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Impact of Pineapple (*Ananas comosus L. Merr*) Stem Extract (PSE) on Ovarian Dysfunction in A Polycystic Ovary Syndrome Rat Model: Insights into Molecular Interactions and Biological Pathways

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Abstract

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder characterized by hyperandrogenism, anovulation, and polycystic ovarian morphology. This study evaluated the therapeutic effects of Pineapple (*Ananas comosus L. Merr*) stem extract (PSE) in a DHEA-induced PCOS rat model. Thirty-six female Sprague-Dawley rats were randomly divided into 6 groups, including a negative control group. Following 7-day acclimatization and PCOS induction with DHEA, treatments were administered from day 43 (H0) to day 70 (H27). Serum levels of LH, FSH, testosterone, and AGEs were measured by ELISA, while ovarian morphology was assessed through H&E staining. The 500 mg/kg BW PSE treatment significantly reduced LH ($p < 0.001$), testosterone ($p < 0.001$), and AGEs ($p < 0.001$) levels while increasing FSH ($p = 0.017$). Furthermore, histopathological examination using hematoxylin-eosin (H&E) staining revealed that PSE administration (500 mg/kg BW) improved follicular development and ovulation compared to the negative control group, as evidenced by a reduction in cystic follicle count and an increase in corpus luteum formation. In conclusion, PSE exhibits potential therapeutic effects in ameliorating PCOS-like symptoms by modulating reproductive hormones and promoting ovarian follicular maturation.

Keywords: *Ananas comosus L. Merr*, Polycystic ovary syndrome (PCOS), Folliculogenesis, Antiandrogenic activity, AGEs, Ovulation

Introduction

Polycystic Ovary Syndrome (PCOS) is a multifaceted illness impacting women of reproductive age, marked by endocrine, metabolic, and reproductive irregularities, and is a primary contributor to prolonged anovulation, potentially leading to infertility [1]. Female infertility is frequently linked to ovulatory dysfunction, tubal abnormalities, or uterine factors, with polycystic ovary syndrome (PCOS) being a main source of ovulatory disorders. The 2003 ESHRE/ASRM consensus stipulates that the diagnosis of PCOS is confirmed when a minimum of 2 of the following 3 criteria are satisfied: Hyperandrogenism, persistent anovulation, and polycystic ovarian morphology observed via ultrasound. The prevalence of PCOS, according to these criteria, varies from 15% to 20%, with fluctuations between 5% and 26% contingent upon the diagnostic guidelines employed [2]. Worldwide, between 4% to 20% of women experience anovulation, while 38% to 88% of people with PCOS are obese, and 50% to 70% demonstrate insulin resistance. In Indonesia, the incidence of central obesity among adolescent females is 46.7%, and over 60% of patients with PCOS are categorized as obese [3].

The pathophysiology of polycystic ovarian syndrome (PCOS) is multifaceted, including a complex interaction of endocrine, metabolic, genetic, and environmental variables that contribute to its rising prevalence. The hypothalamic release of gonadotropin-releasing hormone (GnRH) is pivotal in polycystic ovary syndrome (PCOS) since it inappropriately enhances luteinizing hormone (LH) production, diminishes follicle-stimulating hormone (FSH) levels, and raises the LH/FSH ratio. Increased LH levels promote ovarian androgen synthesis, resulting in hyperandrogenemia, follicular stagnation, and ovulatory impairment [4]. Simultaneously, the overproduction of anti-Müllerian hormone (AMH), diminished FSH levels, increased LH concentrations, and persistent inflammation impede follicular formation, leading to amenorrhea and anovulation [5]. Genetic predisposition and lifestyle factors additionally affect hormonal imbalance and obesity, which exacerbate oxidative stress in visceral adipose tissue, initiate inflammatory responses, and elevate insulin resistance and ovulatory dysfunction [5]. Advanced glycation end products (AGEs), serving as indicators of oxidative stress, trigger signaling pathways that worsen these pathological states in women with PCOS [6].

The management of polycystic ovary syndrome (PCOS) highlighted in numerous studies underscores hyperandrogenism as a key element in the pathophysiology and related metabolic dysfunction of the condition. Individuals with non-hyperandrogenic PCOS may demonstrate distinct etiological mechanisms. fThe Indonesian Society of Reproductive Endocrinology and Fertility and the Indonesian Society of Obstetrics and Gynecology advocate for a management strategy grounded in Levels of Evidence. This approach encompasses patient education, lifestyle modifications, dietary regulation, physical activity, and menstrual cycle regulation. Aromatase inhibitors function by inhibiting the conversion of androgens to estrogens, which results in elevated intraovarian androgen concentrations. This subsequently stimulates the secretion of follicle-stimulating hormone (FSH), which aids in the selection and maturation of dominant follicles. Letrozole, an aromatase inhibitor, is frequently utilized for ovulation induction in patients with PCOS who exhibit resistance to clomiphene citrate. The suggested dosage is 2.5 - 7.5 mg per day for a duration of 5 consecutive days, commencing on the third day of the ovulatory cycle. Concerns have been raised about the potential teratogenic effects of letrozole, especially its association with heightened risks of cardiac and skeletal abnormalities in neonates. Letrozole is not advised for ovulation induction in pregnant women [7].

In line with recommendations for the management of PCOS that focus on hormonal and metabolic regulation, this study also considers the use of pineapple stem extract (PSE) as an adjunctive therapy with potential to complement conventional treatments. PSE, derived from *Ananas comosus* L. Merr, demonstrates potential as a plant-based solution that not only addresses hormonal imbalances in PCOS patients but also utilizes abundant agricultural waste in Indonesia, particularly in Lampung, where it accounts for 65% of the total production. This approach not only offers a therapeutic alternative but also contributes to the sustainable use of environmentally friendly natural resources [8]. PSE comprises a range of bioactive compounds, including phenolics, flavonoids,

terpenoids, and essential minerals like magnesium, zinc, and calcium, which demonstrate antioxidant, antibacterial, and antifungal activities [9]. It contains vitamin E, which prevents cellular damage, along with essential and non-essential amino acids that play roles in protein synthesis and energy metabolism. Previous research indicates that zinc methionine (ZM) may elevate FSH levels, enhance ovulation, and decrease cyst formation in patients with PCOS, thereby reinforcing the potential of PSE as an adjunctive treatment for hormonal imbalances associated with PCOS [10]. This research utilized blood and ovarian tissue sampling, necessitating surgical procedures. Female Sprague-Dawley rats were chosen as the experimental model because of their increased sensitivity relative to Wistar rats, ease of maintenance, high fertility rate, short gestation period, and metabolic, anatomical, and physiological similarities to humans. These traits render Sprague-Dawley rats an appropriate model for investigating PCOS in the context of human physiology.

Building upon this foundation, the present study aims to evaluate the therapeutic effects of PSE on reproductive hormone profiles and ovarian folliculogenesis in a validated polycystic ovary syndrome (PCOS) animal model. This investigation seeks to elucidate the potential of natural botanical compounds as alternative therapeutic interventions for PCOS-related reproductive dysfunction.

Materials and methods

This study is an experimental laboratory study with randomized, controlled trial, pre-test, and post-test design. Thirty-six female Sprague-Dawley rats were randomized into 6 groups (n = 6/group): Normal control/KN (non-induced), negative control /KNeg (DHEA-induced with vehicle: 6 mg/100g BW in sesame oil + 0.2% NaCMC), positive control/KPos (PCOS + letrozole 2.5 mg/kg BW), and 3 treatment groups (KP1, KP2 and KP3) receiving PCOS plus either pineapple stem extract (PSE; 500 or 1,000 mg/kg BW) or combination therapy (letrozole + PSE 500 mg/kg BW). Following 7-day acclimatization, treatments were administered from day 43 (H0). Terminal analyses day 70 (H27) included ELISA quantification of LH, FSH, testosterone, and AGEs, alongside histopathological evaluation of ovarian folliculogenesis (H&E staining).

Pineapple stems (*Ananas comosus L. Merr*)

Pineapple (*Ananas comosus L. Merr*) stems were obtained from PT Great Giant Pineapple (GGP) plantations in Terbanggi Besar, Central Lampung, Indonesia - the primary pineapple cultivation region in Lampung Province. Stem samples were harvested from 14 - 15-month-old plants, selected based on optimal morphological characteristics: Fresh appearance, firm texture, and uniform brownish-green coloration, with exclusion criteria including any signs of pest infestation, microbial infection, or physical damage. The collected stem portions were the aerial segments located above soil level, with standardized dimensions of 5 - 7 cm in diameter and 30 - 40 cm in length (measured from the leaf base). The pineapple stems used in this study underwent formal botanical identification at the accredited Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. Taxonomic identification confirmed the specimens as *Ananas comosus (L.) Merr*, with classification verified according to both the Cronquist system and the Angiosperm Phylogeny Group II (APG II) system.

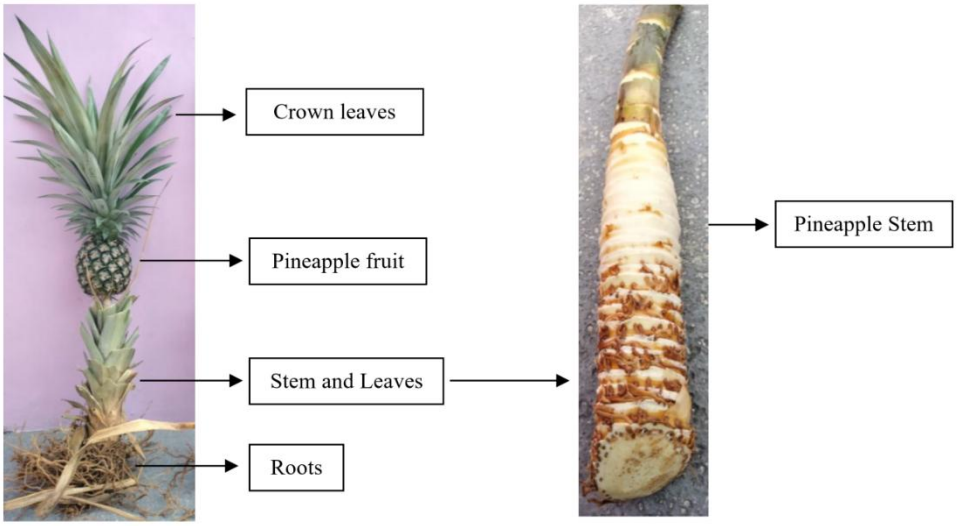


Figure 1 Pineapple (*Ananas comosus* L. Merr) stem.

Simplicia and extraction of pineapple (*Ananas comosus L. Merr*) stem

The preparation of simplicia and extraction of pineapple (*Ananas comosus L. Merr*) stem extract (PSE) was carried out at the Extract Standardization Laboratory of the Faculty of Pharmacy (SEFA) at Universitas Muhammadiyah Surakarta (UMS), under reference number: 00666/SEFA-UMS.B/VIII/22. The simplicia process involved washing approximately 40 kg of pineapple stems with clean water, slicing them thinly, drying them in an oven at 40 - 50 °C for approximately 3 days, and grinding the dried pineapple stems into powder, yielding approximately 5 kg. The extraction method used was maceration, a traditional technique for extracting secondary metabolites with a solvent. In this study, 5 kg of pineapple stem powder was macerated in 7 L of 96% ethanol for 3 days, then filtered using a vacuum Büchner to separate the powder and the first extract. The first extract was evaporated using a rotary evaporator to obtain a semi-thick extract, while the remaining ethanol was used for remaceration of the powder for another 3 days. This process was repeated twice, resulting in the second and third extracts, which were also evaporated to produce semi-thick extracts. The 3 semi-thick extracts were combined and then evaporated over a water bath for 80 h to obtain a thick extract.

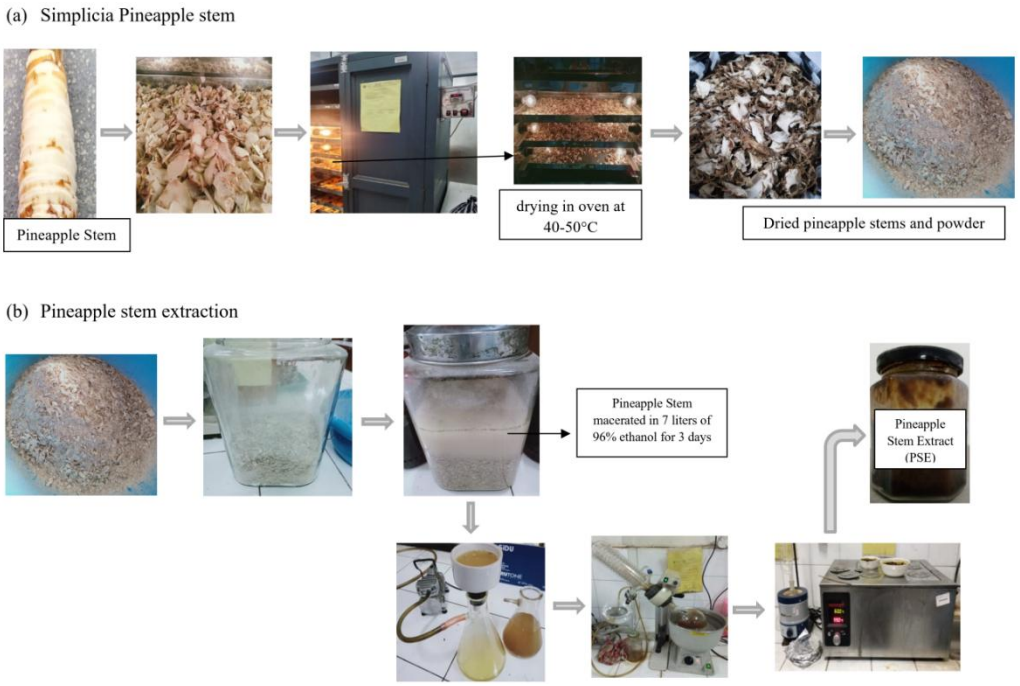


Figure 2 Simplicia preparation and extraction process.

Phytochemical examination of pineapple stem extract (PSE)

The phytochemical study of PSE was performed at the Saraswati Indo Genetech (SIG) Laboratory in Bogor. PT Saraswanti Indo Genetech (SIG), Registration No. LP-184-IDN, is a testing laboratory situated in Bogor, Indonesia. It is the inaugural laboratory in Indonesia to receive ISO/IEC 17025 accreditation from the National Accreditation Committee (KAN) for the assessment of Genetically Modified Organisms (GMO). The analyses conducted on the pineapple stem extract at Saraswati Indo Genetech (SIG) Laboratory in Bogor demonstrated the existence of antioxidant activity (UV-Vis Spectrophotometry), amino acids (UPLC-PDA), vitamins (HPLC-PDA), minerals (ICP OES), and flavonoids (LCMS/MS QTOF).

***In silico* assessment of pineapple stem extract (PSE)**

This study employed *in silico* analysis through molecular docking to investigate ligand-target protein interactions and ADME prediction to evaluate the pharmacokinetic properties and potential toxicity of the drugs. The process encompassed multiple stages: Extraction of target proteins from the Protein Data Bank (PDB), ligand production through PubChem, molecular docking utilizing AutoDock, display of docking results, ADME prediction, and analysis for the identification of probable active molecules. The employed software tools comprised ChemBioOffice 2014 (including ChemBioDraw Ultra and ChemBio3D Ultra), Discovery Studio Visualizer 2016, GaussianView 5.0, and AutoDock 1.5.6 [11].

PSE administrative protocol

The administration of PSE commenced after the DHEA induction was completed at a dosage of 6 mg/100gr body weight on day 43. Thereafter, serum concentrations of LH, FSH, testosterone, and advanced glycation end products (AGEs) were quantified by the ELISA technique. PSE was delivered at dosages of 500 mg/kg body weight (equal to 2.5 mg/kg body weight), 1,000 mg/kg body weight (equivalent to 5 mg/kg body weight), and in a combination therapy of letrozole 2.5 mg/kg BW with PSE 500 mg/kg BW. Dosage 1 and dosage 2 were treated differently to serve as the control and comparison groups. Dosage 1 received only PSE at a lower dose of 500 mg/kg body weight (BW), while dosage 2 received only PSE at a dose of 1,000 mg/kg BW. The combination of PSE and Letrozole in dosage 3 was chosen to determine whether the combined effects are more effective in restoring ovarian function and improving PCOS symptoms. This experimental

design allows us to evaluate the individual and combined effects of both treatments. The therapies were delivered orally through gavage for 27 consecutive days. Subsequent to the treatment period, blood samples were obtained for the evaluation of serum LH, FSH, testosterone, and AGEs levels via ELISA. Ovarian tissues were subsequently collected for histological analysis with hematoxylin-eosin (HE) staining.

Administration of letrozole dosage

Letrozole was provided to the positive control group of Sprague-Dawley rats according to the usual human therapeutic dosage of 2.5 mg/kg BW per day [12] This study's dose calculation was based on the therapy procedure for polycystic ovarian syndrome (PCOS), utilizing letrozole, an aromatase inhibitor, for ovulation induction. Upon converting the human dosage (predicated on a 70 kg body weight) to the corresponding dosage for a 200-gram rat, the determined conversion factor was 0.018 [13]. Consequently, the corresponding dose for a 200 g rat was calculated as: 2.5 mg×0.018 = 0.045 mg per 200 g of body weight. The dose was supplied orally through gavage daily from day 0 to day 27.

Ethical considerations

All techniques in this investigation complied with accepted ethical norms for the treatment and utilization of laboratory animals. Before initiating the study, ethical approval was secured by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret (KEPK FK UNS). The study obtained ethical approval under Ethical Clearance Certificate No. 200/UN27.06.11/KEP/EC/2023, with Protocol No. 177/02/08/2023, and was deemed ethically viable on September 8, 2023.

Polycystic ovary syndrome animal model

This research was performed at the LPPT Unit IV, Faculty of Medicine, Universitas Gadjah Mada (UGM), Yogyakarta, utilizing female Sprague Dawley rats as a model for Polycystic Ovary Syndrome (PCOS). The model was administered dehydroepiandrosterone (DHEA) at a dosage of 6 mg/kg body weight, diluted in 0.2 mL of sesame oil, via subcutaneous injection on the dorsal side of the rats on day 0 following the estrous phase. Sprague Dawley rats, aged 21 days (3 weeks) and weighing between 160 and 200 g, are frequently utilized in DHEA-induced PCOS models [14]. According to prior research [15], 87% of articles utilized a DHEA dosage of 6 mg per 100 g of body weight in rodents, which was also employed in this study. Multiple DHEA delivery techniques and solvents have been documented, with 38% of researchers utilizing sesame oil (0.2 mL) as the solvent [14,15]. This work employed the subcutaneous method to produce the PCOS model [16,17]. This study utilized a 42-day induction period, diverging from the normal 20-day duration employed by 58% of studies, due to preliminary findings suggesting a higher incidence of ovarian cysts compared to a 35-day protocol.

Evaluation of the estrous cycle

The estrous cycle was evaluated using vaginal cytology testing. Sample collection occurred in the morning between 09:00 and 10:00 AM local time. Vaginal smears were collected utilizing sterile cotton swabs softly placed into the vaginal canal. The gathered samples were subsequently placed onto glass slides and analyzed using a light microscope. The phases of the estrous cycle were delineated according to the predominant cell types: Proestrus - dominance of round, nucleated epithelial cells; estrus - presence of cornified squamous epithelial cells; metestrus - a combination of cornified epithelial cells and leukocytes; and diestrus - predominance of nucleated epithelial cells and leukocytes. The examination of the estrous cycle was performed over a period of 12 consecutive days [18].

Assessment of LH, FSH, testosterone and AGEs

Serum hormone concentrations were quantified utilizing commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits. Blood samples were obtained on day 0 after subcutaneous administration of DHEA (6 mg/kg BW in 0.2 mL sesame oil), and subsequently on day 27 post-therapeutic intervention. The concentrations of luteinizing hormone (LH, Cat. No. EA0013Ra), follicle-stimulating hormone (FSH, Cat. No. EA0015Ra), testosterone (Cat. No. EA0023Ra), and

advanced glycation end products (AGEs, Cat. No. 655101) were assessed in accordance with the manufacturer's guidelines.

Histopathological examination of follicle development and corpus luteum

The histopathological examination of follicle development and corpus luteum was performed using Hematoxylin and Eosin (HE) staining, alongside ovulation assessment using vaginal smears to observe the estrus cycle. Female SD rats, induced with 6 mg/100 g body weight (BW) of DHEA dissolved in 0.2 mL sesame oil for 42 days, followed by 27 days of PSE therapy, were euthanized on day 27 after the therapy for ovarian tissue collection. The collected biopsy/operated tissues were first fixed in formalin buffer for at least 48 h. The tissue was then placed in a tissue cassette and immersed in 50% alcohol for 1.5 h, followed by 70% alcohol for 1.5 h, and then 80% alcohol for 1.5 h. Subsequently, the tissue was immersed in 95% alcohol I for 1.5 h and 95% alcohol II for 1.5 h. The tissue was then transferred to absolute alcohol I for 1.5 h, followed by absolute alcohol II for 1.5 h. Next, the tissue was immersed in xylene I for 0.5 h, xylene II for 1.5 h, and xylene III for 1.5 h. After draining, the tissue underwent embedding by immersion in liquid paraffin at a melting point of 58 °C, with incubation at 45 °C overnight. Paraffin blocks were then prepared. Hematoxylin-Eosin staining was performed afterward.

The paraffin blocks were cut to a thickness of 3 - 5 µm, placed on slides, and dried in an incubator at 58 - 60 °C for 20 min. The slides were then immersed in a graded xylene solution (4 stages), each for 5 - 7 min, and pressed with filter paper. The slides were then immersed in a graded alcohol solution (4 stages) for 5 min each, followed by washing with running water. The slides were placed in Hematoxylin solution for 7 - 10 min and washed with running water. They were then immersed in Eosin solution for 2 min, washed with running water, rinsed with 90% alcohol, and dried. The slides were placed in xylene and covered with a deckglass. The observations were conducted using a microscope with 400× magnification (40× objective lens and 10× ocular lens). Follicle development and anovulation were assessed by counting the average number of follicles and corpus luteum in 1 field of view at 400× magnification.

Data analysis

Data analysis was conducted utilizing SPSS for Windows version 29.0 (IBM Corporation, Armonk, NY, USA). A 1-way analysis of variance (ANOVA) was utilized to assess differences among treatment groups. Before performing ANOVA, a normality test of the data was conducted using the Shapiro-Wilk test, and a homogeneity of variance test was performed using Levene's test to ensure that assumptions were met for parameters such as LH, FSH, androgen hormones (testosterone), AGEs, and follicle development parameters. Differences between groups were analyzed using 1-Way ANOVA, and if significant differences were found, a post-hoc Tukey HSD test was performed. The Tukey HSD post-hoc test was applied to control the Type I error rate conservatively and to compare each pair of groups. It is used when the data are normally distributed and variances between groups are equal. The significance level used was $\alpha = 0.05$, with a p-value < 0.05 considered significant. For the LH, testosterone, and AGEs parameters, post-hoc Tukey HSD analysis was applied. For the FSH parameter, since the data did not meet the assumptions for ANOVA, it was analyzed using the non-parametric Kruskal-Wallis test, followed by the post-hoc Dunn's test. Dunn's test is a non-parametric test used to compare pairs of groups after the Kruskal-Wallis test and corrects for Type I error. For the ovarian follicle development and ovulation parameters, the Kruskal-Wallis test was used, and if significant differences were found, the post-hoc Dunn's test was performed, with a significance level of $\alpha = 0.05$ and $p < 0.05$ considered significant. The results were analyzed with a significance level of $p < 0.05$ and presented as mean ± standard deviation (mean ± SD).

Results and discussion

Phytochemical composition of pineapple *(Ananas comosus* L. Merr*)* stem extract

The phytochemical analysis of pineapple stem extract PSE was conducted utilizing several analytical techniques: UV-Vis spectrophotometry for assessing antioxidant activity, UPLC-PDA for amino acid identification, HPLC-PDA for vitamin identification,

ICP-OES for mineral analysis, and LC-MS/MS QTOF for flavonoid compound detection. The outcomes are as follows:

Table 1 Results of antioxidant, vitamin, mineral, and amino acid activity tests on PSE.

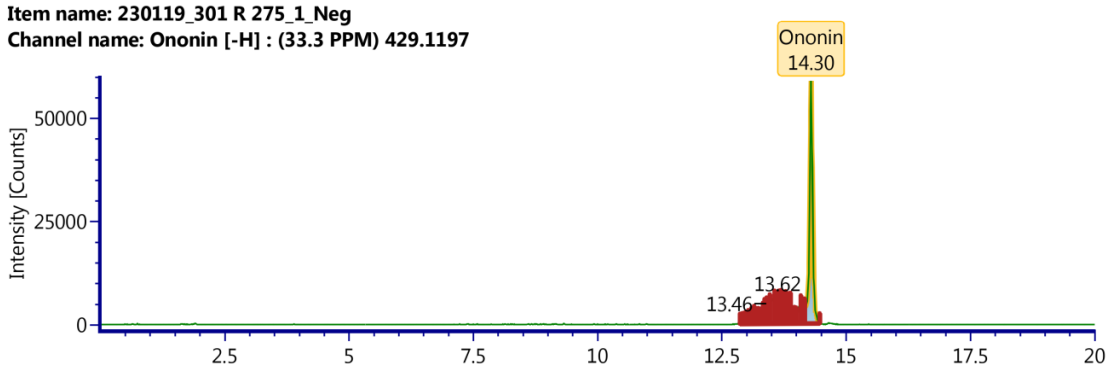
No	Parameter	Molecular Formula	Result (Unit)
1	Antioxidant Activity	-	28,360.38 mg/kg
2	Vitamin B6 (Pyridoxin)	$C_8H_{11}NO_3$	2.32 mg/ 100 g
3	Vitamin E (Alpha Tocopherol)	$C_{29}H_{50}O_2$	11.75 mg/ 100 g
4	Magnesium	Mg	14.64 mg/ 100 g
5	Manganese	Mn	1.96 mg/ kg
6	Zinc	Zn	1.01 mg/ 100 g
7	Calcium	Ca	5.40 mg/ 100 g
8	Copper	Cu	2.18 mg/ kg
9	Chromium	Cr	5.43 mcg/ 100 g
10	L-Methionine	$C_5H_{11}NO_2S$	48.83 g/kg

The antioxidant assessment of the PSE extract produced a result of 28,360.38 mg/kg, determined using the antioxidant activity method 18-9-97/MU utilizing UV-Vis spectrophotometry to ascertain IC50, AEAC, and/or TEAC values. This investigation sought to assess the extract's ability to mitigate cellular damage induced by free radicals, which play a significant role in oxidative stress - a critical element in the etiology of polycystic ovarian syndrome (PCOS) [19]. Additionally, a vitamin analysis of the PSE extract was performed to identify, measure, and assess the levels of vitamin B6/pyridoxine (2.32 mg/100 g) and vitamin E/alpha-tocopherol (11.75 mg/100 g). These vitamins are crucial for sustaining immune system functionality, energy metabolism, collagen production, and cellular defense. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) analysis of mineral and heavy metal content indicated that magnesium (Mg) was the predominant mineral in the PSE extract, with a concentration of 14.64 mg/100g. Magnesium significantly contributes to improving insulin sensitivity, diminishing inflammation, regulating the menstrual cycle, and mitigating stress. The intake of magnesium-rich foods or supplements may mitigate PCOS symptoms and enhance general health [20].

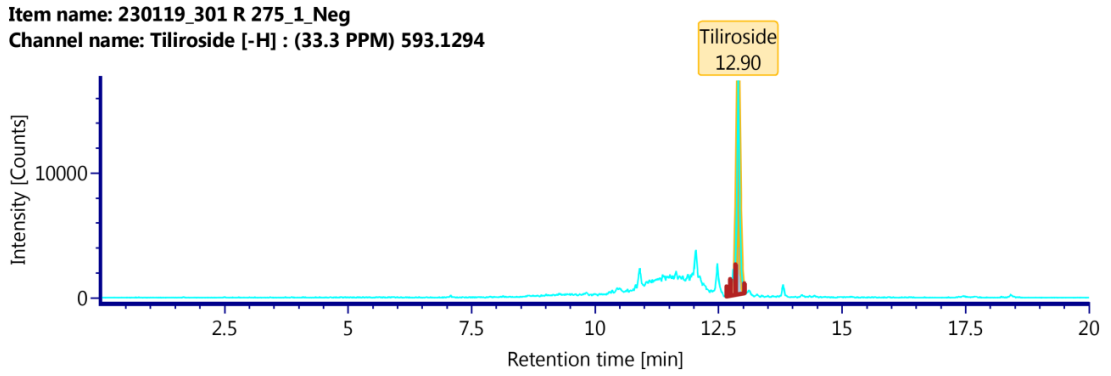
Table 2 Results of antioxidant, vitamin, mineral, and amino acid activity tests on PSE.

No	Compounds	Pubchem id	Formula	Observed RT (min)	Isotope Mathch Mz RMS PPM	Isotope Match Intensity RMS Percent
1	*(-)-Epiafzelechin-3-O-(6''-O-acetyl)-β-D-allosepyranoside*	44257057	$C_{23}H_{26}O_{11}$	10.53	2.40	5.80
2	*3',5-Dihydroxy-7,4'-dimethoxy flavone*	5378823	$C_{17}H_{14}O_6$	8.04	1.06	9.97
3	*Daidzin_1*	107971	$C_{21}H_{20}O_9$	12.63	1.80	5.35
4	*Ononin*	442813	$C_{22}H_{22}O_9$	14.30	3.92	0.72
5	*Pelargonidin 3-glucoside*	443648	$C_{21}H_{21}ClO_{10}$	8.50	1.25	2.89
6	*Tiliroside*	5320686	$C_{30}H_{26}O_{13}$	12.90	0.69	0.77

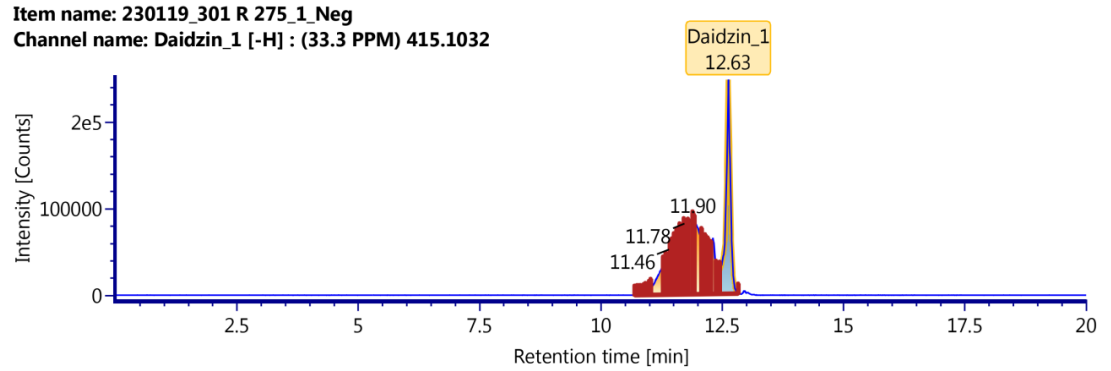
The flavonoid components found in the PSE extract, according to the 3 highest retention times, are Ononin, Tiliroside, and Daidzin_1. Ononin is a glycosylated isoflavone prevalent in several plants of the Leguminosae family, including Trifolium pratense (red clover) and Glycyrrhiza glabra (licorice root) [21]. Tiliroside (TLD) is a glycosylated flavonoid (GFD) sourced from plants, recognized for its extensive array of bioactivities advantageous to human health. These encompass antioxidant, antibacterial, antifungal, antidiabetic, antihyperlipidemic, antiviral, cytotoxic, anti-inflammatory, antirheumatic capabilities, along with the inhibition of neuroinflammation and acute inflammation, and hepatoprotective action [22]. Daidzin is a naturally occurring organic molecule belonging to the phytochemical class of isoflavones, renowned for its biological activity [23].



(a) Ononin



(b) Tiliroside



(c) Daidzin_1

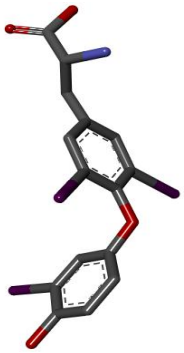
Figure 3 Chromatogram of flavonoid compounds ononin, tiliroside, and daidzin_1.

Gambar 3. memperlihatkan kromatogram 3 senyawa flavonoid PSE. PSE mengandung berbagai senyawa dengan waktu retensi berbeda-beda, mencerminkan keberagaman sifat kimia dalam senyawa tersebut. Pada kromatogram flavonoid, puncak tertinggi muncul pada waktu retensi 14.30 menit, yang diidentifikasi sebagai *Ononin* (A), menunjukkan bahwa senyawa ini merupakan komponen dominan dengan intensitas sinyal yang jauh lebih tinggi dibandingkan puncak lainnya yaitu *Tiliroside* (B) (12.90 menit) dan *Daidzin_1* (C) (12.63 menit).

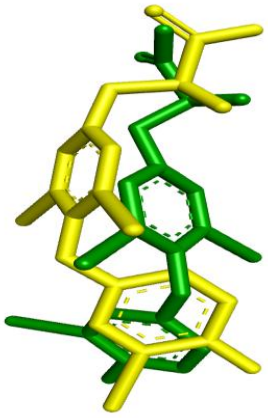
Molecular docking analysis of PSE

In the *in silico* molecular docking analysis of PSE, the following software was installed and utilized: ChemBioOffice 2014 (ChemBioDraw Ultra and ChemBio3D Ultra), Discovery Studio Visualizer 2016, GaussianView 5.0, and AutoDock 1.5.6. The docking target used in this study was the Androgen Receptor, which was retrieved from the Protein Data Bank (PDB) database at https://www.rcsb.org/, maintained by the Research Collaboratory for Structural Bioinformatics (RCSB). The specific enzyme used had the PDB ID: 2PIV, with the following characteristics: PDB DOI: https://doi.org/10.2210/pdb2PIV/pdb, Classification: Hormone Receptor, Organism: Homo sapiens, Expression System: Escherichia coli, Mutations: None, Method: X-ray diffraction, Resolution: 1.95 Å.

(a)



(b)



(c)

Binding affinity (ΔG): –7.42 kcal/mol

Inhibition constant (Ki): 3.65 µM

Docking run number: 25

Root Mean Square Deviation (RMSD): 1.956 Å

Figure 4 (a) Crystallographic structure of the androgen receptor (PDB ID: 2PIV), (b) Structure of the natural ligand, and (c) Visualization of the natural ligand docked to the androgen receptor binding site.

In the docking visualization, the yellow structure represents the native ligand (redocking result), while the green structure depicts the tested natural ligand. The RMSD value of less than 2 Å indicates a high degree of structural similarity between the docked pose and the native ligand, suggesting that the docking

protocol is valid and the ligand binding orientation is reliable. The favorable ΔG and Ki values demonstrate moderate to strong binding affinity of the ligand toward the androgen receptor, further supporting its potential as a candidate for therapeutic modulation in PCOS management.

Table 3 Results of molecular docking analysis on the androgen receptor.

Ligand	Type ligand	Binding energy	Konstanta inhibisi	Number of hydrogen bond (HB)	Number of hydrophobic interaction (HI)
Androgen	Native ligand	−7.42 kcal/mol	3.65 uM	Glu837, Glu829, Arg840, Asn727, Phe673, Ile672	Pro723, Phe673
(-)-Epiafzelechin-3-O-(6”-O-acetyl)-β-D-allosepyranoside	Test ligand	−4.29 kcal/mol	712.55 uM	Glu833, Glu829	Pro723
3’,5-Dihydroxy-7,4’-dimethoxy flavone	Test ligand	−5.17 kcal/mol	162.22 uM	Gly724, Lys720	Pro723, Arg276
Daidzin_1	Test ligand	−5.44 kcal/mol	103.05 uM	Glu837, Ala721	Pro723, Phe673, Val676, Glu837
Ononin	Test ligand	−5.27 kcal/mol	137.17 uM	Arg840, Leu722	Pro723, Phe725, Val676, Ile672
Pelargonidin 3-glucoside	Test ligand	−4.33 kcal/mol	666.08 uM	Glu837, Ile672	Pro723, Val676, Ile672
Tiliroside	Test ligand	−7.41 kcal/mol	3.71 uM	Glu837, Glu829, Ala721	Pro723, Arg726, Leu722
Vitamin E (Alpha Tocopherol)	Test ligand	−6.19 kcal/mol	29.02 uM	Ala721	Pro723, Phe673, Ile672, Val676, Leu830, Tyr834
Vitamin B6 (Pyridoxin)	Test ligand	−4.03 kcal/mol	1.12 mM	Ala721, Gly724, Lys720	Pro723, Arg726
L-Methionine	Test ligand	−3.70 kcal/mol	1.95 mM	Glu837, Arg840, Phe673, Ile672	Pro723, Phe673, Arg840, Val676
L-Aspartic Acid	Test ligand	−3.46 kcal/mol	2.89 mM	Glu837, Phe673, Ile672	Arg840
Metformin	Control ligand	−3.90 kcal/mol	1.37 mM	Asn833	Glu837
Letrozole	Control ligand	−5.60 kcal/mol	78.14 uM	Phe673, Ile672	Glu837, Phe673, Tyr834, Lys836

Results of molecular docking

The principal result of the molecular docking process was the binding affinity, typically represented as a docking score. **Table 3** summarizes the binding affinity values of the test ligands to the androgen receptor, the native ligand, and the positive control

ligands (Letrozole and Metformin). The endogenous ligand demonstrated a docking score of –7.42 kcal/mol. The docking scores for the test ligands were as follows: (-)-Epiafzelechin-3-O-(6”-O-acetyl) -β-D-allosepyranoside: −4.29 kcal/mol**,** 3’,5-Dihydroxy-7,4’-dimethoxyflavone: –5.17 kcal/mol**,** Daidzin_1: –5.44 kcal/mol**,** Ononin: –5.27 kcal/mol**,** Pelargonidin 3-glucoside: –4.33 kcal/mol**,** Tiliroside: –7.41 kcal/mol**.** Tiliroside had the greatest binding affinity to the androgen receptor among the tested ligands, with a docking score of –7.41 kcal/mol. This value is almost identical to that of the native ligand and markedly exceeds the docking scores of the positive control ligands, Letrozole (–5.60 kcal/mol) and Metformin (–3.90 kcal/mol). The data indicate that Tiliroside exhibits a significant binding affinity for the androgen receptor. To verify the docking outcomes, the root mean square deviation (RMSD) was computed for the re-docked native ligand in comparison to its crystallographic position. An RMSD value of ≤2 Å is often deemed acceptable, signifying the structural dependability of the docked complex. This study reports an RMSD value of 1.956 Å for the androgen receptor–ligand complex, affirming the precision and stability of the docking configuration. The hERG channel plays a critical role in cardiac repolarization, and its inhibition can lead to arrhythmias with implications for patient safety. While *in silico* data provide preliminary indications, confirmation through *in vitro* and *in vivo* studies is needed to gain a more comprehensive understanding of its safety profile [24].

Table 4 ADME predictions of tiliroside and vitamin E in PSE.

ADME	Parameter	Tiliroside	Vitamin E
Absorption	Caco2	14.88	29.12
	MDCK	0.04	38.91
	HIA	67.59	97.83
	Skin Permeability	−3.08	−0.52
Distribution	BBB	0.07	19.90
	Pgp inhibition	Inhibitor	Inhibitor
	PPB	100.00	100.00
Metabolisme	CYP_2C19 inhibition	Inhibitor	Inhibitor
	CYP_2C9 inhibition	Inhibitor	Inhibitor
	CYP_2D6 inhibition	Non	Non
	CYP_3A4 inhibition	Inhibitor	Non
	CYP_3A4 substrate	Substrat	Substrat
Elimination	Pure water salobulity (mg/L)	10.9211	0.0006

Table 4 presents the predicted ADME (Absorption, Distribution, Metabolism, and Excretion) parameters of the active compounds Tiliroside and Vitamin E, revealing significant differences in their pharmacokinetic profiles. This analysis provides a preliminary overview of the potential bioavailability and safety of the compounds present in PSE. Evaluation of absorption parameters indicates that Vitamin E exhibits superior oral absorption potential compared to Tiliroside. This is reflected in the Human Intestinal Absorption (HIA) value of 97.83% for Vitamin E, which is classified as very high, while Tiliroside demonstrates a moderate HIA value of 67.59%. These findings are further supported by intestinal permeability data based on Caco-2 and MDCK models, where Vitamin E shows higher permeability values (29.12 and 38.91, respectively) than Tiliroside (14.88 and 0.04), indicating lower transcellular permeability for Tiliroside. In terms of distribution, skin permeability (log Kp) values suggest that Tiliroside (–3.08) possesses very limited dermal penetration compared to Vitamin E (–0.52), making

Tiliroside less suitable for topical applications unless combined with penetration enhancers. Blood-Brain Barrier (BBB) permeability analysis further shows that Vitamin E, with a value of 19.90, has the potential to cross the BBB, while Tiliroside, with a value of 0.07, demonstrates minimal central nervous system penetration. Both compounds are identified as P-glycoprotein (P-gp) inhibitors, which may influence the pharmacokinetics of co-administered drugs through efflux mechanisms. Additionally, both Tiliroside and Vitamin E exhibit a plasma protein binding (PPB) rate of 100%, indicating that the majority of each compound circulates in a bound form, with only a minimal free fraction available for pharmacological activity. Collectively, these findings suggest that while Tiliroside possesses promising biological activities, its pharmacokinetic limitations - particularly in absorption and distribution—may necessitate advanced drug delivery strategies to enhance its therapeutic efficacy.

The metabolic profiling of Tiliroside and Vitamin E reveals notable differences in their interactions with cytochrome P450 (CYP450) enzymes. Tiliroside exhibits a broader inhibitory profile, particularly against CYP2C19, CYP2C9, and CYP3A4, suggesting a higher potential for drug–drug interactions mediated through hepatic metabolism. In contrast, Vitamin E inhibits only CYP2C19 and CYP2C9, indicating a more selective metabolic pathway interference. Notably, neither compound inhibits CYP2D6, reducing the risk of interactions via this isoenzyme. Both compounds are identified as substrates for CYP3A4, indicating that their metabolic clearance is largely dependent on this enzyme. However, Tiliroside's dual role - as both a substrate and an inhibitor of CYP3A4 - raises concerns about auto-inhibition, which could impair its own metabolism and lead to systemic accumulation, particularly under chronic dosing [25]. A significant contrast is seen in aqueous solubility. Tiliroside demonstrates a moderate solubility of 10.92 mg/L, while Vitamin E exhibits extremely poor solubility at 0.0006 mg/L, consistent with its high lipophilicity [26]. Poor water solubility in Vitamin E correlates with limited renal excretion, extended half-life, and potential bioaccumulation in adipose tissue. Additionally, recent advances highlight the use of nanoformulations to enhance the bioavailability of Vitamin E tocotrienols, due to their inherently low systemic absorption [27]. Therefore, while Tiliroside shows promising biological activity, its pharmacokinetic limitations - particularly in absorption and distribution - suggest a need for optimized drug delivery strategies to maximize its therapeutic potential.

Table 5 Toxicity of phytocompounds from pineapple stem.

No	Compound	Ames test	Carsino rat	hERG inhibition	Minnow at
1.	(-)-Epiafzelechin-3-O-(6"-O-acetyl)-β-D-allosepyranoside	Non- mutagen	Negative	High risk	0.613497
2.	3',5-Dihydroxy-7,4'-dimethoxy flavone	Mutagen	Positive	Medium risk	0.0161312
3.	Daidzin_1	Mutagen	Negative	Medium risk	0.676266
4.	Ononin	Mutagen	Negative	Medium risk	0.599931
5.	Pelargonidin 3-glucoside	Non-mutagen	Negative	High risk	1.74613
6.	Tiliroside	Non-mutagen	Negative	High risk	0.0194001

An *in silico* toxicological assessment of phytochemical compounds derived from pineapple stem extract was performed by evaluating several key parameters, including the Ames test (mutagenicity), rat carcinogenicity (Carcino Rat), hERG channel inhibition, and aquatic toxicity prediction using the Minnow Acute Toxicity (Minnow AT) model. The complete results are presented in **Table 5**. Among the compounds tested, Tiliroside demonstrated the most balanced toxicological profile. It was predicted to be non-mutagenic and non-carcinogenic in rodents, which supports its safety in terms of genotoxic and

carcinogenic potential. However, Tiliroside showed a high probability of hERG channel inhibition, indicating a potential risk of cardiotoxicity, particularly related to QT prolongation. Moreover, the compound was also predicted to pose a significant risk for aquatic toxicity, as reflected by its Minnow AT score. While Tiliroside shows promising pharmacological efficacy, these findings suggest that further experimental validation - both *in vitro* and *in vivo* - is essential, especially to evaluate its cardiac safety profile and potential ecotoxicological impacts. A comprehensive toxicological characterization is imperative before Tiliroside can be considered a viable drug candidate for clinical or therapeutic applications.

Effects of pineapple stem extract on LH, FSH, testosterone, and AGEs levels

Descriptive analysis of LH, FSH, testosterone, and AGEs concentrations

This study employed a quantitative experimental approach to evaluate the therapeutic effects of pineapple stem extract on endocrine disruption and oxidative stress in a polycystic ovary syndrome (PCOS) animal model. PCOS was induced by administering dehydroepiandrosterone (DHEA) at a dose of 6 mg/100 g body weight, dissolved in 0.2 mL of sesame oil, and delivered orally for 42 consecutive days. This protocol has been previously validated to mimic the physiological characteristics of PCOS, including elevated androgen levels and histological alterations in the ovaries, which closely resemble the clinical phenotype observed in human PCOS [28]. Subsequent to the establishment of the PCOS model, the subjects were divided into 6 groups: The normal control group (KN), the negative control group (KNeg), the positive control group (KPos), and 3 treatment groups (KP1, KP2 and KP3) administered pineapple stem extract at dosages of 500 and 1,000 mg/kg BW over a duration of 27 days. The biological parameters measured were Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), testosterone, and Advanced Glycation End Products (AGEs), all of which are integral to inflammatory processes and metabolic stress. The parameters were evaluated using the Enzyme-Linked Immunosorbent Assay (ELISA) at 2 time points: Day 0 (H0), immediately post-DHEA induction, and day 27 (H27), subsequent to therapeutic intervention. The findings of this investigation are displayed in **Table 6** below.

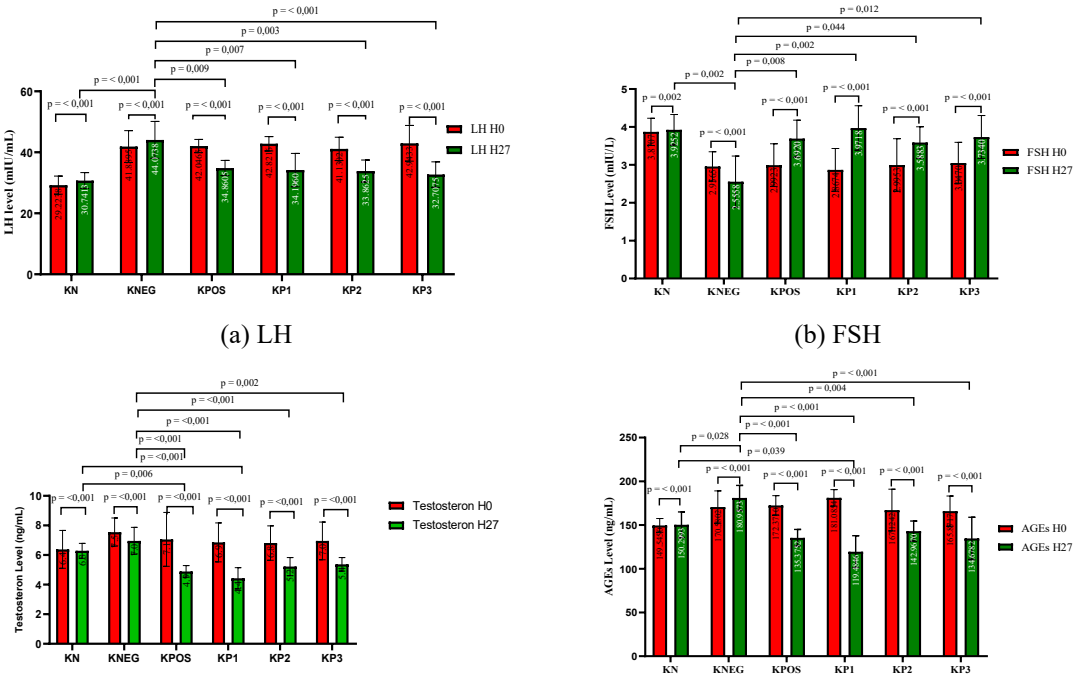
Table 6 Analysis of ELISA results for serum LH, FSH, testosterone, and AGEs levels.

No	Variable	Group						*p-value*
		KN	**KNeg**	**KPos**	**KP1**	**KP2**	**KP3**	
		Mean						
1	**LH H0**	29.223	41.889	42.046	42.821	41.130	42.943	
	Std.deviasi	2.97	5.20	2.17	2.35	3.81	5.86	
	Shapiro wilk	0.232*	0.360*	0.740*	0.063*	0.501*	0.881*	
	Levene Test							0.082**
	Anova							<0.001***
	LH H27	30.741	44.073	34.860	34.196	33.862	32.707	
	Std.deviasi	2.63	6.04	2.48	5.44	3.60	4.18	
	Shapiro Wilk	0.090*	0.076*	0.170*	0.130*	0.413*	0.411*	
	Levene Test							0.052**
	Anova							<0.001***
2	**FSH H0**	3.87	2.95	2.99	2.86	2.99	3.04	
	Std.deviasi	0.35	0.38	0.56	0.56	0.69	0.54	
	Shapiro wilk	0.357*	0.802*	0.268*	0.903*	0.405*	0.321*	

No	Variable	Group						*p-value*
		KN	**KNeg**	**KPos**	**KP1**	**KP2**	**KP3**	
		Mean						
	Levene Test							0.234**
	Anova							0.030***
	FSH H27	3.92	2.55	3.69	3.97	3.58	3.73	
	Std.deviasi	0.40	0.67	0.48	0.58	0.41	0.56	
	Shapiro Wilk	0.379*	0.598*	0.011	0.552*	0.026	0.405*	
	Levene Test							0.428**
	Kruskal-Wallis							0.017***
3	**Testosteron H0**	6.38	7.54	7.05	6.85	6.80	6.95	
	Std.deviasi	1.2	0.95	1.82	1.31	1.16	1.27	
	Shapiro Wilk	0.306*	0.876*	0.938*	0.699*	0.654*	0.473*	
	Levene Test							0.509**
	Anova							0.783
	Testosteron H27	6.28	6.95	4.88	4.43	5.21	5.36	
	Std.deviasi	0.50	0.91	0.39	0.71	0.61	0.45	
	Shapiro Wilk	0.592*	0.938*	0.446*	0.819*	0.143*	0.566*	
	Levene Test							0.618**
	Anova							<0.001***
4	**AGEs H0**	149.54	170.58	172.37	181.08	167.12	165.87	
	Std.deviasi	7.84	18.49	11.17	9.41	23.89	17.29	
	Shapiro Wilk	0.888*	0.180*	0.974*	0.166*	0.562*	0.044*	
	Levene Test							0.083**
	Anova							0.059
	AGEs H27	150.29	180.95	135.37	119.48	142.96	134.67	
	Std.deviasi	14.76	14.34	9.56	18.27	11.57	24.22	
	Shapiro Wilk	0.916*	0.442*	0.950*	0.648*	0.468*	0.178*	
	Levene Test							0.281**
	Anova							<0.001***

Hormonal and AGEs Profiles Following Pineapple Stem Extract Administration. At baseline (H0), there were no statistically significant differences observed in LH levels among the groups (p = 0.082), indicating that all animals were in comparable physiological states following DHEA induction. However, after 27 days of intervention (H27), a significant difference emerged (p < 0.001), suggesting a therapeutic effect of the treatments on the endocrine system. The negative control group (KNeg) exhibited the highest LH levels (44.073 ± 6.04), whereas the treatment group KP3 showed a marked reduction toward physiological levels, indicating partial restoration of pituitary function. Similarly, FSH levels showed no significant differences at baseline (p = 0.234). After treatment, Kruskal–Wallis analysis confirmed a significant difference among groups (p = 0.017). The KP1 group displayed the most notable increase in FSH concentration (3.97 ± 0.58), suggesting a potential stimulatory effect on follicular

maturation by the intervention. Testosterone levels were homogenous across all groups at H0 (p = 0.509). By H27, significant differences were evident (p < 0.001), consistent with the hyperandrogenic profile characteristic of PCOS models. Treatment groups, particularly KP3, demonstrated a substantial decrease in testosterone levels (5.36 ± 0.45), approaching physiological norms and indicating effective hormonal regulation. Advanced Glycation End Products (AGEs), markers of non-enzymatic glycation implicated in oxidative stress and cellular senescence, showed no significant differences at baseline (p = 0.083). However, post-treatment ANOVA analysis revealed a significant reduction across groups (p < 0.001). Notably, KP1 (119.45 ± 18.27) and KP3 (134.67 ± 24.22) exhibited the most pronounced decreases, highlighting the antiglycation and antioxidant potential of bioactive compounds in pineapple stem extract. The significant reductions in LH, testosterone, and AGEs, alongside the elevation of FSH, suggest that phytochemicals present in the extract possess antiandrogenic, gonadotropic, and antioxidative properties. These findings support the hypothesis that pineapple stem extract may serve as a promising alternative therapeutic agent in the management of endocrine and metabolic dysregulation associated with polycystic ovary syndrome (PCOS). A detailed post hoc analysis is visualized in **Figure 4** below.



(a) LH (b) FSH

(c) Testosterone (d) AGEs

Figure 4 Graph of ELISA test results for LH, FSH, testosterone, and AGEs levels.

Figure 4(a) shows that the LH-H0 examination, after the SD rats were induced to the PCOS model, revealed average values in the KNeg, KPos, KP1, KP2, and KP3 groups exceeding the average value of the KN group for LH levels (29.223 ± 2.97). In the LH-27 examination, after the administration of EBN, the average values in the KPos, KP1, KP2, and KP3 groups decreased, approaching the KN group average (30.741 ± 2.63). Meanwhile, the KNeg group showed an increase in the average LH level from H0 (41.889 ± 5.20) to H27 (44.073 ± 6.04). The normality test for the data yielded a Shapiro-Wilk value > 0.05, and the Levene's test gave a p-value of 0.052 (p-value >0.05), meaning the assumptions for the 1-way ANOVA test were met. The results of the ANOVA test showed a p-value <0.001, indicating significant differences among all groups at H27 after the treatment. Statistical analysis was continued with the Paired Sample Test to assess differences between paired groups. A p-value of <0.001 was obtained for each paired group, indicating significant differences between each group before and after treatment. Subsequently, post-hoc Tukey HSD

tests were performed, and the results shown in **Figure 4(a)** revealed significant differences between KN and KNeg ($p < 0.001$), KNeg and KPos (p-value 0.009), KNeg and KP1 (p-value 0.007), KNeg and KP2 (p-value 0.003), and KNeg and KP3 (p-value <0.001).

In PCOS, the frequency of Gonadotropin-Releasing Hormone (GnRH) pulses increases, which leads to increased LH secretion by the pituitary gland and results in a high LH/FSH ratio. Excessive LH stimulates the theca cells of the ovaries to produce large amounts of androgens, while low FSH levels are insufficient to support optimal follicular maturation. Consequently, follicular development halts, and ovulation does not occur. Excess LH can inhibit ovulation and interfere with dominant follicle formation, leading to immature follicles that fail to release eggs. This causes women with PCOS to experience irregular menstrual cycles or even amenorrhea [29]. The administration of mineral supplements can significantly reduce LH levels and improve ovarian quality and the LH/FSH hormonal balance in PCOS rat models [30]. A study published in the Asian Pacific Journal of Reproduction in 2023 showed that vitamin E supplementation in DHEA-induced PCOS rat models significantly reduced LH levels. This decrease was accompanied by an increase in progesterone and estrogen levels, as well as improvements in ovarian morphology, including reduced cystic follicles and enhanced antioxidant enzyme activity, such as SOD, CAT, and GSH [31]. Vitamin E supplementation can reduce LH levels in PCOS rats, but my research focuses on Pineapple Stem Extract (PSE) as a natural alternative. The main difference between the 2 is that vitamin E primarily targets oxidative stress and hormone balance, whereas PSE contains a variety of bioactive compounds that influence multiple pathways, including antioxidant activity, hormone modulation, and follicle development. Therefore, while both address similar issues, PSE has the potential to offer a broader therapeutic effect compared to vitamin E.

The results presented in **Figure 4(b)** show a significant effect on FSH levels after the administration of PSE in the PCOS rat model, particularly in the treatment groups KPos, KP1, KP2, and KP3. The most significant effect was observed in the KP1 group (PSE 500 mg/kg body weight), with a p-value of 0.002. This finding confirms that the treatment or intervention at a dose of 500 mg/kg body weight in the PSE control group has proven to be effective and can serve as a reference in understanding the potential of such interventions in the PCOS rat model. These results also support the hypothesis that the treatment administered influences the increase in FSH levels. In women with PCOS, there is a relative decrease in FSH levels compared to the high levels of LH. This imbalance leads to ovulatory dysfunction, where developing follicles fail to mature adequately for normal ovulation. These immature follicles then form cysts, which are characteristic of the ovaries in women with PCOS [32]. This study is highly relevant to previous research that showed the administration of soybean isoflavones to PCOS rat models increased FSH and estradiol levels, while decreasing LH and testosterone levels. This therapy also improved estrous cycles and ovarian morphology [33]. The flavonoid compounds identified in PSE include Ononin, Tiliroside, and Daidzin_1. Ononin and Daidzin_1 are isoflavonoid flavonoids and are classified as phytoestrogens, which are plant compounds that mimic or modulate the effects of estrogen in the human body. Phytoestrogens exert weaker effects compared to natural estrogen, making them safer for long-term use [34].

Increased testosterone levels in women with PCOS can disrupt ovarian function, causing ovulatory dysfunction and infertility [35]. The results shown in **Figure 4(c)** demonstrate a significant effect on testosterone levels after the administration of PSE in the PCOS rat model, particularly in the treatment groups KPos, KP1, KP2, and KP3. The most significant effects were observed in the KPos, KP1, and KP2 groups, with p-values <0.001. Treatment or intervention that has proven effective in the positive control group (letrozole 2.5 mg/kg body weight), the PSE control group at 500mg/kg body weight, and the PSE control group at 1000mg/kg body weight can serve as a reference for understanding the potential of such interventions in the PCOS rat model. This study is highly relevant to previous research indicating that vitamin E supplementation can reduce testosterone and LH levels while increasing progesterone and FSH levels in women with PCOS [36]. Moreover, a study showed that zinc supplementation in PCOS rat models could reduce testosterone levels and improve egg

quality [37]. Zinc also helps reduce insulin resistance and inflammation, contributing to the decrease in testosterone levels [38]. Another study revealed that magnesium supplementation in women with PCOS could lower testosterone levels and improve insulin sensitivity [39]. Magnesium also helps reduce inflammation and oxidative stress, which contributes to the reduction in testosterone levels [39].

Advanced Glycation End Products (AGEs) are byproducts of the non-enzymatic glycation reaction between sugars and proteins or fats in the body. This process occurs when glucose or fructose binds to proteins, resulting in compounds known as AGEs, which can cause cellular and tissue damage. AGEs have a significant impact on various medical conditions, including diabetes, cardiovascular diseases, and reproductive disorders such as PCOS [40]. The results of this study, shown in **Figure 4(d)**, indicate that after treatment on H27, there were significant differences between KN and KNeg, with a *p*-value of 0.028; KN and KP1, with a *p*-value of 0.039; and KNeg and KPos, with a *p*-value of <0.001. This study is highly relevant to previous research showing that the administration of apigenin and luteolin in PCOS rat models can increase antioxidant levels and reduce AGEs in ovarian tissues, as well as improve ovarian function and decrease testosterone levels [41]. The phytochemical analysis of PSE revealed a high antioxidant activity of 28,360.38 mg/kg. The flavonoid compounds in PSE, including Ononin, Tiliroside, and Daidzin_1, have the ability to scavenge free radicals and protect cells from oxidative damage, which may contribute to various degenerative diseases, including cancer, heart disease, and premature aging [42]. Furthermore, therapies involving vitamin E, minerals such as zinc and magnesium, and certain amino acids have been shown to reduce AGEs levels and improve hormonal profiles in women with PCOS [43]. Magnesium also helps reduce oxidative stress and inflammation [44].

Effect of pineapple stem extract on follicular development

Histopathological examination was performed using Hematoxylin-Eosin (HE) staining, which highlights the cell nuclei in dark purple (due to hematoxylin) and the cytoplasm and extracellular matrix in pink (due to eosin). This examination aimed to observe the morphological features of the ovaries. The analysis of the average number of follicular development revealed the following results: Primordial follicles (7.17), primary follicles (5.54), secondary follicles (5.91), tertiary follicles (2.29), Graafian follicles (2.66), cystic follicles (0.94), atretic follicles (3.20), and corpus luteum (4.06).

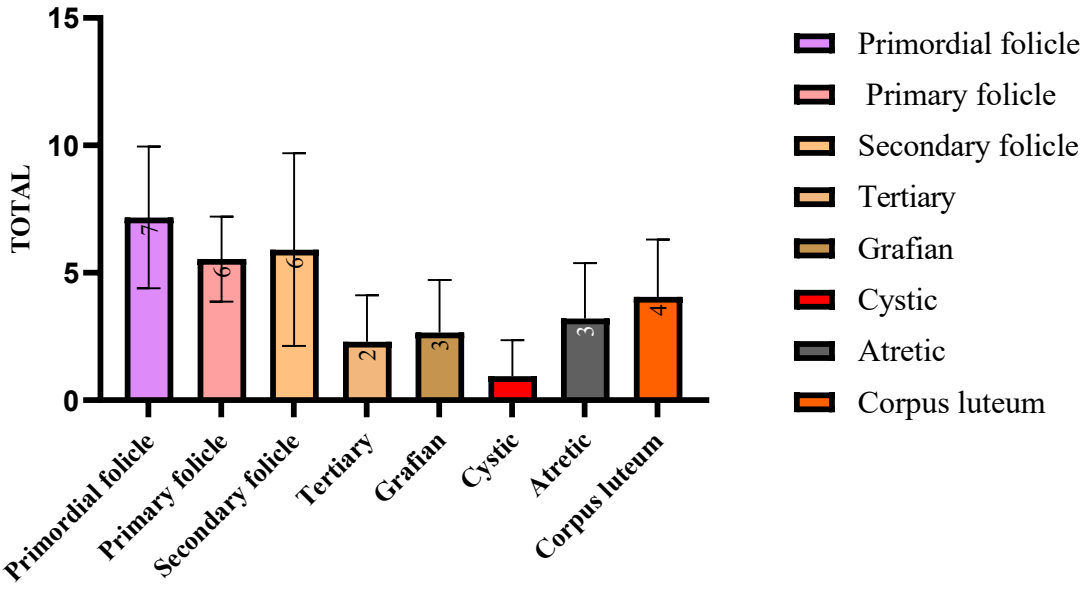


Figure 5 Average follicular development after PSE administration.

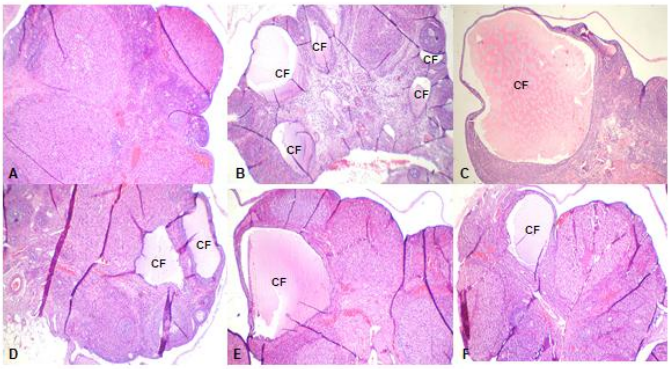


Figure 6 Histopathological examination results of the ovary.

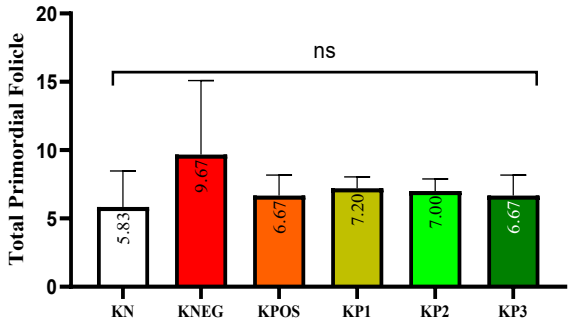
The histopathological examination of the ovary in **Figure 6** shows the absence of cystic follicles (CF) in the control group (A). Multiple cystic follicles were observed in the Kneg group (B). The number of cystic follicles decreased in the KPos group (C), KP1 group (D), KP2 group (E), and KP3 group (F) (Magnification: 40×).

Table 7 Mean rank of the number of primordial, primary, secondary, tertiary, graafian, cystic, atretic follicles, and corpus luteum in female SD rats treated with KN, KNeg, KPos, KP1, KP2, KP3 after 27 days of treatment.

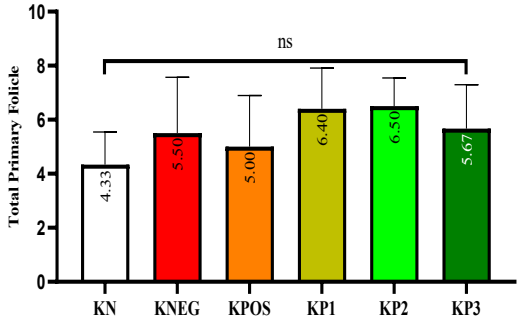
Follicular development	KN	KNeg	KPos	KP1	KP2	KP3	*p*-value
Primordial	10.25	21.42	18.25	21.50	20.00	17.17	0.400
Primary	10.50	18.08	15.17	22.70	23.75	18.58	0.233
Secondary	30.42	12.75	12.17	20.50	18.50	14.08	0.017*
Tertiary	19.58	8.92	27.92	14.60	13.83	22.58	0.015*
Graafian	16.92	6.75	26.75	15.10	14.92	27.08	0.003*
Cystic	10.50	28.42	17.17	20.20	17.67	14.42	0.024*
Atretic	16.92	31.92	14.58	9.00	15.33	18.75	0.005*
Corpus Luteum	21.00	20.67	22.50	9.20	12.42	20.75	0.143

Table 7 mean rank of the number of primordial, primary, secondary, tertiary, graafian, cystic, atretic follicles, and corpus luteum in female SD rats treated with control, PCOS, PCOS + letrozole, PCOS + PSE dose 1, PCOS + PSE dose 2, and PCOS + combination of PSE and letrozole after 27 days of treatment. The mean rank for the number of follicles in each group was calculated using the Kruskal-Wallis test. The results of the mean rank for each follicle type in each group are as follows: KN Group: Primordial (10.25), Primary (10.50), Secondary (30.42), Tertiary (19.58), Graafian (16.92), Cystic (10.50), Atretic (16.92), Corpus Luteum (21.00). KNeg Group: Primordial (21.42), Primary (18.08), Secondary (12.75), Tertiary (8.92), Graafian (6.75), Cystic (28.42), Atretic (31.92), Corpus Luteum (20.67). KPos Group: Primordial (18.25), Primary (15.17), Secondary (12.17), Tertiary (27.92), Graafian (26.75), Cystic (17.17), Atretic (14.58), Corpus Luteum (22.50). KP1 Group: Primordial (21.50), Primary (22.70), Secondary (20.50), Tertiary (14.60), Graafian (15.10), Cystic (20.20), Atretic (9.00), Corpus Luteum (9.20). KP2 Group: Primordial (20.00), Primary (23.75), Secondary (18.50), Tertiary (13.83), Graafian (14.92), Cystic (17.67), Atretic (15.33), Corpus Luteum (12.42). KP3 Group: Primordial (17.17), Primary (18.58), Secondary

(14.08), Tertiary (22.58), Graafian (27.08), Cystic (14.42), Atretic (18.75), and Corpus Luteum (20.75). The results of the Kruskal-Wallis statistical test for all groups on each follicle showed the following *p*-values: Primordial follicle: *p*-value = 0.400, Primary follicle: *p*-value = 0.233, Secondary follicle: *p*-value = 0.17, Tertiary follicle: *p*-value = 0.015, Graafian follicle: *p*-value = 0.003, Cystic follicle: *p*-value = 0.24, Atretic follicle: *p*-value = 0.005, Corpus luteum: *p*-value = 0.143. Therefore, statistically, it can be concluded that there is no significant difference in primordial follicles, primary follicles, and corpus luteum (*p*-value >0.05), while significant differences were observed in secondary follicles, tertiary follicles, Graafian follicles, cystic follicles, and atretic follicles (*p*-value <0.05). For the variables with significant differences, further analysis was performed using the Post Hoc Dunn test.



(A)



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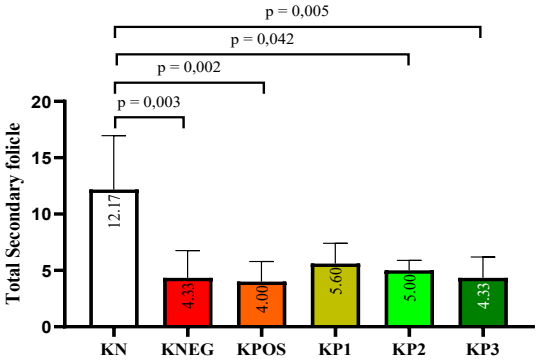

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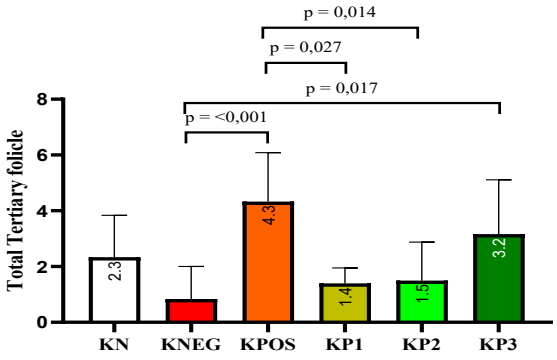

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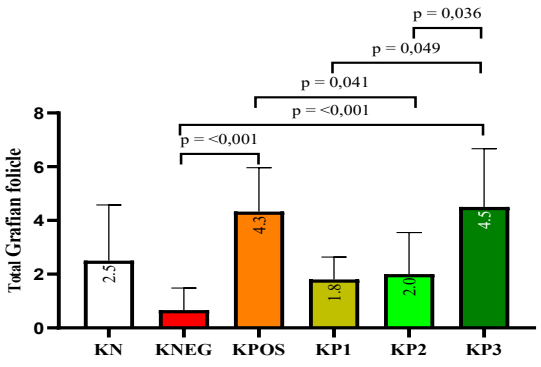

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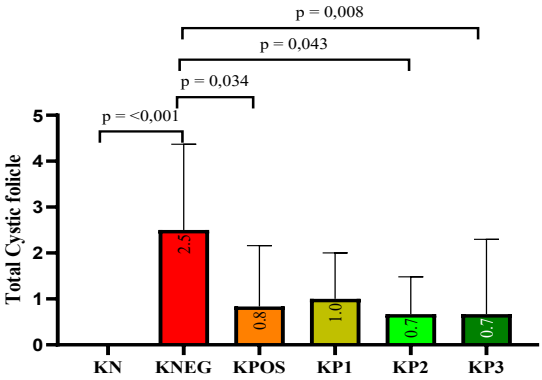

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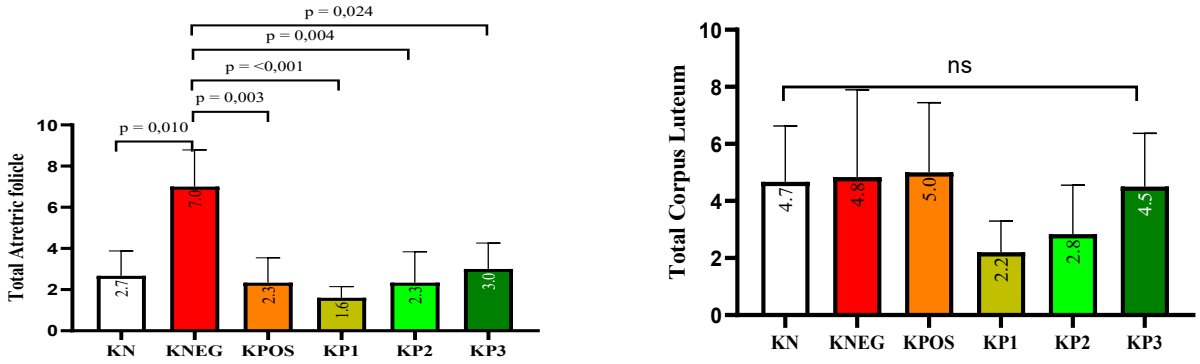


Figure 7 Graph of analysis of the number of ovarian follicles after being given PSE.

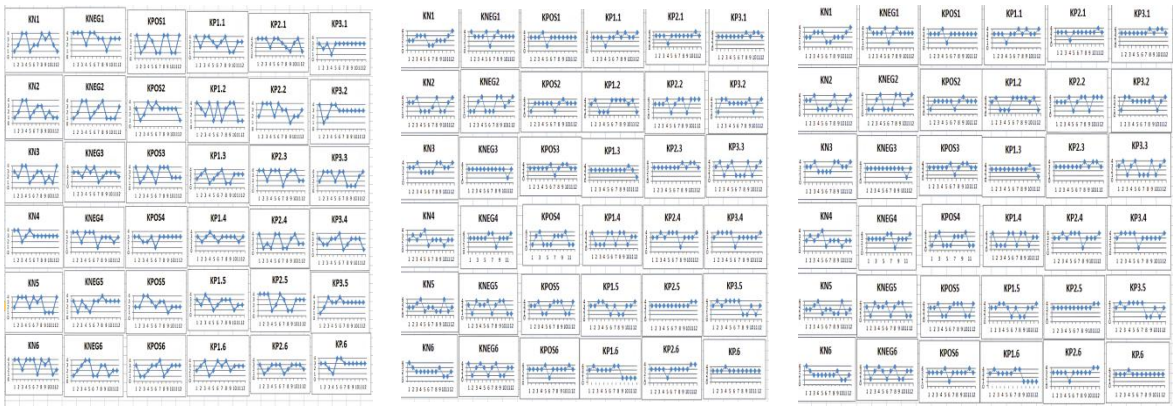
High primordial follicle counts in PCOS indicate a blockade in development, with improvement marked by a reduction in the number of primordial follicles, although these results were not statistically significant [45]. Primary follicles are often trapped at this stage and fail to further develop. This is due to hormonal imbalances, such as elevated levels of LH (Luteinizing Hormone), which inhibits follicular maturation. As a result, the number of primary follicles remains higher than in normal ovaries [46]. In PCOS, secondary follicles may accumulate in the ovaries without progressing to tertiary follicles or ovulation. The hormonal imbalance prevents these follicles from developing into dominant follicles that are capable of ovulating [47]. Tertiary follicles are more developed, with a clear antral cavity and surrounded by a thicker layer of granulosa cells. In women with PCOS, many follicles become trapped at the tertiary follicle stage but are unable to progress to Graafian follicles or ovulate [46]. Graafian follicles are fully mature and ready for ovulation, characterized by a thick granulosa layer, a large antral fluid cavity, and a fully developed oocyte. In women with PCOS, these follicles rarely form because most developing follicles do not reach this stage of maturity. Cystic follicles are a hallmark of polycystic ovaries (PCOS), where follicles fail to develop and eventually turn into cysts. PCOS increases the number of cystic follicles, reflecting anovulation and ovarian dysfunction. Atretic follicles are those that degenerate or fail to mature, usually due to hormonal disturbances or an ovarian environment that is not conducive to their development. The corpus luteum is the structure formed after ovulation when the mature Graafian follicle ruptures and releases the oocyte. The corpus luteum produces progesterone, which is essential for preparing the endometrium for implantation.

Therapy with flavonoids, vitamin E, minerals, and amino acids has shown potential in improving follicular development in the PCOS rat model. The administration of these compounds can regulate hormonal balance, increase insulin sensitivity, reduce inflammation, and improve egg quality, all of which contribute to the improvement of follicular development [49]. Administration of vitamin E can enhance the corpus luteum, increase the number of primary follicles, and improve the key structural parameters of follicles at the later stages of follicular development [50].

Effect of pineapple stem extract on ovulation as observed from estrous cycle examination

The estrous cycle examination was performed after a 7-day adaptation period. A total of 36 female SD rats from all groups underwent daily vaginal swabbing between 09:00 and 10:00 AM WIB for 12 consecutive days. Vaginal swabs were collected using cotton buds inserted into the vagina, after which the samples were placed on glass slides and analyzed under a light microscope. The examination results, shown in **Figure 8(a)**, indicate that all groups experienced the estrous cycle (proestrus, estrus, metestrus, and diestrus). The results from **Figure 8(b)** show that after induction with DHEA (6 mg per 100 g body weight) dissolved in 0.2 mL sesame oil for 12 consecutive days, changes in the cycle were observed. In the KN group (non-DHEA induced), the cycle was normal, with the rats going through proestrus, estrus,

metestrus, and diestrus phases. In contrast, the KNeg group, induced with DHEA, showed no estrus phase, with only proestrus, metestrus, and diestrus phases present. The estrous cycle examination after DHEA induction (6 mg per 100 g body weight) dissolved in 0.2 mL sesame oil for 42 days, followed by oral administration of PSE for 27 days, showed changes as seen in **Figure 8(c)**. In the KN group (no DHEA+PSE), no changes in the cycle were observed. The KNeg group experienced an irregular estrous cycle, while the KPos, KP1, KP2, and KP3 groups exhibited a normal cycle. The SD rats with PCOS, after receiving PSE, experienced a normal estrous cycle (proestrus, estrus, metestrus, and diestrus). Calcium plays a crucial role, especially during the ovulation phase, as it influences the release of gonadotropin-releasing hormone (GnRH), which controls ovulation by stimulating the release of LH [51]. This function is different from other minerals in PSE that support overall ovarian health. While calcium is essential for ovulation, it works in conjunction with other minerals to support ovarian function and the treatment of PCOS.



(a) Normal (b) PCOS Model (c) Post PSE

Figure 8 Estrous cycle examination results.

Conclusions

This study demonstrates that pineapple stem extract (PSE) exhibits antiandrogenic properties with potential application as an alternative therapy for polycystic ovary syndrome (PCOS). Administration of PSE significantly reduced luteinizing hormone (LH) and testosterone levels, increased follicle-stimulating hormone (FSH) levels, and decreased advanced glycation end-products (AGEs). Moreover, PSE effectively improved follicular development and ovulatory processes in female Sprague Dawley rats with a DHEA-induced PCOS model. These findings suggest that PSE may serve as a promising therapeutic candidate for managing PCOS. *In silico* analysis of the active compound tiliroside - present in PSE - revealed a strong binding affinity to the androgen receptor. Tiliroside also exhibited a relatively balanced toxicity profile, being non-mutagenic and non-carcinogenic, although it demonstrated potential risks related to hERG channel inhibition and aquatic toxicity. The administration of PSE at a dose of 500 mg/kg body weight has been shown to be effective in restoring hormonal balance and improving ovarian function, with minimal side effects. Based on extrapolation for human use, the recommended clinical dose is estimated to be 280 mg/70 kg body weight, [13] administered orally once daily. The importance of allergy testing in the methodology should be emphasized to ensure the safety of PSE administration before human trials. Further studies are also required to evaluate the absorption, distribution, metabolism, and excretion (ADME) of Pineapple Stem Extract (PSE) in humans. Additional clinical studies are needed to comprehensively assess the efficacy and safety of PSE in human populations for the management of PCOS.

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Declaration of Generative AI in Scientific Writing

During the preparation of this work, the authors used generative AI (ChatGPT, OpenAI's GPT-4) solely for language polishing, specifically for editing and checking grammar. We unequivocally state that this tool was not used to generate scholarly material, develop concepts, analyze data, or interpret findings. The authors bear complete responsibility for the work's intellectual substance, validity, and integrity.

CRediT Author Statement

Desi Kumalasari: Conceptualization, Methodology, Formal analysis, Resources, Investigation, Validation, Project administration, and writing-original draft. **Sri Sulistiyowati:** Project administration, Data curation, Formal analysis, Investigation. **Reviono Reviono:** Data curation, Formal analysis, Investigation. **Uki Retno Budihastuti:** Data curation, Formal analysis, Investigation, and writing-original draft. **Soetrisno Soetrisno:** Formal analysis, Investigation, and writing-original draft. **Brian Wasita:** Methodology, Data curation, Formal analysis, Investigation, and writing-original draft. **Eti Poncorini Pamungkasari:** Methodology, Data curation, Formal analysis, Investigation, and writing-original draft. **Paramasari Dirgahayu:** Formal analysis, Investigation, and writing-original draft.

References

[1] S Amanat, F Ashkar, MH Eftekhari, N Tanideh, S Doaei, M Gholamalizadeh, F Koohpeyma and M Mokhtari. The effect of genistein on insulin resistance, inflammatory factors, lipid profile, and histopathologic indices in rats with polycystic ovary syndrome. *Clinical and Experimental Reproductive Medicine* 2021; **48(3)**, 236.

[2] R Rani, YA Hajam, R Kumar, RA Bhat, S Rai and MA Rather. A landscape analysis of the potential role of polyphenols for the treatment of Polycystic Ovarian Syndrome (PCOS). *Phytomedicine Plus* 2022; **2(1)**, 100161.

[3] H Said, RW Fedre, S Hernandez, SL Rodriguez, F Mursyid and I Nettles. The prevalence and risk factors for Polycystic Ovary Syndrome (PCOS) among adolescents in indonesia: Implications for early intervention. *Sriwijaya Journal of Obstetrics and Gynecology* 2024; **2(1)**, 34-47.

[4] CZ Pei, L Jin and KH Baek. Pathogenetic analysis of polycystic ovary syndrome from the perspective of omics. *Biomedicine & Pharmacotherapy* 2021; **142**, 112031.

[5] M Rostamtabar, S Esmaeilzadeh, M Tourani, A Rahmani, M Baee, F Shirafkan, K Saleki, SS Mirzababayi, S Ebrahimpour and HR Nouri. Pathophysiological roles of chronic low-grade inflammation mediators in polycystic ovary syndrome. *Journal of Cellular Physiology* 2021; **236(2)**, 824-838.

[6] E Rudnicka, AM Duszewska, M Kucharski, P Tyczyński and R Smolarczyk. Oxidative stress and reproductive function: Oxidative stress in polycystic ovary syndrome. *Reproduction* 2022; **164(6)**, F145-F154.

[7] K Eskandar, JA Oliveira, SA Ribeiro, MP Chavez, AIA Zotti, YJM Dias and AMM Novellino. Review article Letrozole and clomiphene versus letrozole alone for ovulation induction in women with PCOS: A systematic review and meta-analysis. *Revista Brasileira de Ginecologia e Obstetrícia* 2025; **47**, e-rbgo21.

[8] WM Hikal, AA Mahmoud, HAH Said-Al Ahl, A Bratovcic, KG Tkachenko, M Kačániová and RM Rodriguez. Pineapple (*Ananas comosus* L. Merr.), waste streams, characterisation and valorisation: An overview. *Open Journal of Ecology* 2021; **11(9)**, 610-634.

[9] N Istiqomah, AH Ramadhani, RS Ningrum and E

Purwati. Ethanol extract analysis of steam pineapple (*Ananas comosus.* L) and its application as antibacterial agent: *In vitro* and *silico* studies. *IOP Conference Series: Earth and Environmental Science* 2021; **886(1)**, 012019.

[10] Y Öztürkler and A Güven. *The role of exogenous antioxidants in enhancing reproductive*. Livre de Lyon, Lyon, France, 2022.

[11] A Unar, M Imtiaz, TT Trung, M Rafiq, MQ Fatmi and TH Jafar. Structural and functional analyses of SARS COV-2 RNA-dependent RNA Polymerase protein and complementary vs. synthetic drugs against COVID-19 and the exploration of binding sites for docking, molecular dynamics simulation, and density functional theory studies. *Current Bioinformatics* 2022; **17(7)**, 632-656.

[12] LJ Chen, Y Liu, L Zhang, JY Li, WQ Xiong, T Li, H Ding and BJ Li. Sequential 2.5 mg letrozole/FSH therapy is more effective for promoting pregnancy in infertile women with PCOS: A pragmatic randomized controlled trial. *Frontiers in Endocrinology* 2024; **14**, 1294339.

[13] AB Nair and S Jacob. A simple practice guide for dose conversion between animals and human. *Journal of Basic and Clinical Pharmacy* 2016; **7(2)**, 27.

[14] ZEU Korun, SS Gocmez, S Furat, KK Sarihan, FC Eraldemir, HA Akpulat, D Sahin and S Yildiz. Beneficial effects of *Alchemilla vulgaris* in DHEA - induced rat model of polycystic ovary syndrome. *Reproductive Sciences* 2025; **32**, 2453-2466.

[15] S Koçak. PCOS animal models: An approach induced by dehydroepiandrosterone. *Experimental and Applied Medical Science* 2021; **2(1)**, 136-145.

[16] J Rudic, V Jakovljevic, N Jovic, M Nikolic, J Sretenovic, S Mitrovi, S Bolevich, S Bolevich, M Mitrovic, S Raicevic, K Andric, AD Milenkovic, D Rakic and JJ Jovic. Antioxidative effects of standardized *Aronia melanocarpa* extract on reproductive and metabolic disturbances in a rat model of polycystic ovary syndrome. *Antioxidants* 2022; **11(6)**, 1099.

[17] D Rakic, J Joksimovic Jovic, V Jakovljevic, V Zivkovic, M Nikolic, J Sretenovic, M Nikolic, N Jovic, MB Ilic, P Arsenijevic, A Dimitrijevic, T Vulovic, N Ristic, K Bulatovic, S Bolevich, L Stijak and S Pantovic. High fat diet exaggerate metabolic and reproductive PCOS features by promoting oxidative stress: An improved EV model in rats. *Medicina* 2023; **59(6)**, 1104.

[18] AF Ajayi and RE Akhigbe. Staging of the estrous cycle and induction of estrus in experimental rodents: An update. *Fertility Research and Practice* 2020; **6(1)**, 5.

[19] YF Chiang, IC Lin, KC Huang, HY Chen, M Ali, YJ Huang and SM Hsia. Caffeic acid's role in mitigating polycystic ovary syndrome by countering apoptosis and ER stress triggered by oxidative stress. *Biomedicine & Pharmacotherapy* 2023; **166**, 115327.

[20] AA Muhammed Saeed, S Noreen, FH Awlqadr, MI Farooq, M Qadeer, N Rai, HA Farag and MN Saeed. Nutritional and herbal interventions for polycystic ovary syndrome (PCOS): A comprehensive review of dietary approaches, macronutrient impact, and herbal medicine in management. *Journal of Health, Population and Nutrition* 2025; **44(1)**, 143.

[21] I Adam-Dima, AA Olteanu, OT Olaru, DE Popa and C Purdel. Methods of analysis of phytoestrogenic compounds: An up-to-date of the present state. *Separations* 2024; **11(7)**, 205.

[22] L Hu, Y Luo, J Yang and C Cheng. Botanical flavonoids: Efficacy, absorption, metabolism and advanced pharmaceutical technology for improving bioavailability. *Molecules* 2025; **30(5)**, 1184.

[23] S Ahmad, F Ahsan, JA Ansari, T Mahmood, A Shamim, S Bano, R Tiwari, VA Ansari, Shafiurrahman and M Kesari. A review on daidzein as food supplement: Exploring its phytopharmacological and preclinical status. *EFood* 2024; **5(5)**, e70008.

[24] M Vagos, IGMV Herck, J Sundnes, HJ Arevalo, AG Edwards and JT Koivumäki. Computational modeling of electrophysiology and pharmacotherapy of atrial fibrillation: Recent advances and future challenges. *Frontiers in Physiology* 2018; **9**, 1221.

[25] NV Mohamad. Strategies to enhance the solubility and bioavailability of tocotrienols using

self-emulsifying drug delivery system. *Pharmaceuticals* 2023; **16(10)**, 1403.

[26] AS Mohd Zaffarin, SF Ng, MH Ng, H Hassan and E Alias. Pharmacology and pharmacokinetics of vitamin E: Nanoformulations to enhance bioavailability. *International Journal of Nanomedicine* 2020; **15**, 9961-9974.

[27] CN Wong, SK Lee, YM Lim, SB Yang, YL Chew, AL Chua and KB Liew. Recent advances in vitamin E TPGS-based organic nanocarriers for enhancing the oral bioavailability of active compounds: A systematic review. *Pharmaceutics* 2025; **17(4)**, 485.

[28] KJ Chang, JH Chen and KH Chen. The pathophysiological mechanism and clinical treatment of polycystic ovary syndrome: A molecular and cellular review of the literature. *International Journal of Molecular Sciences* 2024; **25(16)**, 9037.

[29] S Singh, N Pal, S Shubham, DK Sarma, V Verma, F Marotta and M Kumar. Polycystic ovary syndrome: Etiology, current management, and future therapeutics. *Journal of Clinical Medicine* 2023; **12(4)**, 1454.

[30] E Günalan, A Yaba and B Yılmaz. The effect of nutrient supplementation in the management of polycystic ovary syndrome-associated metabolic dysfunctions: A critical review. *Journal of the Turkish German Gynecological Association* 2018; **19(4)**, 220-232.

[31] OT Olaniyan, A Dare, CO Adetunji, GE Okotie, JB Dare, BM Adigun and F Adebayo. Vitamin E modulates androgen receptor gene expression to attenuate ovarian dysfunctions in a rat model of dehydroepiandrosterone-induced polycystic ovary. *Asian Pacific Journal of Reproduction* 2023; **12**, 81-89.

[32] YA Hajam, HA Rather, R Kumar, M Basheer and MS Reshi. A review on critical appraisal and pathogenesis of polycystic ovarian syndrome. *Endocrine and Metabolic Science* 2024; **14**, 100162.

[33] X Ma, X Li, LL Ma, Y Chen and S He. Soy isoflavones alleviate polycystic ovary syndrome in rats by regulating NF- κB signaling pathway. *Bioengineered* 2021; **12(1)**, 7204-7212.

[34] VP Chavda, AZ Chaudhari, PC Balar, A Gholap and LK Vora. Phytoestrogens: Chemistry, potential health benefits, and their medicinal importance. *Phytotherapy Research* 2024; **38(6)**, 3060-3079.

[35] A Bushell and BJ Crespi. The evolutionary basis of elevated testosterone in women with polycystic ovary syndrome: An overview of systematic reviews of the evidence. *Frontiers in Reproductive Health* 2024; **6**, 1475132.

[36] G Tefagh, M Payab, M Qorbani, F Sharifi, Y Sharifi, MS Ebrahimnegad Shirvani, F Pourghazi, R Atlasi, Z Shadman, N Rezaei, E Mohammadi-Vajari, B Larijani and M Ebrahimpur. Effect of vitamin E supplementation on cardiometabolic risk factors, inflammatory and oxidative markers and hormonal functions in PCOS (polycystic ovary syndrome): A systematic review and meta - analysis. *Scientific Reports* 2022; **12(1)**, 5770.

[37] FF Torshizi, M Chamani, HR Khodaei, AA Sadeghi, SH Hejazi and RM Heravi. Therapeutic effects of organic zinc on reproductive hormones, insulin resistance and mTOR expression, as a novel component, in a rat model of Polycystic ovary syndrome. *Iranian Journal of Basic Medical Sciences* 2020; **23(1)**, 36-45.

[38] R Ahmad, R Shaju, A Atfi and MS Razzaque. Zinc and diabetes: A Connection between micronutrient and metabolism. 2024; **13(16)**, 1359.

[39] X Luo, WY Cai, HL Ma, J Cong, H Chang, JS Gao, WJ Shen, Y Wang, XM Yang and XK Wu. Associations of serum magnesium with insulin resistance and testosterone in women with polycystic ovary syndrome. *Frontiers in Endocrinology* 2021; **12**, 683040.

[40] J Chaudhuri, Y Bains, S Guha, A Kahn, D Hall, N Bose, A Gugliucci and P Kapahi. The role of advanced glycation end products in aging and metabolic diseases: Bridging association and causality. *Cell Metabolism* 2019; **28(3)**, 337-352.

[41] AV Sirotkin and AH Harrath. Apigenin as a promising agent for enhancing female reproductive function and treating associated disorders. *Biomedicines* 2024; **12(10)**, 2405.

[42] H Abrahamse and BP George. Flavonoids: Antioxidant powerhouses and their role in nanomedicine. *Antioxidants* 2024; **13(8)**, 922.

[43] S Alesi, C Ee, LJ Moran, V Rao and A Mousa. Nutritional supplements and complementary therapies in polycystic ovary syndrome. *Advances in Nutrition* 2022; **13(4)**, 1243-1266.

[44] N Zeber-Lubecka, M Ciebiera and EE Hennig. Polycystic ovary syndrome and oxidative stress - from bench to bedside. *International Journal of Molecular Sciences* 2023; **24(18)**, 14126.

[45] E Gershon and N Dekel. Newly identified regulators of ovarian folliculogenesis and ovulation. *International Journal of Molecular Sciences* 2020; **21(12)**, 4565.

[46] S Longobardi, FG Klinger, W Zheng, MR Campitiello, T D'Hooghe and A La Marca. Gonadotropin activity during early folliculogenesis and implications for polycystic ovarian syndrome and premature ovarian insufficiency: A narrative review. *International Journal of Molecular Sciences* 2024; **25(14)**, 7520.

[47] ASK Jones and A Shikanov. Follicle development as an orchestrated signaling network in a 3D organoid. *Journal of Biological Engineering* 2019; **13(1)**, 2.

[48] LA Owens, SG Kristensen, A Lerner, G Christopoulos, S Lavery, AC Hanyaloglu, K Hardy, CY Andersen and S Franks. Gene expression in granulosa cells from small antral follicles from women with or without polycystic ovaries. *The Journal of Clinical Endocrinology & Metabolism* 2019; **104(12)**, 6182-6192.

[49] V Calcaterra, E Verduci, H Cena, VC Magenes, CF Todisco, E Tenuta, C Gregorio, R De Giuseppe, A Bosetti, E Di Profio and G Zuccotti. Polycystic ovary syndrome in insulin-resistant adolescents with obesity: The role of nutrition therapy and food supplements as a strategy to protect fertility. *Nutrients* 2021; **13(6)**, 1848.

[50] SM Aburawi, SA Treesh, HA El Jaafari, MT El Ghedamsi, NA Nafati, OA Benmahmoud, M Almajry and N Shebani. Effect of vitamin E on polycystic ovary syndrome induced by dehydroepiandrosterone in female albino mice: Histological study. *Malaysian Journal of Pharmaceutical Sciences* 2021; **19(2)**, 111-130.

[51] C Kapper, P Oppelt, C Ganhör, AA Gyunesh, B Arbeithuber, P Stelzl and M Rezk-Füreder. Minerals and the menstrual cycle: Impacts on ovulation and endometrial health. *Nutrients* 2024; **16(7)**, 1008.