Ind. J. Pharm. Edu. Res., 2026; 60(4s):s1544-s1552.
https://www.ijper.org

Original Article

Chromatographic and Spectroscopic Characterisation of Bioactive Compounds from *Diplocyclos palmatus* for Polycystic Ovary Syndrome Management

Richa Yadav[1], Jaya Parkash Yadav[1,2,*]

[1]Department of Genetics, Maharshi Dayanand University, Rohtak, Haryana, INDIA.
[2]Ex-Vice Chancellor, Indira Gandhi University, Meerpur, Rewari, Haryana, INDIA.

ABSTRACT

Aim: Polycystic ovary syndrome is a prevalent endocrine disorder that arises from hormonal imbalance, typically presenting with excessive androgen production, disrupted ovulatory cycles, and various metabolic abnormalities. Current pharmacological options often cause side effects and show inconsistent efficacy, highlighting the need for safer alternatives. The present study aimed to investigate the phytoconstituents of *Diplocyclos palmatus* (L.) C. Jeffrey methanolic seed extract and evaluate their therapeutic efficacy against Polycystic Ovary Syndrome in a letrozole-induced rat model. **Materials and Methods:** Methanolic seed extract of *D. palmatus* was subjected to phytochemical screening, chromatographic separation, and structural characterisation using Reverse Phase High-Performance Liquid Chromatography, Gas Chromatography–Mass Spectrometry and Nuclear Magnetic Resonance techniques. The isolated bioactive compounds, quercetin and vanillic acid, were evaluated in a letrozole-induced rat model of Polycystic Ovary Syndrome to assess their effects on hormonal balance, ovarian histoarchitecture, and metabolic parameters. **Results:** Both quercetin and vanillic acid demonstrated significant therapeutic activity by restoring hormonal balance, improving ovarian morphology, and normalizing metabolic parameters through modulation of endocrine function and reduction of oxidative stress. Among the two, quercetin exhibited a more pronounced effect, indicating superior efficacy in alleviating Polycystic Ovary Syndrome-associated alterations compared to vanillic acid. **Conclusion:** The findings validate the traditional use of *D. palmatus* and identify its bioactive constituents as effective candidates for Polycystic Ovary Syndrome therapy. Quercetin and vanillic acid are both found to be promising compounds for the development of safe, plant-based therapeutics in PCOS management.

Keywords: Polycystic Ovarian Syndrome (PCOS), *Diplocyclos palmatus*, Quercetin, Vanillic acid

Correspondence:
Prof. Jaya Parkash Yadav, PhD
Department of Genetics, Maharshi Dayanand University, Rohtak-124001, Haryana, INDIA.
Email: yadav1964@rediffmail.com
ORCID: 0000-0002-3500-6811

Received: 15-06-2026;
Revised: 03-07-2026;
Accepted: 21-08-2026.

INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a major cause of infertility in women of reproductive age, with a global prevalence ranging from 5% to 25%.[1] It is characterised by hyperandrogenism and dysregulation of reproductive hormones, including Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), estrogen, and progesterone, and is often associated with insulin resistance, menstrual irregularities, and obesity.[2-4] The complex and multifactorial nature of PCOS makes its management challenging, as current therapies such as hormonal contraceptives, anti-androgens, and insulin sensitisers are frequently limited by adverse effects and poor long-term outcomes.[5-7] Medicinal plants offer a promising source of safer bioactive compounds for PCOS management. *Diplocyclos palmatus* (Cucurbitaceae), traditionally used in Ayurveda and folk medicine for reproductive and metabolic disorders, has been reported to contain flavonoids, phenolic acids, alkaloids, and terpenoids with notable antioxidant and hormone-modulating potential.[8-11] Isolation and characterisation of such bioactive constituents using advanced chromatographic and spectroscopic techniques, including HPLC, GC–MS, and NMR, are critical for pharmacological validation.[12,13] Accordingly, the present study aims to isolate and characterise bioactive compounds from the methanolic seed extract of D. palmatus and evaluate their efficacy in an experimental PCOS rat model, thereby supporting its potential role in the management of PCOS.

DOI: 10.5530/ijper.20263948

MATERIALS AND METHODS

Plant Collection and Authentication

Plants were collected between September and November 2022 from Jalukari, Chamba, Himachal Pradesh, India (32°33′09.2″ N, 76°07′48.7″ E). A voucher specimen (Accession No. 14405) was prepared and authenticated at the Patanjali Research Institute, Haridwar, Uttarakhand, India, and deposited for future reference.

Chemicals used

Analytical-grade standards and reagents were sourced from Sigma Aldrich Chemical Pvt. Ltd., India.

Preparation of Plant Extracts

The collected plant material was thoroughly washed, shade-dried, and powdered. The MSE was prepared using a Soxhlet extraction method. The extract was concentrated under reduced pressure at a temperature of 35-45°C using a rotary evaporator and then stored at 4°C until further analysis.[14]

Preliminary Phytochemical Screening

The MSE was subjected to qualitative phytochemical tests to detect alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids following standard protocols.[15,16]

Chromatographic Separation and Isolation of Bioactive Compounds

Column Chromatography (CC)

The crude MSE was separated using silica gel column chromatography (100–200 mesh size), eluting the column with a gradient of n-hexane and methanol of increasing polarity (from 90:10 to 70:30, v/v).[17]

Thin Layer Chromatography (TLC)

Fractions were screened by TLC on silica gel plates (254 nm fluorescent indicator). Quercetin and vanillic acid were used as reference standards. For quercetin, the mobile phase consisted of distilled water, acetic acid, and methanol (700:300:10), with bands visualised in an iodine chamber (R_f = 0.585).[18] For vanillic acid, the mobile phase consisted of toluene, ethyl acetate, and formic acid (5:4:1), with bands visualised under UV light (R_f = 0.762).[19] The R_f value is calculated using the following formula:

R*f* = Distance travelled by the solvent front/ Distance travelled by the compound

Preparative TLC (Prep-TLC)

Fractions of interest were further purified by preparative TLC on silica gel GF_{254}–coated TLC plates (20 × 20 cm, 2.5 mm thickness). Developed bands were visualised, scraped, and extracted with volatile solvents by centrifugation.

Preparative RP-HPLC

Quantitative analysis was conducted using an Agilent 1260 Infinity RP-HPLC system equipped with a Diode Array Detector (DAD). Calibration curves for quercetin and vanillic acid were established in the concentration range of 0.10–25 µg/mL, with a correlation coefficient (R^2) greater than 0.99. Seed extract at a concentration of 10 mg/mL was filtered through a 0.22 µm membrane before injection. Quercetin was analysed using a solvent mixture of acetonitrile and 0.3% trichloroacetic acid (50:50, v/v) at a flow rate of 0.9 mL/min, with detection monitored at a wavelength of 254 nm over a run time of 13 min.[20] Analysis of vanillic acid was carried out using a reverse-phase C18 (ODS) preparative column with a mobile phase consisting of water (69.3%), methanol (29.7%), and acetic acid (1.0%) (v/v), corresponding to a ratio of 700:300:10 (v/v/v) at a flow rate of 1.0 mL/min, with detection monitored at 260 nm over a run time of 14 min.[21] Method validation was carried out according to ICH Q2(R^1) guidelines.

Structural Characterisation of Isolated Compounds

GC–MS

Metabolite profiling of the seed extract was performed using a BRUKER SCION GC-MS system equipped with an Rtx®-5 capillary column (30 m × 0.25 mm, with a 0.25 µm film thickness). The oven temperature was programmed to increase from 75°C (held for 5 min) to 320°C at a rate of 7-8°C per minute. Helium served as the carrier gas at a flow rate of 0.99 mL/min.[22] Compounds were identified by comparing their fragmentation patterns with those in the NIST library database.

NMR Spectroscopy

Structural characterisation of quercetin and vanillic acid was carried out using a Bruker AVANCE-III 400 MHz FT-NMR spectrometer. The isolated compounds (10 mg each) were dissolved in 0.6 mL of DMSO-d_6, and one-dimensional (^{1}H and ^{13}C) NMR spectra were recorded at 25 ± 0.1°C. Chemical shifts from both spectra, along with coupling constants obtained from ^{1}H NMR, confirmed the structural identities of quercetin and vanillic acid.

Experimental Animals

The study protocol was approved by the Institutional Animal Ethical Committee (IAEC/2023/46-60, dated 02/03/2023) in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. Female Wistar rats (150–180 g) were obtained from the Disease-Free Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India. Animals were housed at Maharshi Dayanand University, Rohtak, India, under controlled conditions (25 ± 2°C, 50–60% humidity, 12 hr light/dark cycle) with free access to food and water.

PCOS Induction and Treatment Protocol

Fifteen rats were randomly divided into a negative control group (n=3) and a PCOS induction group (n=12). The use of n=3 animals per group was based on ethical, logistical, and scientific considerations. PCOS induction in rats produces consistent and reproducible endocrine and histopathological alterations, as established in previous studies, allowing meaningful assessment of treatment effects even with a small sample size. Limiting the number of animals aligns with the 3Rs principle (Replacement, Reduction, and Refinement) in animal research, minimising animal use while still generating statistically significant outcomes. Moreover, each animal served as a biological replicate with multiple endpoints measured (hormonal, oxidative stress, and histopathological parameters), enhancing the robustness of the dataset. Despite the small sample size, clear and consistent trends were observed across all measured variables, supporting the validity of the conclusions. PCOS was induced by oral administration of letrozole (1 mg/kg/day)[23] for 21 days, while controls received 0.5% Carboxymethyl Cellulose (CMC). Estrous cycle disruption, confirmed by a persistent diestrus phase in vaginal cytology, indicated successful induction.

Post-induction, the PCOS group was subdivided into four groups: (i) PCOS control, (ii) metformin (500 mg/kg),[24] (iii) quercetin (30 mg/kg),[25] and (iv) vanillic acid (30 mg/kg).[26] Body weight and estrous cycle progression were monitored throughout treatment.

Biochemical and Hormonal Assays

At the end of treatment, blood samples were collected via direct cardiac puncture and the separated serum was later analysed for major reproductive hormones using respective ELISA kits. oxidative stress markers SOD (Superoxide Dismutase),[27] catalase,[28] and GSH[29] (reduced glutathione) were analysed in liver tissue homogenates.

Histological Examination

Ovaries were removed, fixed in 10% formalin, dehydrated, and embedded in paraffin. Staining of sections (5 µm thickness) was done using H and E (haematoxylin and eosin) stain and examined microscopically for follicular development, cyst formation, and corpus luteum.

Statistical Analysis

Data were expressed as mean ± Standard Deviation (SD). Statistical comparisons were performed using Student's t-test, ANOVA, and the Duncan post hoc test, as implemented in GraphPad (Version 3), with $p<0.05$ considered statistically significant.

RESULTS

Phytochemical Screening

Preliminary qualitative phytochemical analysis of MSE of *Diplocyclos palmatus* revealed the presence of tannins, proteins, flavonoids, phenols, cardiac glycosides, steroids, saponins, diterpenoids, triterpenoids, and alkaloids. These results indicate that the extract is rich in diverse bioactive constituents, particularly polyphenols and flavonoids, which may contribute to its pharmacological potential.

Separation and Identification of Bioactive Compounds

Column Chromatography (CC)

Column chromatography of the MSE yielded approximately 27 fractions (2 mL each) collected sequentially.

TLC

TLC analysis revealed that some fractions showed distinct separation when developed using a solvent system consisting of distilled water (69.3%), acetic acid (29.7%), and methanol (1.0%) (v/v) on silica gel GF_{254} plates, showed bands with R_f values comparable to those of the quercetin standard (R_f =0.585) when visualised in an iodine chamber, confirming the presence of quercetin. While some fractions showed improved separation when developed in a solvent system consisting of toluene (50%), ethyl acetate (40%), and formic acid (10%) (v/v) on silica gel GF_{254} TLC plates exhibited bands with R_f values similar to the vanillic acid standard (R_f = 0.762) under UV light, indicating the presence of vanillic acid.

Prep-TLC

Preparative TLC enabled the isolation of target compounds from the extract. After development, distinct bands corresponding to quercetin and vanillic acid were identified and marked. The desired bands were carefully scraped, and silica was separated by centrifugation in a volatile solvent. The supernatant obtained contained the purified compounds, while the silica settled as a pellet, confirming the successful recovery of quercetin- and vanillic acid-enriched fractions.

RP-HPLC Quantification of Bioactive Compounds

The developed RP-HPLC method was validated for linearity, specificity, sensitivity, accuracy, precision, and robustness. Calibration curves for quercetin and vanillic acid showed good linearity over 0.10–25 µg/mL with R^2 values of 0.994 and 0.997, respectively. Sharp, well-resolved peaks confirmed specificity, with no interference from other phytoconstituents. LOD and LOQ were 0.184 ± 0.6 and 0.723 ± 0.9 µg/mL for quercetin, and 0.171 ± 0.2 and 0.732 ± 0.5 µg/mL for vanillic acid, demonstrating high sensitivity. Recovery ranged from 99.53–99.90% for quercetin and 99.40–99.91% for vanillic acid, while %RSD for intra- and inter-day precision was <0.20%, confirming repeatability. Robustness testing showed no significant changes under minor variations in flow rate, temperature, pH, or wavelength. Quantitative analysis of MSE revealed quercetin at 2.643 min (peak area 24,424; 3.34% of extract) and vanillic acid

at 6.258 min (peak area 3,283; 2.31% of extract). Chromatograms are shown in Figure 1a-1d.

GC–MS Analysis

GC–MS profiling of MSE revealed the presence of multiple phytoconstituents with potential bioactivity. The GC–MS chromatogram of the SME is presented in Figure. 2. Notably, key identified compounds included quercetin (Rt 20.109 min, peak area 1.30×10^6, Mol. Wt. 302) and vanillic acid (Rt 24.16 min, peak area 2.13×10^6, Mol. Wt. 168). Other major components detected were chlorogenic acid, 9,12,15-octadecatrienoic acid, 4,7,10-hexadecatrienoic acid, 7,10,13-eicosatrienoic acid, 10,12-tricosadiynoic acid, pregnenolone acetate, arachidonic acid, and formic acid. The presence of these polyphenols, fatty acids, and steroids suggests a rich chemical profile that may contribute to the observed pharmacological activities of the extract.

NMR Spectroscopy

The ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of quercetin (3,3′,4′,5,7-pentahydroxyflavone, $C_{15}H_{10}O_7$) showed hydroxyl protons at δ 12.46 (5-OH), 10.75 (7-OH), 9.57 (3′-OH), 9.34 (4′-OH), and 9.26 (3-OH), while aromatic B-ring protons resonated at δ 7.65 (d, J ≈ 2 Hz, H-2′), 7.53 (dd, J ≈ 8, 2 Hz, H-6′), and 6.87 (d, J ≈ 8 Hz, H-5′), and A-ring protons appeared at δ 6.41 (d, J ≈ 2 Hz, H-8) and 6.18 (d, J ≈ 2 Hz, H-6) (Figure 3a). The ^{13}C NMR spectrum (100 MHz, DMSO-d_6) exhibited a carbonyl at δ 176.0 (C-4), oxygenated aromatics at δ 164.3 (C-7), 161.1 (C-5), 156.5 (C-9/C-2), 148.1 (C-4′), 145.4 (C-3′), and 147.25 (C-3), with additional signals at δ 136.1 (C-6′), 122.4 (C-1′), 120.4 (C-2′), 115.7 (C-5′), 115.4 (C-10), 103.4 (C-8), and 97.9 (C-6) (Figure 3b). Vanillic acid (4-hydroxy-3-methoxybenzoic acid, $C_8H_8O_4$) ^{1}H NMR displayed a phenolic OH at δ 9.9, aromatic protons at δ 7.36 (d, J ≈ 2 Hz, H-6), 7.04 (d, J ≈ 8 Hz, H-5), 6.89 (dd, J ≈ 8, 2 Hz, H-2), and a methoxy singlet at δ 3.79 (–OCH_3) (Figure 3c), while ^{13}C NMR showed a carboxyl carbon at δ 167 (C-1), oxygenated aromatics at δ 148 (C-4) and 146 (C-3), aromatic CHs at δ 123–126 (C-2, C-5, C-6), additional quaternary carbons at δ 114–120, and the methoxy carbon at δ 55 (Figure 3d). These NMR data confirm the structural integrity and identity of quercetin and vanillic acid, consistent with the literature.[29,30]

In vivo Efficacy in PCOS Rat Model

Modulation of Reproductive Hormones Following Treatment in PCOS Rats

PCOS induction elevated serum LH, FSH, and testosterone, while decreasing estrogen and progesterone compared to the negative control (healthy, non-PCOS rats). Treatment significantly modulated these hormonal disturbances. Metformin showed the strongest effects, reducing LH (p=0.003) and testosterone (p=0.0001) and increasing FSH (p=0.006), estrogen (p=0.0001), and progesterone (p=0.008). Quercetin also produced marked improvements: LH (p=0.004), testosterone (p=0.0004), FSH

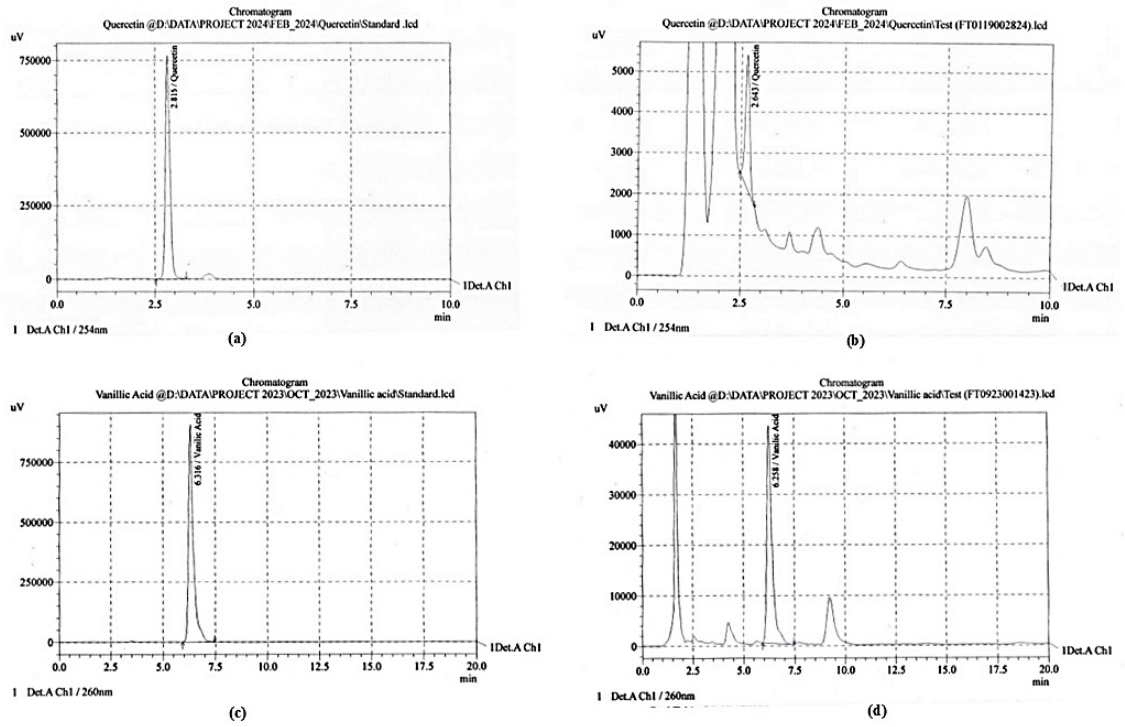

Figure 1: RP-HPLC chromatograms illustrating (a) Quercetin standard, (b) MSE with the corresponding quercetin peak, (c) Vanillic acid standard, and (d) MSE with the corresponding vanillic acid peak.

(p=0.04), estrogen (p=0.0001), and progesterone (p=0.009). Vanillic acid induced moderate yet significant changes: LH (p=0.006), testosterone (p=0.0005), FSH (p=0.05), estrogen (p=0.0003), and progesterone (p=0.01). Overall, metformin was most effective, followed closely by quercetin, with vanillic acid showing milder responses (Table 1).

Oxidative Stress Parameters

Oxidative stress analysis revealed a marked reduction in antioxidant markers SOD, GSH, and catalase in the liver tissues of all PCOS-induced groups compared to the negative control (The negative control group comprised healthy, non-PCOS-induced rats maintained under normal conditions, serving as the baseline for physiological antioxidant enzyme levels). Treatment with metformin and the bioactive compounds significantly restored these antioxidant enzyme activities compared to the PCOS control group. In the metformin group, SOD, GSH, and catalase levels improved with p values of 0.002, 0.003, and 0.005, respectively. Quercetin also demonstrated strong antioxidant activity, significantly increasing SOD (p=0.009), GSH (p=0.0001), and catalase (p=0.001). The vanillic acid-treated group showed notable improvements, with SOD (p=0.01), GSH (p=0.0001), and catalase (p=0.0001). Among the treatments, metformin produced the strongest overall effects across all markers, while the bioactive compounds exhibited a dose-dependent response, with Quercetin being more effective than vanillic acid (Table 2).

Effect of Treatment on Ovarian Histopathology in Adult Female Rats

Histological analysis of ovarian tissue displayed significant variations among the different experimental groups. The negative control group showed a typical ovarian structure, including primary, secondary, and Graafian Follicles (GF), along with a properly developed Corpus Luteum (CL), (Figure 4a). In contrast, the PCOS control group displayed characteristic PCOS features, including multiple Cystic Follicles (CF) and disrupted ovarian structure (Figure 4b). Following 21 days of treatment, the metformin group showed restoration of normal ovarian architecture with healthy follicles and CL (Figure 4c). In a similar way, treatments with Quercetin and vanillic acid led to recovery, as demonstrated by the lack of cystic follicles and the presence of healthy follicles and CL (Figure 4d and 4e, respectively).

DISCUSSION

The present study demonstrated that the Methanolic Seed Extract (MSE) of *Diplocyclos palmatus* is rich in diverse phytochemicals, including tannins, flavonoids, phenols, saponins, alkaloids, terpenoids, and cardiac glycosides, many of which possess documented pharmacological activities.[30-32] High levels of quercetin (3.34%) and vanillic acid (2.31%) were confirmed by RP-HPLC, TLC, GC–MS, and NMR analyses, consistent with previous reports on cucurbitaceous plants.[33-36] Quercetin is well recognised for its antioxidative, anti-inflammatory, and hormone-regulatory effects, while vanillic acid exhibits antioxidant, anti-glycation,

Table 1: Effects of 21-day treatment on hormonal levels as measured in serum samples: Testosterone, FSH, LH, Progesterone, and Estrogen.

Experimental Groups	Testosterone (ng/mL)	FSH (mIU/mL)	LH (ng/mL)	Progesterone (ng/mL)	Estrogen (pg/mL)
Negative control	0.58 ± 0.04*	4.42 ± 0.02*	2.9 ± 0.8*	7.3 ± 0.1*	49.25 ± 1.0*
PCOS control	1.56 ± 0.1*	4.27 ± 0.01*	12.96 ± 0.4*	2.73 ± 0.1*	10.89 ± 0.08*
Metformin	0.6 ± 0.07*†	4.39 ± 0.01*#	5.06 ± 0.5*†	7.46 ± 0.8*#	47.46 ± 1.05*#
Vanillic acid	0.59 ± 0.04*†	4.51 ± 0.03*#	3.93 ± 0.15*†	7.28 ± 0.08*#	48.24 ± 0.96*#
Quercetin	0.7 ± 0.02*†	4.39 ± 0.03*#	4.66 ± 0.35*†	7.53 ± 0.05*#	40.99 ± 0.51*#

* Data expressed as Mean ± SEM (n=3). Treatment with Metformin, Vanillic acid and Quercetin resulted in a significant (p≤0.05) reduction in testosterone and LH, along with a significant (p≤0.05) increase in FSH, progesterone and estrogen compared to the PCOS control group. † Indicates a significant decrease, and # indicates a significant increase relative to the PCOS control group.

Table 2: Effects of 21-day treatment on oxidative stress markers: Super Oxide Dismutase (SOD), Glutathione (GSH) and catalase.

Experimental Groups	Average U/mg Protein (Liver Tissue Homogenate)		
	SOD	GSH	Catalase
Negative control	12.23 ± 1.11*	124.46 ± 0.04*	3.54 ± 0.3*
PCOS Control	5.47 ± 1.2*	35.50 ± 0.03*	1.70 ± 0.02*
Metformin	10.96 ± 0.5*#	106.31 ± 0.08*#	3.49 ± 0.3*#
Vanillic acid	12.43 ± 0.10*#	82.68 ± 0.003*#	2.90 ± 0.07*#
Quercetin	12.85 ± 0.23*#	95.76 ± 0.004*#	3.04 ± 0.09*#

* Data presented as Mean ± SEM (n=3). Treatment with Metformin, Vanillic acid and Quercetin resulted in a significant increase in antioxidant marker levels compared with the PCOS control group (p≤0.05). # denotes a significant increase relative to the PCOS control group.

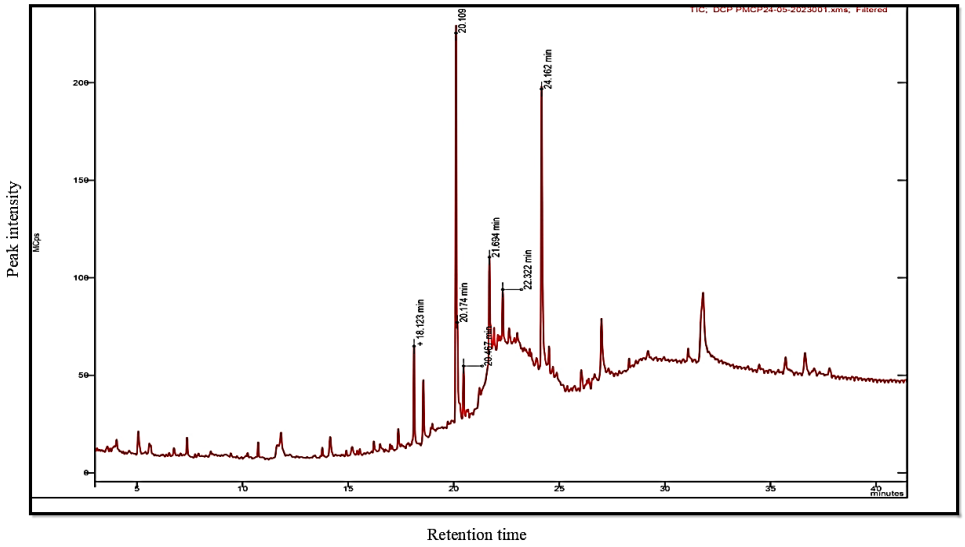

Figure 2: GC–MS chromatogram of the MSE showing the presence of key bioactive compounds, including quercetin (Rt 20.109 min) and vanillic acid (Rt 24.262 min).

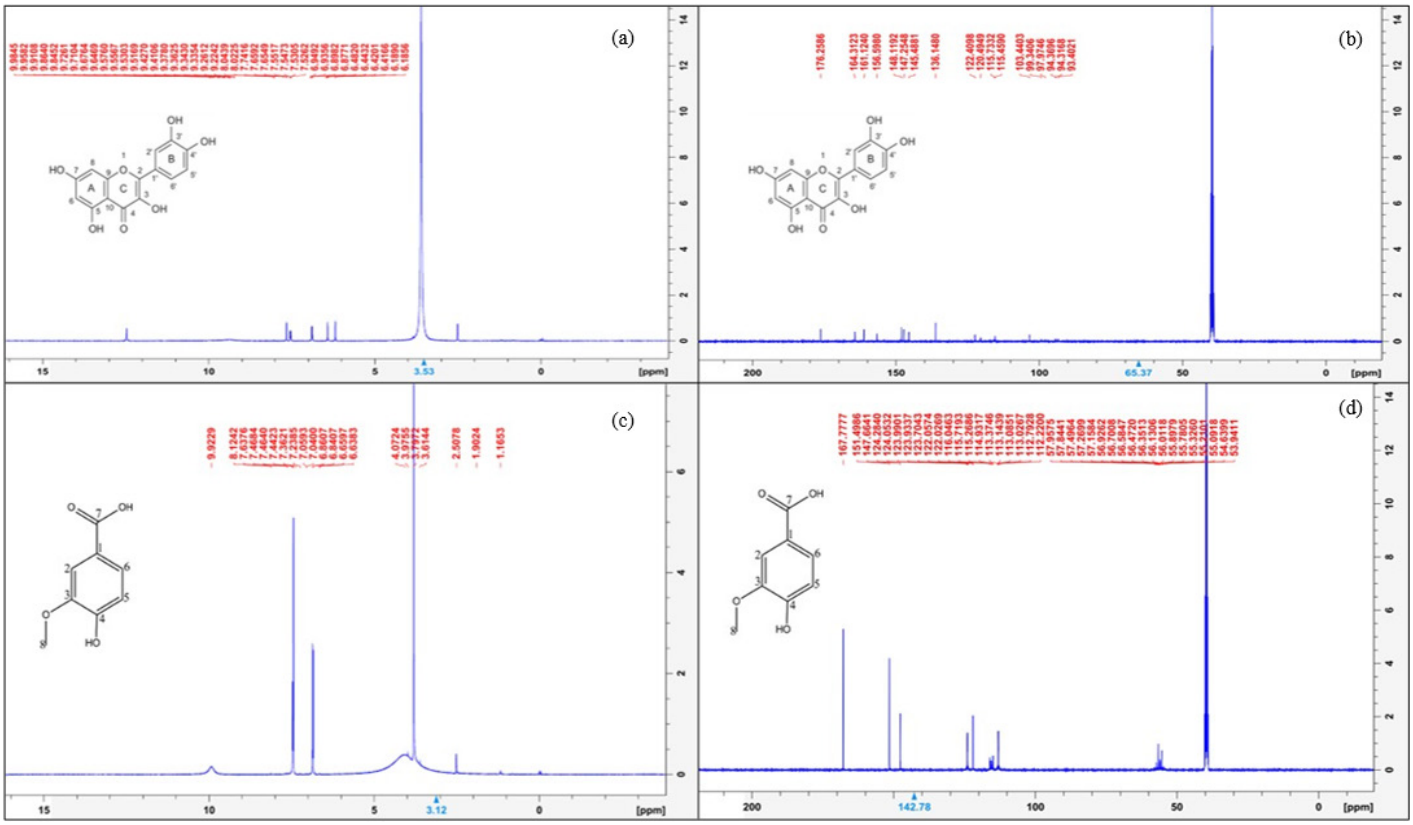

Figure 3: NMR spectra of quercetin and vanillic acid: (a) ^{1}H NMR spectrum of quercetin (400 MHz, DMSO-d_6) showing characteristic proton signals of the flavonoid structure; (b) ^{13}C NMR spectrum of quercetin (100 MHz, DMSO-d_6) displaying characteristic carbon resonances confirming its flavonoid framework; (c) ^{1}H NMR spectrum of vanillic acid (400 MHz, DMSO-d_6) showing characteristic proton signals corresponding to a 1,2,4-trisubstituted aromatic ring system; (d) ^{13}C NMR spectrum of vanillic acid (100 MHz, DMSO-d_6) confirming the presence of aromatic, carboxyl, and methoxy carbons consistent with its chemical structure.

and anti-inflammatory properties.[37-40] In the PCOS rat model, induction resulted in elevated LH, FSH, and testosterone levels, along with reduced estrogen and progesterone, consistent with established PCOS pathology.[41,42] Metformin treatment effectively restored hormonal balance, validating the experimental model.[43] Quercetin produced comparable therapeutic effects by normalising gonadotropins, reducing androgen levels, and improving estrogen and progesterone concentrations, corroborating earlier studies.[44,32] Vanillic acid also improved the hormonal profile, though its efficacy was comparatively moderate [32]. Both compounds significantly ameliorated oxidative stress by restoring endogenous antioxidant enzymes such as SOD, GSH,

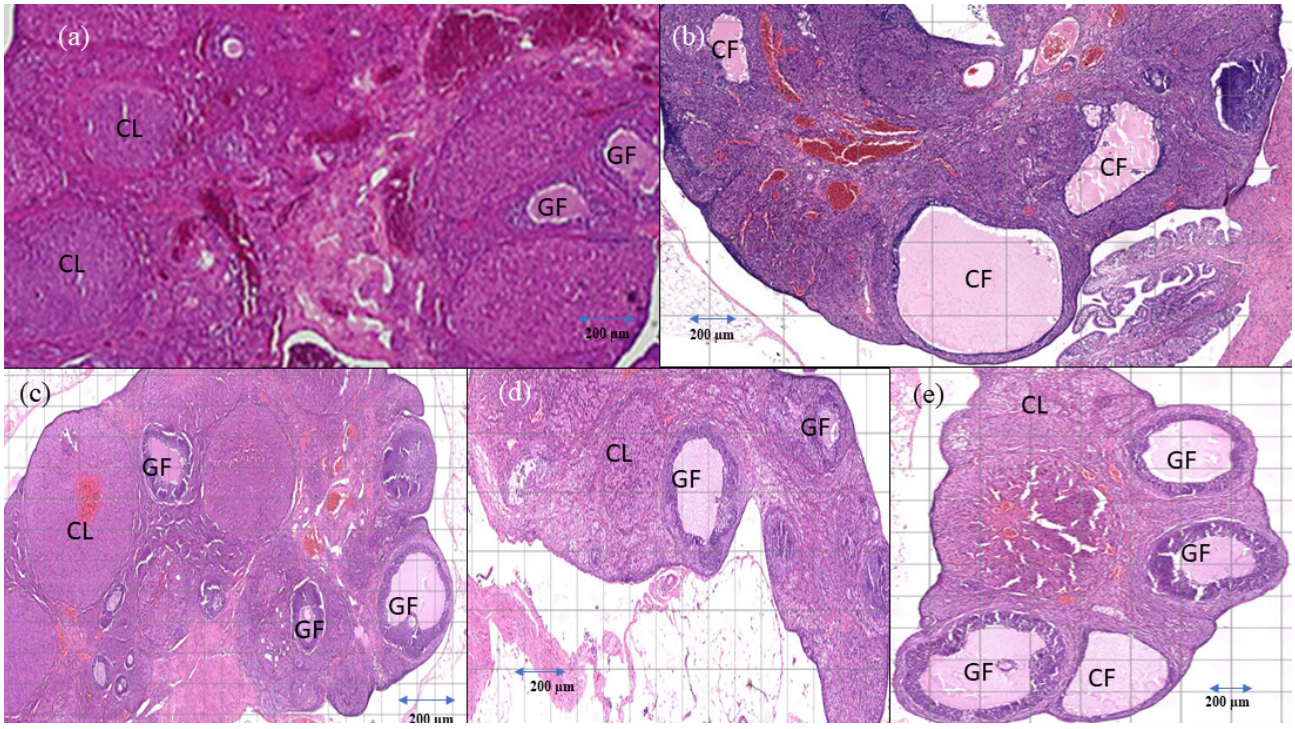

Figure 4: Histopathological sections of rat ovaries (H&E stain; scale bar = 200 µm). (a) Negative control showing normal ovarian architecture with corpora lutea (CL) and growing follicles (GF). (b) PCOS control exhibiting multiple large cystic follicles (CF) and absence of CL. (c) The metformin-treated group showed restoration of ovarian morphology with CL and GF. (d) Quercetin-treated group demonstrating normal follicular development with CL and GF. (e) Vanillic acid–treated group showing reduced cystic follicles with the presence of CL and GF. Treated groups exhibited a marked reduction in cystic follicles compared to the PCOS control.

and catalase, supporting previous reports highlighting the role of polyphenols in combating oxidative damage associated with PCOS.[45-47] Histopathological evaluation further confirmed recovery of ovarian architecture, with reduced cystic follicles and improved folliculogenesis in treated groups, particularly with quercetin.[39] Despite these promising findings, the study has certain limitations. The evaluation was restricted to a single-dose regimen and short-term treatment duration, which may not fully capture dose-dependent or long-term therapeutic effects. Additionally, molecular mechanisms underlying the observed endocrine and antioxidant effects were not explored, limiting mechanistic interpretation. Furthermore, the findings are based on an animal model, and direct extrapolation to human PCOS requires cautious interpretation. Future investigations should therefore focus on dose–response studies, molecular pathway elucidation, gene and protein expression analyses related to steroidogenesis, oxidative stress, and inflammation, as well as long-term safety and toxicity profiling. Clinical studies are also warranted to validate the translational potential of *D. palmatus*–derived bioactive compounds for PCOS management.

LIMITATIONS AND FUTURE PROSPECTS

The major limitations of this study include the use of a single-dose treatment, lack of mechanistic molecular investigations, and reliance on an animal model. Future research should emphasize dose optimization, mechanistic pathway studies, long-term safety evaluation, and clinical validation to comprehensively establish the therapeutic potential of *Diplocyclos palmatus*–derived bioactive compounds in the management of PCOS.

CONCLUSION

This study demonstrates that the methanolic seed extract of *Diplocyclos palmatus* is rich in bioactive phytoconstituents, with quercetin and vanillic acid identified as major components through chromatographic and spectroscopic analyses. The validated RP-HPLC method confirmed their reliable quantification in the extract. In a PCOS-induced rat model, both compounds significantly improved hormonal imbalance, enhanced antioxidant defence, and restored normal ovarian architecture. Quercetin exhibited therapeutic efficacy comparable to metformin, while vanillic acid showed moderate yet consistent benefits, supporting the ethnopharmacological relevance of *D. palmatus* as a potential source of safer plant-based interventions for PCOS.

ACKNOWLEDGEMENT

Richa Yadav is grateful to the CSIR-UGC, New Delhi, for the award of a JRF/SRF.

ABBREVIATIONS

PCOS: Polycystic Ovarian Syndrome; **MSE:** Methanolic Seed Extract; **LH:** Luteinizing Hormone; **FSH:** Follicle-Stimulating Hormone; **HPLC:** High-Performance Liquid Chromatography; **GC-MS:** Gas Chromatography–Mass Spectrometry; **NMR:** Nuclear Magnetic Resonance; **CC:** Column Chromatography; **TLC:** Thin-Layer Chromatography; **Prep-TLC:** Preparative Thin-Layer Chromatography; **SOD:** Superoxide Dismutase; **GSH:** Glutathione; **SD:** Standard Deviation; **ANOVA:** Analysis of Variance; **R_f:** Retardation Factor; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **Mol. Wt:** Molecular Weight; **Rt:** Retention Time; **CL:** Corpus Luteum; **CF:** Cystic Follicle; **GF:** Graffian Follicle.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study investigates the bioactive composition and anti-PCOS potential of *Diplocyclos palmatus* Methanolic Seed Extract (MSE). The methanolic extract was prepared via Soxhlet extraction and subjected to phytochemical screening, revealing flavonoids, phenolics, tannins, and saponins. Chromatographic separation (CC, TLC, Prep-TLC) and RP-HPLC identified and quantified quercetin (3.34%) and vanillic acid (2.31%) as major constituents. Structural confirmation was achieved using GC–MS and $^{1}H/^{13}C$ NMR analyses. In a letrozole-induced PCOS rat model, treatment with quercetin (30 mg/kg), vanillic acid (30 mg/kg), or metformin (500 mg/kg) for 21 days significantly restored hormonal balance (reduced LH, testosterone; increased FSH, estrogen, progesterone; $p<0.05$) and improved antioxidant markers (SOD, GSH, catalase). Histopathology showed normalisation of ovarian architecture with healthy follicles and corpus luteum. The findings suggest *Diplocyclos palmatus* is a rich source of polyphenolic bioactive compounds with potent hormone-regulating and antioxidant effects. Quercetin exhibited efficacy comparable to metformin, indicating its potential as a natural therapeutic agent for PCOS management.

REFERENCES

1. Deswal R, Narwal V, Dang A, Pundir CS. The prevalence of polycystic ovary syndrome: a brief systematic review. J Hum Reprod Sci. 2020;13(4):261-71. doi: 10.4103/jhrs.JHRS_95_18, PMID 33627974.
2. Ding H, Zhang J, Zhang F, Zhang S, Chen X, Liang W, *et al.* Resistance to the insulin and elevated level of androgen: A major cause of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2021;12:741764. doi: 10.3389/fendo.2021.741764, PMID 34745009.
3. Shamran SG, Hammood SA. A review: the relationships between ovarian disease and tumour marker. *Al-Kufa Univ J Biol*. 2022;14(3):1-15.
4. Kumar AN, Naidu JN, Satyanarayana U, Ramalingam K, Anitha M. Metabolic and endocrine characteristics of Indian women with polycystic ovary syndrome. Int J Fertil Steril. 2016;10(1):22-8. doi: 10.22074/ijfs.2016.4764, PMID 27123196.
5. Bai H, Ding H, Wang M. Polycystic ovary syndrome (PCOS): symptoms, causes, and treatment. Clin Exp Obstet Gynecol. 2024;51(5):1-12. doi: 10.31083/j.ceog5105126.
6. Wawrzkiewicz-Jałowiecka A, Kowalczyk K, Trybek P, Jarosz T, Radosz P, Setlak M, *et al.* In search of new therapeutics—molecular aspects of the PCOS pathophysiology: genetics, hormones, metabolism and beyond. Int J Mol Sci. 2020;21(19):7054. doi: 10.3390/ijms21197054, PMID 32992734.
7. Adam LN, Adam LN. Comprehensive overview of polycystic ovary syndrome: pathophysiology, clinical features, and emerging therapeutic approaches. Obes Med. 2025;55:100606. doi: 10.1016/j.obmed.2025.100606.
8. Pisa Beni MY, Kumar P, Lal B. *Putranjiva roxburghii* Wall. and *Diplocyclos palmatus* (L.) C. Jeffrey as the potential sources of future drugs for infertility: a review. J Bioresour. 2021;8(2):10-8.
9. Attar UA, Ghane SG. Phytochemicals, antioxidant activity and phenolic profiling of *Diplocyclos palmatus* (L.) C. Jeffery. Int J Pharm Pharm Sci. 2017;9(4):101. doi: 10.22159/ijpps.2017v9i4.16891.
10. Patel SB, Attar UA, Sakate DM, Ghane SG. Efficient extraction of cucurbitacins from *Diplocyclos palmatus* (L.) C. Jeffrey: optimization using response surface methodology, extraction methods and study of some important bioactivities. Sci Rep. 2020;10:1-12.
11. Wang X, Zhang W, Zhou S. Multifaceted physiological and therapeutical impact of curcumin on hormone-related endocrine dysfunctions: a comprehensive review. Phytother Res. 2024;38(7):3307-36. doi: 10.1002/ptr.8208, PMID 38622915.
12. Mutha RE, Kalaskar M, Khan ZG. Modern analytical techniques for quality control and chemical identification of phytochemicals. J Pharmacogn Phytochem. 2025; 2025:167-88.
13. Barthwal R, Mahar R. Exploring the significance, extraction, and characterisation of plant-derived secondary metabolites in complex mixtures. Metabolites. 2024;14(2):119. doi: 10.3390/metabo14020119, PMID 38393011.
14. Santhosam SD, Selvam P, Danodia A. Phytochemical analysis of *Abrus precatorius* seeds: a review. Int J Pharm Sci Rev Res. 2023;79(2):122-9. doi: 10.47583/ijpsrr.2023.v79i02.020.
15. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J Exp Pharmacol. 2023;15:51-62. doi: 10.2147/JEP.S379805, PMID 36789235.
16. Ndezo Bisso B, Njikang Epie Nkwelle R, Tchuenguem Tchuenteu R, Dzoyem JP. Phytochemical screening, antioxidant, and antimicrobial activities of seven under-investigated medicinal plants against microbial pathogens. Adv Pharmacol PharmSci. 2022; 2022:1-8.
17. Susanti I, Pratiwi R, Rosandi Y, Hasanah AN. Separation methods of phenolic compounds from plant extract as antioxidant agents candidate. Plants (Basel). 2024;13(7):965. doi: 10.3390/plants13070965, PMID 38611494.
18. Amir M, Mujeeb M, Ahmad S, Akhtar M, Ashraf K. Design expert-supported development and validation of HPTLC method: an application in simultaneous estimation of quercetin and rutin in *Punica granatum*, *Tamarindus indica* and *Prunus domestica*. Pharm Methods. 2013;4(2):62-7. doi: 10.1016/j.phme.2013.12.004.
19. Huang Z, Dostal L, Rosazza JP. Mechanisms of ferulic acid conversions to vanillic acid and guaiacol by *Rhodotorula rubra*. J Biol Chem. 1993;268(32):23954-8. doi: 10.1016/S0021-9258(20)80477-3, PMID 8226936.
20. Javed M, Mal T. A review on estimation of quercetin, rutin, apigenin, naringin and hesperidin from citrus plant using HPLC. J Pharmacogn Phytochem. 2025;14(4):469-79. doi: 10.22271/phyto.2025.v14.i4g.15506.
21. Verma S, Gupta A, Ramana MV, Rawat AK. High-performance thin-layer chromatographic analysis for the simultaneous quantification of gallic acid, vanillic acid, protocatechuic acid, and quercetin in the methanolic fraction of *Limonia acidissima* L. fruits. JPC J Planar Chromatogr Mod TLC. 2016;29(5):356-60. doi: 10.1556/1006.2016.29.5.5.
22. Sani MS, Bakar J, Rahman RA, Abas F. Effects of coated capillary column, derivatization, and temperature programming on the identification of *Carica papaya* seed extract composition using GC/MS analysis. J Anal Test. 2020;4(1):23-34. doi: 10.1007/s41664-020-00118-z.
23. Areloegbe SE, Abugu IA, Ajadi IO, Aturamu A, Ajadi MB, Atuma CL, *et al.* Probiotics mitigate ovarian angiogenic disturbance in letrozole-induced polycystic ovarian syndrome. BMC Complement Med Ther. 2025;25(1):1-9.
24. Desai BN, Maharjan RH, Nampoothiri LP. *Aloe barbadensis* Mill. formulation restores lipid profile to normal in a letrozole-induced polycystic ovarian syndrome rat model. Pharmacognosy Res. 2012;4(2):109-15. doi: 10.4103/0974-8490.94736, PMID 22518083.
25. Jahan S, Abid A, Khalid S, Afsar T, Shaheen G, Almajwal A et al. Therapeutic potentials of quercetin in management of polycystic ovarian syndrome using letrozole-induced rat model: a histological and biochemical study. J Ovarian Res. 2018;11:1-10.
26. Vinothiya K, Ashokkumar N. Modulatory effect of vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensive rats. Biomed Pharmacother. 2017;87:640-52. doi: 10.1016/j.biopha.2016.12.134, PMID 28088113.
27. Bandeira SD, Guedes GD, Fonseca LJ, Pires AS, Gelain DP, Moreira JC, *et al.* Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity. Oxid Med Cell Longev. 2012; 2012:1-8.
28. Ershova OA, Bairova TA, Kolesnikov SI, Kalyuzhnaya OV, Darenskaya MA, Kolesnikova LI. Oxidative stress and catalase gene. Bull Exp Biol Med. 2016;161(3):400-3. doi: 10.1007/s10517-016-3424-0, PMID 27496033.
29. Schmitt B, Vicenzi M, Garrel C, Denis FM. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers: A comparative crossover study. Redox Biol. 2015;6:198-205. doi: 10.1016/j.redox.2015.07.012, PMID 26262996.

30. Fard Tabrizi F, Hajizadeh-Sharafabad F, Vaezi M, Jafari-Vayghan H, Alizadeh M, Maleki V. Quercetin and polycystic ovary syndrome, current evidence and future directions: a systematic review. Pourteymour. J Ovarian Res. 2020;13:1-10.
31. Gao J, Huang Y, Song Y, Huang X, Zhang D, Hou Z. Therapeutic effects of *Althaea officinalis* L. and metformin on estradiol-induced polycystic ovary syndrome in rats: insights into the PI3K/AKT pathway, inflammation, and oxidative stress. Cell J. 2024;26(8):473-86. doi: 10.22074/cellj.2024.2026329.1550, PMID 39380478.
32. Wang P, Wang Y, Liu S, Wang K, Yao Y, Liu W, *et al.* Bioactive metabolites of *Dioscorea* species and their potential applications in functional food development. Foods. 2025;14(4):1-29.
33. Irshad M, Ahmad I, Mehdi SJ, Goel HC, Rizvi MM. Antioxidant capacity and phenolic content of the aqueous extract of commonly consumed cucurbits. Int J Food Prop. 2014;17(1):179-86. doi: 10.1080/10942912.2011.619025.
34. Ibrahim TA, El-Hefnawy HM, El-Hela AA. Antioxidant potential and phenolic acid content of certain cucurbitaceous plants cultivated in Egypt. Nat Prod Res. 2010;24(16):1537-45. doi: 10.1080/14786419.2010.489049, PMID 20835955.
35. Sambandam B, Thiyagarajan DE, Ayyaswamy AR, Raman P, Kulasekaran J, Venkatasamy H. Extraction and isolation of flavonoid quercetin from the leaves of *Trigonella foenum-graecum* and their antioxidant activity. Int J Pharm Pharm Sci. 2016;8(6):1-5.
36. González-Baró AC, Parajón-Costa BS, Franca CA, Pis-Diez R. Theoretical and spectroscopic study of vanillic acid. J Mol Struct. 2008;889(1-3):204-10. doi: 10.1016/j.molstruc.2008.01.049.
37. Bhardwaj JK, Panchal H, Saraf P. Ameliorating effects of natural antioxidant compounds on female infertility: a review. Reprod Sci. 2021;28(5):1227-56. doi: 10.1007/s43032-020-00312-5, PMID 32935256.
38. Sirotkin AV. Quercetin action on health and female reproduction in mammals. Crit Rev Food Sci Nutr. 2024;64(33):12670-84. doi: 10.1080/10408398.2023.2256001, PMID 37698182.
39. Gadhave PV, Sutar GV, Sajane SJ, Redasani VK, Das K, Alobid S, *et al.* Protective effects of vanillic acid on letrozole-induced polycystic ovarian syndrome: a comprehensive study in female Wistar rats. Saudi Pharm J. 2024;32(2):1-9.
40. Wang W, Barkat MQ, Amin S, Alshammari A, Albekairi NA, Manzoor H, *et al.* Pharmacological appraisal of aqueous ethanolic extract of *Cuminum cyminum* (cumin seeds) in obesity and endocrinopathies associated with polycystic ovarian syndrome in animal models. J Food Biochem. 2024;2024(1):1-17. doi: 10.1155/jfbc/1184854.
41. Yang J, Chen C. Hormonal changes in PCOS. J Endocrinol. 2024;261(1):1-14. doi: 10.1530/JOE-23-0342.
42. Emanuel RH, Roberts J, Docherty PD, Lunt H, Campbell RE, Möller K. A review of the hormones involved in the endocrine dysfunctions of polycystic ovary syndrome and their interactions. Front Endocrinol (Lausanne). 2022;13:1017468. doi: 10.3389/fendo.2022.1017468, PMID 36457554.
43. Palomba S, Falbo A, Zullo F, Orio F Jr. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 2009;30(1):1-50. doi: 10.1210/er.2008-0030, PMID 19056992.
44. Wang Z, Zhai D, Zhang D, Bai L, Yao R, Yu J, *et al.* Quercetin decreases insulin resistance in a polycystic ovary syndrome rat model by improving inflammatory microenvironment. Reprod Sci. 2017;24(5):682-90. doi: 10.1177/1933719116667218, PMID 27634381.
45. Rudnicka E, Duszewska AM, Kucharski M, Tyczyński P, Smolarczyk R. Oxidative stress and reproductive function: oxidative stress in polycystic ovary syndrome. Reproduction. 2022;164(6):F145-54. doi: 10.1530/REP-22-0152, PMID 36279177.
46. Khamineh Y, Ghiasvand M, Panahi-Alanagh S, Rastegarmand P, Zolghadri S, Stanek A. A narrative review of quercetin's role as a bioactive compound in female reproductive disorders. Nutrients. 2025;17(7):1118. doi: 10.3390/nu17071118, PMID 40218878.
47. Surya S, Sampathkumar P, Sivasankaran SM, Pethanasamy M, Elanchezhiyan C, Deepa B, *et al.* Vanillic acid exhibits potent antiproliferative and free radical scavenging effects under in vitro conditions. Int J Nutr Pharmacol Neurol Dis. 2023;13(3):188-98. doi: 10.4103/ijnpnd.ijnpnd_29_23.

Cite this article: Yadav R, Yadav JP. Chromatographic and Spectroscopic Characterisation of Bioactive Compounds from *Diplocyclos palmatus* for Polycystic Ovary Syndrome Management. Indian J of Pharmaceutical Education and Research. 2026;60(4s):s1544-s1552.