ethical Clearance Approval Letter was issued by the Nasarawa State University, Keffi (NSUK) Animal Care and Use Research Ethics Committee (NSUK-ACUREC), Directorate of Research, Innovation and Enterprise, Nasarawa State University, Keffi (Approval No: NSUK-ACUREC/BCH/25/02 – 29-05-2025). All experimental procedures were conducted in accordance with established guidelines for the care and use of rat models for the experiment, ensuring humane handling and minimal suffering throughout the study.

Collection and Extraction of Sample

The fruits of *P. nitida* were bought in late May from the Ultra-Modern New Market, Borikiri, Port Harcourt Local Government Area of Rivers State. It was identified and authenticated by Josiah C. Onovo ^{MACSS, MGSN} (Professor of Cytogenetics and Plant Breeding), Nasarawa State University, Keffi, Nasarawa State. The fruits were sliced, diced, macerated and dried using an automated food dehydrator (Model: F9770-AC220-240V 50/60Hz 350W) at 50°C, after which it was grinded using an automatic blender (AKAI blender). 50g of the grinded sample was weighed using a manual weighing balance (Scout Pro SPU401) and put in a 1000ml beaker. 1000ml distilled water was added to the sample and soaked for 72hrs which was intermittently shaken, stirred at 12hrs intervals and was stored in the refrigerator at 18°C. The sample was filtered using Whatman filter papers and further subjected for extraction using a rotary evaporator (Pyrex Quick-fit RE 200/RC) to obtain a concentrated yield.

Experimental Design

Both qualitative, quantitative and HPLC analyses were carried out to characterize the samples:

Qualitative Phytochemical Screening

Qualitative analysis of bioactive constituents was performed using standard procedures as described by Balamurugan *et al.*, (2019). Tests carried out included:

- i. **Test for Alkaloids:** 0.1g of the extract was boiled with 5ml of 1% aqueous HCl on a steam bath. The mixture was filtered and 1ml of the filtrate was treated with 2 drops of Dragendorff's reagent.
- ii. **Test for Flavonoids:** 0.1g of the extract was dissolved in water and filtered. 3ml of lead ethanoate solution was added to 1ml of the filtrate.
- iii. **Test for Terpenoids:** 0.1g of the extract was dissolved in ethanol. 1ml of Acetic anhydride was added, followed by the addition of concentrated H₂SO₄.
- iv. **Test for Tannins:** 0.1g of the extract was stirred with 10ml of distilled water and then filtered. 4 drops of 1% ferric chloride solution was added to 2ml of the filtrate.
- v. **Test for Saponins:** 0.1g of the extract was boiled with 5ml of distilled water and filtered. 3ml of distilled water was further added to the filtrate and shaken vigorously for about 5 minutes.
- vi. **Test for Phenols:** 0.1g of the extract was boiled with distilled water and then filtered. 4 drops of 10% ferric chloride solution was added to 2ml of the filtrate.
- vii. **Test for Glycosides:** 0.1g of the extract was mixed with 30ml of distilled water and heated on a water bath for 5 minutes. 0.2ml of Fehling's solution A & B was added to 5ml of the filtrate and it turned alkaline. The solution was heated on a water bath for 2 minutes.

- viii. **Test for Steroids:** 0.1g of the extract was mixed with 2ml of acetic acid. The solution was allowed to cool in the freezer for 5 minutes, followed by the careful addition of concentrated H_2SO_4 .

Quantitative Analysis of the Plant Extract

- i. **Test for Alkaloids:** 0.1g of the extract was weighed out and macerated with 20ml of 20% H_2SO_4 in ethanol (1:1) and was filtered. 1ml of the filtrate was pipetted out and 5ml of 60% H_2SO_4 was added. After 5 minutes, 5ml of 0.5% formaldehyde in 60% H_2SO_4 was also added. They were mixed together and allowed to sit for 3 minutes. Absorbance was measured at 550nm.
- ii. **Test for Flavonoids:** 0.1g of the extract was weighed and macerated with 20ml of ethyl acetate and filtered. 5ml of the filtrate was pipetted and then mixed with 5ml of dilute ammonia. It was shaken vigorously and the upper layer of the mixture was collected. Absorbance was measured at 490nm.
- iii. **Test for Tannins:** 0.1g of the extract was weighed. 50ml of distilled water was added and filtered. 5ml of the filtrate was pipetted out and mixed with 0.1ml and 0.008ml Potassium ferricyanide. Absorbance was measured at 720nm.
- iv. **Test for Saponins:** 0.1g of the extract was weighed and macerated with 20ml of petroleum ether. It was decanted into a beaker and washed again with 10 ml of petroleum ether. The filtrate was combined and evaporated to dryness. The residue was dissolved in 6ml of ethanol. Then 2ml of the dissolved residue was put in a test tube and 2ml of chromogen solution (4ml iron stock made up to 24ml with concentrated H_2SO_4) was added to it. It was allowed to sit for 30 minutes. Absorbance was measured at 550nm.
- v. **Test for Phenol:** 0.1g of the extract was weighed and macerated with 20ml of 80% ethanol and filtered. 5ml of the filtrate was pipetted out and 5ml of Folin–ciocalteu reagent was added. After 3 minutes, 2ml of 20% sodium carbonate (iv) was added and the mixture was allowed to sit for 10 minutes. Absorbance was measured at 650nm.
- vi. **Test for Glycoside:** 0.1g of the extract was weighed and macerated with 20ml of distilled water and 2.5ml of 1.5% lead acetate was added and filtered. 2.5ml of chloroform was added to the filtrate and shaken vigorously. The lower layer was collected and evaporated to dryness. 3ml of Glacial acetic acid was added together with 0.1ml of 5% ferric chloride and 0.25ml of concentrated H_2SO_4 . The mixture was shaken and put in the dark for 2hrs. Absorbance was measured at 530nm.

HPLC Analysis of the Plant Extract

HPLC analysis of bioactive constituents was performed using standard procedures as described by Odubango, (2019). Separation was carried out on a Phenomenex C18 column measuring 4.6 mm × 250 mm with 5 µm particle size. The mobile phase consisted of solvent A, made up of acidified water systems including water containing 1% formic acid, water–

acetic acid, 0.5% aqueous formic acid, and water containing 1% phosphoric acid, while solvent B consisted of acetonitrile, methanol, and 1% acetic acid in acetonitrile. Gradient elution was applied, beginning with 13% solvent B up to 10 minutes and increasing progressively to 20%, 30%, 50%, 60%, 70%, and 80% solvent B at 20, 30, 40, 50, 60, 70, and 80 minutes, respectively. The flow rate was 0.6 mL/min, and the injection volume was 40 µL. Detection was carried out within 250–366 nm, while confirmation of compounds was done using retention time. The standards used for quantification included gallic acid, ferulic acid, caffeic acid, maleic acid, quercetin, naringin, kaempferol, tannic acid, salicylic acid, saponin and apigenin.

Animal Studies

Twenty (20) adult female and five (5) adult male albino rats were obtained from B.S.U, Makurdi and bred in the animal house of the Department of Biochemistry, Nasarawa State University and were used for the experiment. They were maintained under standard conditions where they were fed with rat pellets and were allowed unrestricted access to water. The animals were acclimatized for two (2) weeks and weighed between 200–250g at the commencement of the experiment and were subjected to hormonal assay to affirm fertility before treatment. All experimental procedures involving the animals were conducted in accordance with established institutional ethical guidelines for the care and use of laboratory animals.

Grouping of Animals/Treatment

The albino rats were divided into five (5) groups of four (4) animals per group. The use of four animals per group across five experimental groups was considered adequate for this study based on the resource equation approach for determining sample size in animal experiments. According to the resource equation method, the error degree of freedom E , should fall between 10 and 20 to ensure an appropriate balance between statistical validity and ethical animal use. In this study, the total number of animals were 20 distributed across five groups, yielding an E value of 15 ($E = 20 - 5$), which falls within the recommended range. Therefore, the use of four animals per group was considered sufficient to detect treatment-related effects while adhering to ethical principles that advocate the reduction of animal use in scientific research (Arifin & Zahiruddin, 2017).

- i. **Group 1 (Control Group):** In this group, the animals served as control and were not administered any form of medication/treatment.
- ii. **Group 2 (100mg/kg Extract Treatment Group):** In this group, the animals received *P. nitida* aqueous fruit extract at a dose of 100 mg/kg body weight. This dose was achieved using a prepared concentration of 25 mg/mL, with administration volumes adjusted according to individual body weights (200–250g) to ensure accurate dose delivery.
- iii. **Group 3 (200mg/kg Extract Treatment Group):** In this group, animals received *P. nitida* aqueous fruit extract at a dose of 200 mg/kg body weight. This dose was

achieved using a prepared concentration of 50mg/mL, with administration volumes adjusted according to individual body weights (200–250g) to ensure accurate dose delivery.

- iv. **Group 4 (300mg/kg Extract Treatment Group):** In this group, the animals received *P. nitida* aqueous fruit extract at a dose of 300 mg/kg body weight. This dose was achieved using a prepared concentration of 100mg/mL, with administration volumes adjusted according to individual body weights (200–250g) to ensure accurate dose delivery.
- v. **Group 5 (LNG/EE Treatment Group):** In this group, the animals received LNG/EE at a dose of 0.005mg/kg body weight. This dose was achieved using a prepared concentration of 0.001mg/mL, with administration volumes ranging from 0.8mL–1mL according to individual body weights (200–250g) to ensure accurate dose delivery. The dose calculations were based on the relationship between administered concentration, volume administered, and body weight to ensure dose standardization across all treatment groups.

Mating Protocol

After seven (7) days of oral administration of *P. nitida* aqueous crude extract, one sexually mature male albino rat was cohabited with four female rats per group (1:4) for twenty-one (21) consecutive days to allow for mating. Daily vaginal smears were examined for the presence of sperm to confirm successful copulation.

Blood Collection and Hormonal Assay

Following the twenty-eight (28) day experimental period, female rats were fasted overnight and euthanized by cervical dislocation under mild diethyl ether anesthesia in accordance with Nasarawa State University, Keffi (NSUK) Animal Care and Use Research Ethics Committee (NSUK-ACUREC) (Approval No: NSUK-ACUREC/BCH/25/02 – 29-05-2025).

Blood samples were obtained via cardiac puncture into plain sample bottles and allowed to clot for 30 minutes at room temperature. Serum was harvested after centrifugation at 3000 * g for 15 minutes and stored at -20°C prior to analysis.

Serum levels of Estradiol, Progesterone, and Prolactin were quantified using the Hipro PalmF Fluorescence Immunoassay Analyzer (Hipro Biotechnology Co., Ltd., Guangzhou, China).

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation (SD). Quantitative Data from laboratory analyses were processed using statistical tests such as One-way ANOVA and Tukey's post-hoc test. Statistical significance was set at ($p < 0.05$).

Results and Discussion

Results

Qualitative Phytochemical Composition

The qualitative phytochemical screening of the extract revealed the presence and absence of key bioactive constituents as presented in Table 3.1. The results indicated the presence of a broad spectrum of phytochemicals, particularly polyphenols, saponins, tannins, flavonoids, alkaloids and glycosidic compounds, while terpenoids and steroids were not detected.

Table 3.1 Qualitative Phytochemical Composition

Phytochemical	Inference
Saponins	+
Phenols	+
Tannins	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Terpenoids	–
Steroids	–

+ = present and - = absent

Quantitative Phytochemical Composition

The quantitative analysis of the phytochemicals is presented in Table 3.2. Saponins (0.695 mg/g) and glycosides (0.509 mg/g) were the most abundant phytochemicals, while phenols (0.067 mg/g), tannins (0.076 mg/g) and flavonoids (0.104 mg/g) were in moderate concentrations, and alkaloids (0.027 mg/g) were present in the lowest concentration.

Table 3.2: Quantitative Phytochemical Composition

Phytochemical	Conc (mg/g)
Saponins	0.695 ± 0.003
Phenols	0.067 ± 0.002
Tannins	0.076 ± 0.001
Alkaloids	0.027 ± 0.002
Flavonoids	0.104 ± 0.001
Glycosides	0.509 ± 0.002
Terpenoids	Not Detected
Steroids	Not Detected

HPLC Phytochemical Profile of the Extract

The HPLC chromatographic analysis identified specific bioactive compounds as shown in Table 3.3. The HPLC chromatogram in Table 3.3 revealed a diverse spectrum of phytochemicals dominated by phenolic acids and flavonoids. Ferulic acid (146.43 mg/L) was

the most abundant compound, followed by Salicylic acid (84.16 mg/L) and Saponins (75.13 mg/L). Moderate concentrations of Gallic acid (21.07 mg/L), Tannic acid (29.19 mg/L), Apigenin (15.43 mg/L) and Naringin (10.85 mg/L) were also detected. Trace levels of key flavonoids such as Quercetin (0.34 mg/L) and Kaempferol (0.46 mg/L) were observed.

Table 3.3: HPLC Phytochemical Profile of the Extract

S/N	Compound	RT (min)	Peak Area (mAU.s)	Conc (mg/L)
1	Caffeic Acid	1.322	44.925	0.0202
2	Ferulic Acid	1.675	324.141	146.435
3	Maleic Acid	1.813	40.963	2.233
4	Gallic Acid	3.216	683.552	21.071
5	Salicylic Acid	3.429	288.704	84.164
6	Saponins	4.795	18.068	75.135
7	Tannic Acid	4.862	16.023	29.189
8	Apigenin	5.315	28.414	15.434
9	Naringin	7.042	0.483	10.851
10	Kaempferol	10.195	0.0237	0.462
11	Quercetin	12.177	0.0320	0.340
12	p-Coumaric Acid	14.329	0.0339	0.165

Effect of Plant Extract on Female Reproductive Hormones

The effects of the plant extract and LNG/EE on circulating prolactin, progesterone, and estrogen levels in female rats are summarized in Table 3.4. There was a significant difference in serum prolactin levels among experimental groups ($p < 0.05$) compared to the control group rats that showed a prolactin concentration of 20.00 ± 0.68 ng/mL. Administration of the extract at 100, 200, and 300 mg/kg resulted in a dose-dependent reduction in prolactin levels (17.20 ± 0.54 , 14.52 ± 0.45 , and 12.10 ± 0.43 ng/mL, respectively), all of which were significantly lower than control values. The LNG/EE treated group also demonstrated a significant decrease in prolactin (15.08 ± 0.31 ng/mL), relative to the control group.

Serum progesterone levels differed significantly across groups ($p < 0.05$). Control animals exhibited a progesterone concentration consistent with basal luteal activity. Extract treated groups showed a dose-dependent elevation in progesterone, with the 200 and 300 mg/kg doses producing the highest values, which were significantly greater than control and the 100 mg/kg group ($p < 0.05$). In contrast, the LNG/EE group displayed marked suppression of progesterone, significantly lower than all extract treated groups.

The Estrogen concentrations were significantly altered by treatment ($p < 0.05$). Control rats recorded 40.02 ± 1.01 pg/mL. Extract administration significantly increased estradiol levels in a dose-dependent manner, with values of 48.60 ± 0.98 , 61.90 ± 0.95 , and 70.40 ± 1.48 pg/mL at 100, 200, and 300 mg/kg, respectively. Conversely, the LNG/EE treated group

exhibited a significant reduction in estrogen (24.48 ± 0.60 pg/mL) compared with all other groups.

Table 3.4: Effect of Plant Extract on Female Reproductive Hormones

Group	Prolactin (ng/mL)	Progesterone (ng/mL)	Estradiol (pg/mL)
Control	20.00 ± 0.68^a	12.10 ± 0.74^c	40.02 ± 1.01^c
100 mg/kg Extract	17.20 ± 0.54^b	15.40 ± 0.81^b	48.60 ± 0.98^b
200 mg/kg Extract	14.52 ± 0.45^c	18.90 ± 0.69^a	61.90 ± 0.95^a
300 mg/kg Extract	12.10 ± 0.43^d	21.30 ± 0.88^a	70.40 ± 1.48^a
LNG/EE	15.08 ± 0.31^c	6.20 ± 0.52^d	24.48 ± 0.60^d

Data are expressed as Mean $\pm$ SD (n = 4). Different superscript letters within the same column indicate significant differences following one-way ANOVA and Tukey's post-hoc test ($p < 0.05$).

Discussion

This study investigated the effects of a phytochemical-rich extract on female reproductive hormones, using LNG/EE regimen as a standard hormonal control. The findings demonstrate that the extract possesses significant fertility-supportive properties, mediated through its rich phytochemical compositions and dose-dependent hormonal restoration.

Qualitative and quantitative phytochemical analyses revealed that the extract is rich in saponins, glycosides, phenols, flavonoids, tannins, and alkaloids, with saponins (0.695 ± 0.003 mg/g) and glycosides (0.509 ± 0.002 mg/g) being the most abundant. This profile is consistent with previous reports on medicinal plants used in reproductive health, where polyphenols and saponins were shown to have contributed to antioxidant and endocrine-modulatory activities (Akbaribazm *et al.*, 2021; Vasková *et al.*, 2023). The absence of terpenoids and steroids, coupled with low alkaloid content, suggests a favourable safety profile, as high alkaloid concentrations have been linked to reproductive toxicity (Adamski, 2020).

HPLC analysis provided compound-specific evidence, identifying ferulic acid (146.43 mg/L) as the dominant constituent, followed by salicylic acid (84.16 mg/L) and saponins (75.13 mg/L). Other notable compounds included gallic acid, tannic acid, apigenin, naringin, quercetin, and kaempferol. The consistency between qualitative, quantitative, and HPLC results strengthens the reliability of the phytochemical characterization and supports the biological activity observed. The observed hormonal changes could have been attributed to the synergistic action of the identified bioactive compounds. For instance, ferulic acid, the predominant compound, is a well-established phytoestrogen with antioxidant and anti-inflammatory properties. It enhances estrogen receptor activity and protects ovarian granulosa cells from oxidative stress, thereby supporting estradiol synthesis and follicular development (Purushothaman *et al.*, 2024; Zduńska *et al.*, 2018). Similarly, saponins have been reported to stimulate estrogen production and enhance follicle-stimulating hormone

(FSH) and luteinizing hormone (LH) secretion, thereby promoting ovulation (Chen *et al.*, 2016).

Flavonoids such as apigenin, quercetin, naringin, and kaempferol exhibited phytoestrogenic activity by binding to estrogen receptors and modulating progesterone signaling (Panche *et al.*, 2016; Wang *et al.*, 2018). These compounds are known to contribute to antioxidant defence, protecting ovarian cells from oxidative damage and maintaining steroidogenic function (Vasková *et al.*, 2023). Gallic acid and caffeic acid further support ovarian health by scavenging reactive oxygen species and regulating progesterone secretion (Abarikwu *et al.*, 2014; Kumar *et al.*, 2019).

The extract produced a dose-dependent reduction in serum prolactin levels, with the 300 mg/kg dose showing the most pronounced effect (12.10 ± 0.43 ng/mL). Elevated prolactin is known to suppress GnRH secretion and impair ovarian steroidogenesis (Melmed *et al.*, 2022). The reduction in prolactin observed in this study may be mediated by flavonoids that enhance dopaminergic signaling, thereby exerting tonic inhibition on prolactin released from the anterior pituitary (Iancu *et al.*, 2023). Although, LNG/EE also reduced prolactin, this effect is likely due to exogenous steroid feedback rather than neuroendocrine modulation.

Serum progesterone levels were significantly elevated in extract-treated groups, particularly at 200 and 300 mg/kg (18.90 ± 0.69 and 21.30 ± 0.88 ng/mL, respectively). Progesterone is critical for corpus luteum function and endometrial receptivity during implantation. The increase suggests enhanced luteal steroidogenesis, possibly through upregulation of 3β -hydroxysteroid dehydrogenase by saponins and flavonoids (Dixon, 2020). In contrast, LNG/EE caused marked progesterone suppression (6.20 ± 0.52 ng/mL), consistent with its contraceptive mechanism of inhibiting ovulation and corpus luteum formation (Teal & Edelman, 2021).

Estradiol levels were also significantly increased in a dose-dependent manner following extract administration, reaching 70.40 ± 1.48 pg/mL at 300 mg/kg. This indicates stimulation of ovarian estrogen biosynthesis, likely through enhanced aromatase activity and protection of granulosa cells by phenolic acids and flavonoids (Patisaul & Jefferson, 2010). The concurrent elevation of estradiol and progesterone reflects synchronized follicular development and luteal maturation, which are essential for fertility. Conversely, LNG/EE significantly reduced endogenous estradiol (24.48 ± 0.60 pg/mL) via negative feedback on the hypothalamic-pituitary-gonadal (HPG) axis, confirming its contraceptive action.

The hormonal profile produced by *P. nitida* aqueous fruit extract is fundamentally different from that of LNG/EE. While LNG/EE suppresses endogenous hormone production to prevent ovulation, *P. nitida* aqueous fruit extract enhances hormonal balance, suggesting an endocrine-supportive rather than contraceptive effect.

The low alkaloid content and absence of terpenoids and steroids indicate minimal risk of reproductive toxicity at the tested doses. The dominance of antioxidant compounds such as ferulic acid, gallic acid, and flavonoids may suggest the extract's ability to reduce

prolactin and enhance estradiol and progesterone levels observed in this study. This also shows the plant's potential protective effect on oocyte quality and overall reproductive health.

The strong agreement between HPLC and quantitative analyses further validates the extract as a bioactive, fertility-supporting phytochemical source, with potential applications in managing infertility and improving reproductive outcomes. Therefore, the extract demonstrated a fertility-supportive hormonal profile, characterized by reduced prolactin (relief of reproductive axis inhibition), elevated progesterone (enhanced luteal function) and increased estradiol (improved ovarian steroidogenesis).

Conclusion

P. nitida is a highly valuable medicinal plant with significant pharmacological properties. The aqueous fruit extract of the plant contains significant amounts of saponins, glycosides, and phenolic compounds, with ferulic acid, salicylic acid identified as key bioactive ingredients. Administration of the extract to female albino rats produced significant, dose-dependent alterations in reproductive hormone levels, characterized by reduced prolactin and elevated progesterone and estradiol.

Furthermore, the findings of this research demonstrate its potential utility as fertility-supportive, enhancing reproductive health, regulating and maintaining hormonal balance and adequately promoting enhanced luteal function and improving ovarian steroidogenesis. However, further studies are needed to elucidate the underlying mechanisms and to evaluate its overall safety and efficacy in human subjects.

Recommendations

Drawing from the findings, the following recommendations are put forward:

1. Further studies should be conducted to determine safe dosage range of *P. nitida* aqueous fruit extract for long-term use.
2. Clinical trials should be carried out on human subjects to validate the hormonal effects observed in animal models.
3. Advanced molecular studies should be undertaken to elucidate the mechanisms through which the extract influences reproductive hormones.
4. Pharmacokinetic studies should be carried out to understand its metabolism and excretion pathways.
5. Standardization and formulation studies should be encouraged to develop *P. nitida*-based therapeutic products for reproductive health management.
6. Comparative studies involving other medicinal plants and conventional contraceptive drugs should be conducted to establish relative efficacy.
7. Government and health agencies should support research into plant-based alternatives to synthetic reproductive drugs to enhance healthcare accessibility.

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Conflict of Interest

The authors declare that they have no financial or other relationships that might lead to any conflict.

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