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Phytochemical characterization and antioxidant potential of *Flacourtia indica* (Burm. f.) Merr. root extracts: A GC-MS based validation of traditional medicinal use

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Abstract

Flacourtia indica (Burm.f.) Merr. is a plant of notable ethnomedicinal value traditionally used to treat inflammatory, infectious, and cardiovascular ailments. This study assessed the phytochemical composition and antioxidant potential of root extracts prepared using ethanol (80%), methanol and water. Qualitative analysis confirmed the presence of polyphenols, flavonoids, anthocyanins and quinones, the ethanolic extract showing the most varied phytochemical profile. Quantitative assays showed that the alcoholic extract had the highest total phenolic content (316 mg GAE/100 g), flavonoid content (128 mg QE/100 g), anthocyanins (18.26 mg/100 g) and ascorbic acid (23.16 mg/100 g). Antioxidant activities were solvent-dependent; the aqueous extract gave the strongest DPPH scavenging effect (9.10% inhibition), while the alcoholic extract recorded the highest ferric-reducing antioxidant power value (472.14 µMol $Fe^{2?}$/g). TLC studies confirmed the presence of quercetin-like flavonoids (Rf = 0.97), while GC-MS profiling identified a pool of diverse bioactives comprising flavonoids, terpenes, alkaloids, and organosiloxanes. These findings validate the traditional use of *F. indica* roots and highlight that the ethanolic root extract as a promising compound for further pharmacological exploration.

1. Introduction

Medicinal plants remain key basis of therapeutic agents particularly in traditional medicine across Asia and Africa. *Flacourtia indica* (Burm.f.) Merr. Salicaceae or Governor's plum has been traditionally used to manage fever, inflammation, infections and cardiovascular disorders (Eramma, 2016). Commonly distributed in tropical and subtropical Africa and Asia. In India, it occurs mainly in sub Himalayan tracts, central regions and the Western Ghats, thriving in dry deciduous to semi evergreen forests, village boundaries, degraded forests and scrublands (Dharmappa, 2022). Its ecological adaptability supports its ethnomedicinal use especially among rural and tribal populations. The plant reaches 3 to 10 m bears spiny branches, greenish flowers and dark purple drupes (Verdcourt, 2017). Leaves, fruits, bark and roots contain bioactive phytochemicals such as flavonoids, phenolic acids, tannins and alkaloids. Root based preparations are traditionally used for hypertension, microbial infections and oxidative stress related conditions. Polyphenols and flavonoids confer antioxidant properties aiding in cellular protection, cardioprotection and metabolic regulation (Eramma and Patil, 2023).

Traditional medicinal claims do increasingly need scientific substantiation by phytochemical and pharmacological studies. Plants like *F. indica* contain a rich repository of secondary metabolites responsible for executing several therapeutic effects including free radical scavenging and enzyme modulation. A central mechanism in the pathogenesis of neurodegeneration, atherosclerosis and diabetes includes oxidative stress brought about by excessive reactive oxygen species (ROS).The antioxidant defense property of bioactives from plants, especially phenolics, flavonoids and ascorbic acid, plays a vital role in mitigating oxidative damage and restoring cellular homeostasis (Dumanovic *et al*., 2021). Hence, the antioxidant profile of root extracts from *F. indica* provides a scientific justification for its traditional uses and helps in the identification of bioactive leads for natural antioxidant formulations.

However, detailed biochemical profiling and solvent based comparative antioxidant evaluation of *F. indica* roots are limited. Ethanol, methanol and water extract different phytocompounds based on polarity and combined analyses using thin-layer chromatography

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(TLC) and gas chromatography/mass spectrometry (GC-MS) provide comprehensive identification and characterization of phyto-constituents (Mutha *et al*., 2025). This study evaluated qualitative and quantitative phytochemicals of ethanol (80%), methanol and aqueous root extracts, assessed antioxidant capacity *via* 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays, estimated total phenolic and ascorbic acid content, confirmed flavonoids by TLC and identified individual compounds using GC-MS to validate ethnopharmacological relevance and to determine the most bioactive extract for further pharmacological development.

2. Materials and Methods

2.1 Sampling and sample preparation

Healthy mature roots of *Flacourtia indica* (Burm.f.) Merr. Figure 1 was collected during the flowering season from wild populations in Shengottai, Tenkasi district, Tamil Nadu, India (8.9725° N, 77.2488° E). The species was authenticated by a taxonomist at the Department of Botany and a Voucher Specimen (FI-Root-2025) was deposited in the Institutional Herbarium. Roots were washed with tap and distilled water, chopped, shade dried at 25 to 28^{0}C for 10 to 12 days then ground to a fine powder was sieved through a 60 mesh screen and stored in airtight containers for extraction (Eramma and Gayathri, 2021).

Figure 1: *F. indica* plant and roots.

2.2 Preparation of root extracts

Approximately, 25 g of the dried root powder was extracted with three solvents of increasing polarity, *viz.,* 80% ethanol, methanol and distilled water. Each solvent extraction was carried-out separately in a Soxhlet apparatus for 6 h. After extraction, the solvents were filtered through whatman No.1 filter paper and concentrated in a rotary vacuum evaporator (Buchi, Switzerland) at 40^{0}C under reduced pressure. The concentrated extracts were weighed and stored in amber coloured glass vials at 4^{0}C until further analysis (Eramma and Gayathri, 2013).

2.3 Qualitative phytochemical screening

Qualitative screening of ethanolic, methanolic and aqueous *F. indica* root extracts was performed following Shaikh and Patil (2020) to detect major secondary metabolites. Polyphenols were identified by adding 5% ferric chloride producing a dark green or blue black coloration. Flavonoids were detected using ethanol, 1% aluminium chloride and concentrated sulphuric acid (H_2SO_4), yielding a yellow to orange color. Anthocyanins were confirmed by treatment with chloroform ($ChCl_3$) and aqueous ammonia (NH_3) which resulted in a pink or reddish layer, while quinones were confirmed by the formation of red, blue or green precipitates upon addition of concentrated sulphuric acid.

2.4 Quantitative estimation of phytochemicals

2.4.1 Total phenolic content (TPC)

TPC of *F. indica* root extract was determined according to the folin-ciocalteu method following of Kupina *et al*. (2017). One ml of diluted extract was mixed with 5 ml of folin-ciocalteu reagent (10%) and kept for incubation at RT for 5 min. Subsequently, 4 ml of sodium carbonate (Na_2CO_3) 7.5% was added to it and further incubated in the dark at 37^{0}C for 30 min. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). Gallic acid was used as the standard and results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry root extract.

2.4.2 Total flavonoid content (TFC)

TFC of *F. indica* root extract was measured by the aluminium chloride colorimetric method using quercetin as the standard following Tristantini and Amalia (2019). Briefly, 1 ml of extract was mixed with 4 ml of distilled water and 0.3 ml of sodium nitrite ($NaNO_3$) 5% and incubated for 5 min at RT. Then, 0.3 ml of aluminium chloride (Al_2Cl_3)(10%) was added and incubated for 6 min, followed by 2 ml of sodium hydroxide (NaOH) (1 M). The final volume was adjusted to 10 ml with distilled water, vortexed and absorbance was measured at 510 nm. TFC was calculated using a quercetin calibration curve and expressed as mg quercetin equivalent (QE) per gram of extract.

2.4.3 Ascorbic acid (titrimetric method)

The ascorbic acid content was quantified by titration using 2,6-dichlorophenolindophenol (DCPIP) dye. A known volume of extract was mixed with 5 ml of 3% metaphosphoric acid (HPO_3) and titrated against standard DCPIP until a light pink endpoint persisted for 30 sec. A parallel titration with standard ascorbic acid solution was used for calibration. The amount of ascorbic acid was calculated and expressed in mg/100 g of dry root powder (Vahid *et al*., 2012).

2.4.4 Anthocyanins (colorimetric method)

Anthocyanin content was determined using the pH differential method (Lee *et al*., 2005). The extract was diluted in two separate buffers: potassium chloride buffer (pH 1.0) and sodium acetate (CH_3COONa) buffer (pH 4.5). The absorbance of both solutions was measured at 520 and 700 nm. Anthocyanin concentration was calculated using the formula 1.

$$A = (A_{520} - A_{700})\ pH\ 1.0 - (A_{520} - A_{700})\ pH\ 4.5 \quad \ldots\ldots\ldots\ldots\ (1)$$

The results were expressed as mg cyanidin-3-glucoside equivalents per 100 g.

2.5 Antioxidant activity

2.5.1 DPPH radical scavenging assay

The free radical scavenging activity of *F. indica* root extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay following a modified protocol of Eramma and Gayathri, (2021). Briefly, 0.25/ g of dried extract was homogenized in 5/ ml of 0.1% (w/v) trichloroacetic acid (CCl_3COOH) and centrifuged at 10,000/

rpm for 5/ min. For the assay, 100/ µl of the sample was mixed with 90/ µl of extraction solvent (80% ethanol, methanol or water) and 1/ ml of 0.1/ mm DPPH in methanol. The mixture was incubated in the dark at room temperature for 20/ min. Control comprised 100/ µl ethanol with 1/ ml DPPH. Absorbance was recorded at 517/ nm and radical scavenging activity was calculated using the standard formula 2.

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100 \quad \ldots\ldots\ldots\ldots (2)$$

where, A_0 is the control absorbance and A is the sample absorbance.

2.5.2 FRAP assay

The antioxidant activity of *F. indica* root extract was evaluated using the FRAP assay following Rajurkar and Hande (2011) with minor modifications. The FRAP reagent was freshly prepared by mixing 300 m mofacetate buffer (pH 3.6), 10 mm TPTZ 2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mm HCl (Hydrochloric acid) and 20 mm $FeCl_3 \cdot 6H_2O$ (Ferric chloride hexa hydrate) in a ratio of 10:1:1 and then incubated at 37^{0}C for 10 min in the dark. Methanolic root extracts (10-600/ µg/ml) were mixed with 3 ml of FRAP reagent vortexed and incubated at 37^{0}C in dark for 30 min. The formation of the Fe^{2+}-TPTZ complex was measured at 593 nm. Ascorbic acid (10-100/ µg/ml) served as the standard and methanol as the blank. Results were expressed as ascorbic acid equivalents (AAE) using a standard calibration curve.

2.6 Thin layer chromatography (TLC)

TLC was used to identify flavonoids in ethanolic, methanolic and aqueous root extracts of *F. indica* following the method of Eramma and Patil (2023). About 20 to 50/ µl of each concentrated extract was applied onto pre-coated silica gel TLC plates. Plates were developed in a solvent of n-butanol, acetic acid and water (4:1:5) and subsequently exposed to ammonia fumes overnight for enhanced visualization. Flavonoid compounds were indicated by dark orange bands and the retention factor (R_f) for each band was calculated using the standard formula 3.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} \quad \ldots (3)$$

2.7 GC-MS analysis

GC-MS analysis of the ethanolic root extract of *F. indica* was performed following a slightly modified protocol of Eramma and Gayathri (2013). About 1/ ml of concentrated extract was re-dissolved in HPLC grade methanol centrifuged at 10,000/ rpm for 5/ min at 4^{0}C and filtered through a 0.22/ µm PTFE syringe filter. The filtered solution was transferred to GC-MS vials and analyzed using an Agilent 7890A gas chromatograph coupled to a 5975C mass selective detector with a DB-5MS capillary column (30/ m × 0.25/ mm; ID × 0.25/ µm). The oven temperature was set at 60^{0}C for 2/ min, ramped at 10^{0}C/min to 30^{0}C and held for 10/ min. Helium was used as the carrier gas at 1.0/ ml/min. The injection volume was 1/ µl in split mode (10:1). The mass spectrometer operated in electron impact mode at 70/ eV scanning m/z 40-600 with ion source and interface temperatures of 200^{0}C and 250^{0}C, respectively. Phytochemicals were identified by comparison with the NIST Mass Spectral Library classified by structural group and interpreted for potential bioactivities such as antioxidant, antimicrobial and cardioprotective effects.

2.8 Statistical analysis

All experiments were performed in triplicates and data were expressed as mean ± standard deviation (SD) using descriptive statistics.

3. Results

3.1 Qualitative phytochemical screening

Preliminary phytochemical screening of *F. indica* root extracts in ethanolic, methanolic and aqueous solvents revealed solvent dependent composition. Polyphenols (deep green with $FeCl_3$) and anthocyanins (pink in chloroform ammonia test) were present in all extracts, while flavonoids (orange yellow with $AlCl_3/H_2SO_4$) and quinones (red bluish precipitates with H_2SO_4) were detected only in ethanolic and methanolic extracts (Table 1). The ethanolic extract showed the richest profile, reflecting ethanol's intermediate polarity and superior extraction efficiency (Ferreira and Sarraguca, 2024).

3.2 Quantitative estimation of phytochemical

3.2.1 Total phenolic content

The Table 2 and Figure 2 shows the TPC of *F. indica* root extracts measured by the folin-ciocalteu method (mg GAE/100 g) varied with solvent. The ethanolic extract had the highest phenolic concentration (316 mg GAE/100 g) followed by methanolic (236 mg GAE/100 g) and aqueous extracts (92 mg GAE/100 g).

Table 1: Qualitative phytochemical screening of *F. indica* root extracts

S.No.	Phytochemicals	Ethanolic extract	Methanolic extract	Aqueous extract
1	Polyphenols	+	+	+
2	Flavonoids	+	+	–
3	Quinones	+	+	–
4	Anthocyanins	+	+	+

Table 2: Total phenolic content of *F. indica* root extracts

S. No.	Extract type	OD (mean ± SD)	TPC (mg GAE/100 g ± SD)
1	Ethanol (80%)	2.201 ± 0.082	316 ± 10.58
2	Methanol	1.664 ± 0.167	236 ± 11.14
3	Aqueous	0.664 ± 0.041	92 ± 4.16

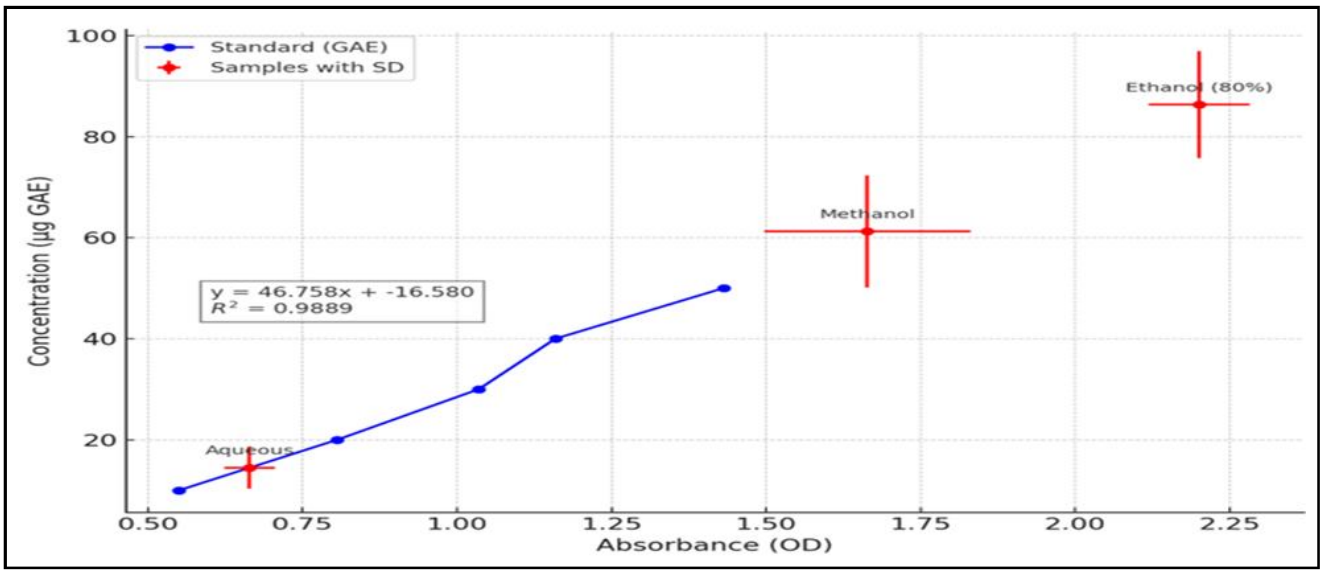

Figure 2: Standard curve for estimation of total phenolic content (TPC) using gallic acid equivalents (GAE).

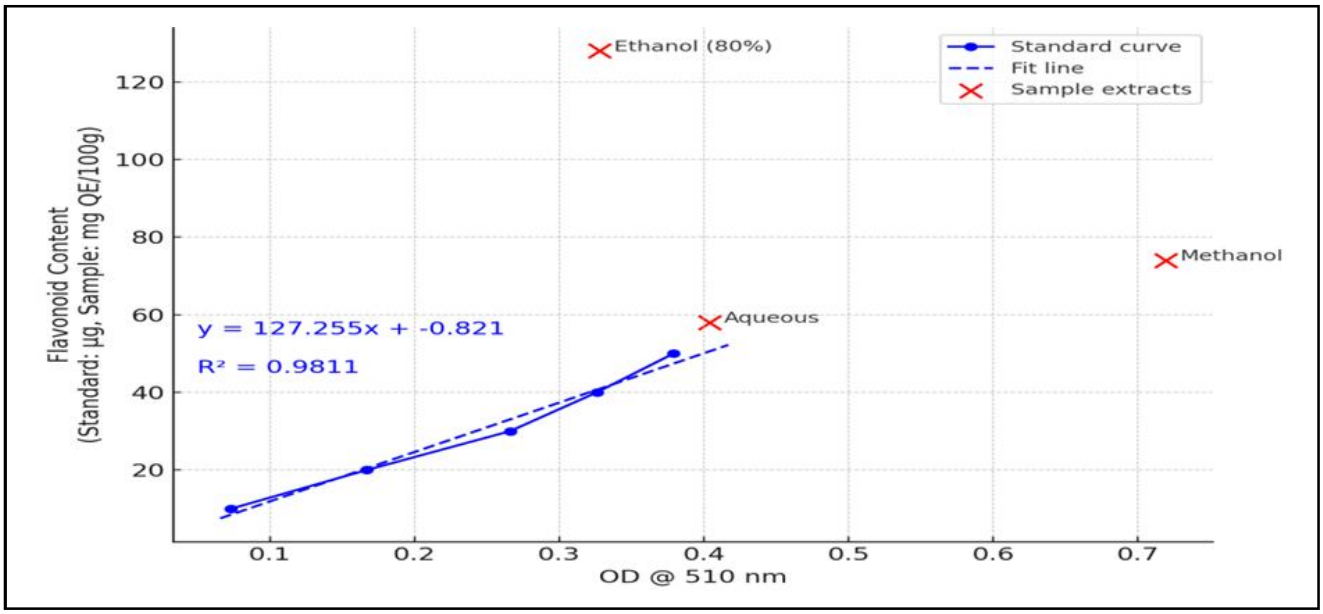

Figure 3: Standard curve for estimation of total flavonoid content (TFC) using quercetin equivalents (QE).

3.2.2 Total flavonoid content

Table 3 and Figure 3 explained the TFC of *F. indica* root extracts measured using the aluminium chloride method (mg QE/100 g) varied with solvent. The 80% ethanolic extract had the highest TFC (128 mg QE/100 g; OD 0.328), followed by methanolic (74 mg QE/100 g; OD 0.719) and aqueous extracts (58 mg QE/100 g; OD 0.404).

Table 3: Total flavonoid content of *F. indica* root extracts

S.No.	Extract type	OD (mean ± SD)	TFC (mg QE/100 g ± SD)
1.	Ethanol (80%)	0.328 ± 0.028	128 ± 10.58
2.	Methanol	0.719 ± 0.007	74 ± 11.14
3.	Aqueous	0.404 ± 0.013	58 ± 4.16

3.2.3 Ascorbic acid

The ascorbic acid content of *F. indica* root extracts measured by titration (mg/100 g) given in Table 4 was indicated by a pale pink endpoint. The ethanolic extract showed the highest concentration (23.16 mg/100 g), followed by the aqueous (14.74 mg/100 g) and methanolic extracts (6.31 mg/100 g).

Table 4: Ascorbic acid content of *F. indica* root extracts

S.No.	Extract type	Ascorbic acid content (mg/100 g ± SD)
1	Methanol	6.31 ± 0.5
2	Ethanol	23.16 ± 1.2
3	Aqueous	14.74 ± 0.8

3.2.4 Anthocyanin

The anthocyanin content of *F. indica* root extracts measured by the pH differential method (mg cyanidin-3-glucoside equivalents/100 g) varied with solvent. The ethanolic extract showed the highest concentration (18.26 mg/100 g), followed by methanolic (14.73 mg/100 g) and aqueous extracts (11.21 mg/100 g).

3.3 Antioxidant activity

3.3.1 DPPH radical scavenging assay

The DPPH assay showed solvent dependent variations in antioxidant activity of *F. indica* root extracts. The aqueous extract exhibited the highest radical scavenging activity (9.10% ± 0.46), followed by ethanolic (4.39% ± 0.22), methanolic (2.92% ± 0.15), and direct samples (0.48% ± 0.02) (Table 5).

Table 5: DPPH radical scavenging activity of *F. indica* root extracts

S.No.	Sample type	OD (Mean ± SD)	% DPPH inhibition (Mean ± SD)
1	Direct sample	0.618 ± 0.028	0.48 ± 0.02
2	Ethanolic extract	0.588 ± 0.010	4.39 ± 0.22
3	Methanolic extract	0.633 ± 0.067	2.92 ± 0.15
4	Aqueous extract	0.559 ± 0.080	9.10 ± 0.46

3.3.2 FRAP assay

The FRAP assay showed solvent dependent variations in the reducing potential of *F. indica* root extracts with the ethanolic extract exhibiting the highest activity (472.14 µMol Fe²z /g), followed by methanolic (398.76 iMol Fe²z /g) and aqueous extracts (301.25 µMol Fe²z /g).

3.3.3 TLC analysis

TLC of the ethanolic *F. indica* root extract revealed (Figure 4) a prominent spot (R_f = 0.97) corresponding to the standard quercetin indicating its presence as a major flavonoid component.

3.4 GC-MS analysis

The GC-MS analysis of the ethanolic root extract of *F. indica* revealed a mixture of 15 phytochemical constituents eluting between 2.26 and 18.17 min (Figure 5 and Table 6). Early retention peaks corresponded to simple esters, whereas the mid-region of the chromatogram was dominated by organosiloxanes, which contributed the largest proportion of the total peak area. Among these, octasiloxane, hexadecamethyl- (~9%), heptasiloxane, tetradecamethyl- (~8%) and cyclononasiloxane, octadecamethyl- (~7%) represented the major components. At higher retention times, several biologically relevant metabolites were detected including indole-2-carboxylic acid derivatives (~6%), gibb-3-ene dicarboxylic acid lactone methyl ester (~4.5%), benzylamine derivatives, adamantane-based compounds, imidazole-related structures and the isoquinoline alkaloid glaucine (~3%). Overall, the extract exhibited a chemically rich profile consisting of esters, siloxanes, alkaloids, diterpenoids and diverse heterocyclic compounds. The complete list of identified metabolites along with their retention times, molecular formulas, peak areas and pharmacological relevance is presented in Table 6.

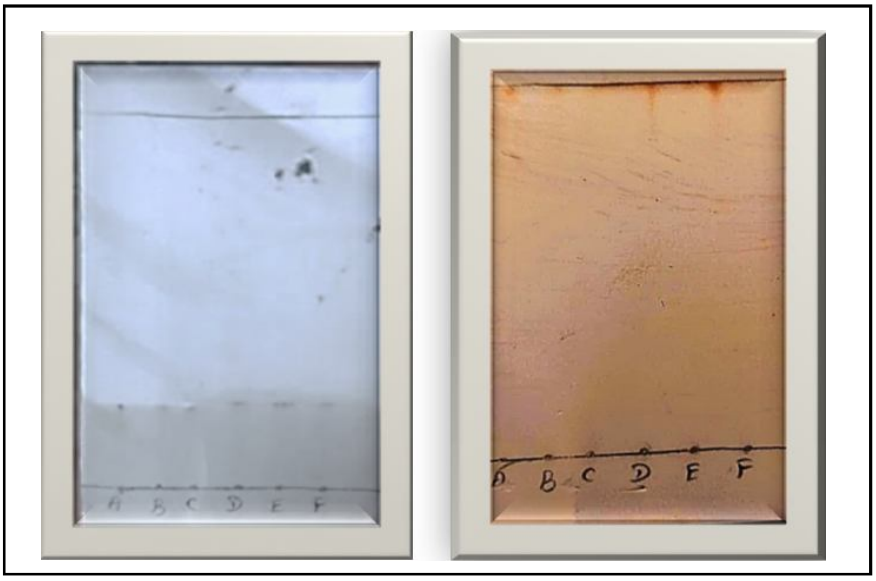

Figure 4: TLC profile of *F. indica* root extracts in ethanol, methanol and water showing prominent spot at R_f 0.97 corresponding to quercetin.

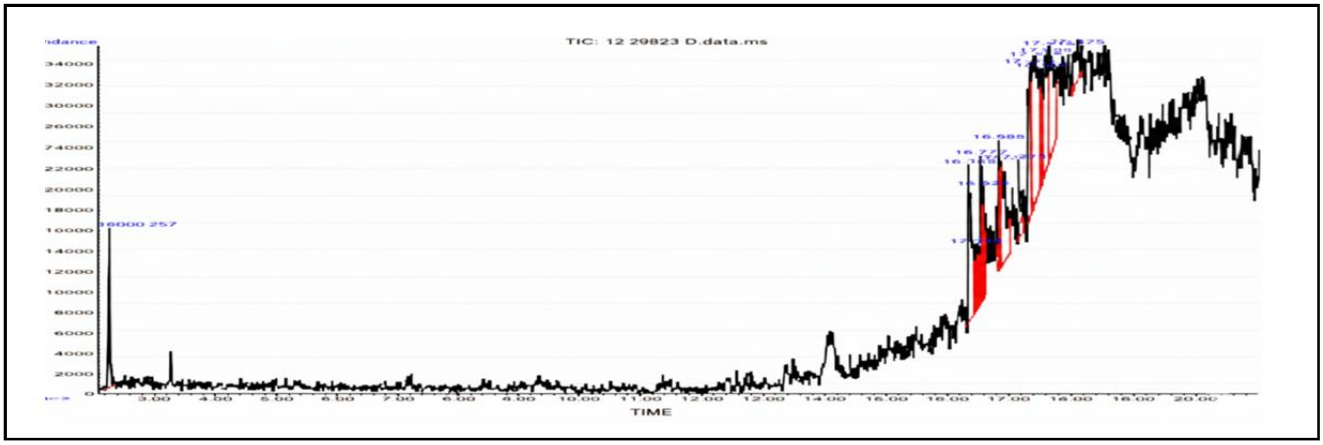

Figure 5: GC-MS chromatogram of the ethanolic root extract of *F. indica* showing major phytoconstituents.

Table 6: GC-MS based phytochemical profiling of ethanolic root extract of *F. indica*

S.No.	Compound name	Retention time (min)	Area %	Molecular formula	Compound category	Pharmacological activities	References
1	Cyclopropanecarboxylic acid, 2-ethenyl-, methyl ester	2.26	~1.5%	$C_6H_8O_2$	Fatty acid ester	Antimicrobial	Akhmetova *et al*., 2018
2	1-Cyclopentene-1-carboxylic acid, methyl ester	2.26	~1.3%	$C_7H_{10}O_2$	Monocarboxylic acid ester	Anti-inflammatory	Sadek *et al.,* 2013
3	Heptasiloxane, tetradecamethyl-	16.38	~8%	$C_{14}H_{44}O_6Si_7$	Organosiloxane	Cosmetic additive	Johnson *et al*., 2011
4	Octasiloxane, hexadecamethyl-	16.52	~9%	$C_{16}H_{50}O_7Si_8$	Organosiloxane	EmollientInert	Bhavani *et al.,* 2025
5	Silicic acid, diethyl bis (trime-thylsilyl) ester	16.57	~5%	$C_{10}H_{28}O_4Si_3$	Silicon ester	Intermediate Hydrophobic agent	Thiemann, 2025
6	1H-Indole-2-carboxylic acid, substituted	16.62	~6%	$C_{19}H_{23}NO_4$	Indole alkaloid derivative	Antioxidant, Anticancer	Youssif *et al.,* 2018
7	trans-3-Ethoxy-β-methyl-β-nitrostyrene	16.88	~4%	$C_{11}H_{13}NO_3$	Nitroaromatic	Antibacterial	Milhazes *et al.,* 2006
8	N-Methyl-N-acetyl-3,4-methy-lenedioxy benzylamine	17.20	~3.5%	$C_{11}H_{13}NO_3$	Benzylamine derivative	Neuromodulatory	Colado *et al*., 2001
9	Gibb-3-ene dicarboxylic acid lactone methyl ester	17.58	~4.5%	$C_{21}H_{28}O_5$	Diterpenoid	Plant hormone, antimicrobial	Petracek *et al*., 2003; Karthikeyan *et al.,* 2016
10	N-Methyl-1-adamantaneacetamide	17.71	~3%	$C_{13}H_{21}NO$	Adamantane derivative	Antiviral,CNS active	Popiolek *et al*., 2024; Balakrishnan *et al*., 2025
11	Glaucine	17.80	~3%	$C_{21}H_{25}NO_4$	Isoquinoline alkaloid	Antitussive, Antihypersensitive, Antispasmodic	Kusman Saygi *et al.,* 2023.
12	Cyclononasiloxane, octadecamethyl-	17.71	~7%	$C_{18}H_{54}O_9Si_9$	Siloxane polymer	Antifungal	Mustafa *et al.,* 2013
13	Phenol, 2-[4-(2-hydroxyethylamino)-2-quinazolinyl]-	17.88	~2.5%	$C_{16}H_{17}N_3O_2$	Quinazoline derivative	Anti-inflammatory	Zayed, 2022
14	2-(4-Chlorophenyl)-5,7-dimethylimidazo[1,2-a]pyridine	18.17	~3%	$C_{16}H_{12}ClN_3$	Imidazopyridine	Antitumor	Aliwaini *et al.,* 2019
15	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid ester	17.71	~2.5%	$C_{10}H_9N_5O_2$	Triazole derivative	Antimicrobial, Antifungal	Abd El-Aleam *et al.,* 2020

4. Discussion

The pharmacological significance of the widespread occurrence of polyphenols and anthocyanins is represented by their antioxidant and anti-inflammatory activities (Garg *et al*., 2022), while anthocyanins have been known to modulate oxidative stress and cellular defense (Teng *et al*., 2022) and thus, support traditional uses in diarrhea, wounds and inflammation. The selective occurrence of flavonoids and quinones only in alcoholic extracts underlines the higher efficacy of these solvents in recovering bioactives. Flavonoids

possess activities such as antimicrobial, hepatoprotective and cytoprotective effects (Sangeetha *et al*., 2016), while the presence of quinones contributes through redox cycling and the generation of ROS (Franza and Gaudu, 2022). The predominance of these phytoconstituents in ethanolic extracts agrees well with traditional preparations involving alcohol and also parallels other Salicaceae species, establishing its ethnomedicinal relevance (Prashith Kekuda *et al*., 2017).

Ethanol is an intermediate polarity solvent that can therefore facilitate the solubilization of both hydrophilic and moderately lipophilic phenolics, which in turn enhances the antioxidant potential (Devi *et al*., 2023). Phenolics may exhibit their antioxidant action as hydrogen or electron donors that neutralize free radicals, along with the chelation of pro-oxidant metals (Castaneda-Arriaga *et al*., 2018). Their abundance in *F. indica* roots has supported their traditional use against conditions related to oxidative stress, such as wounds, fever and inflammation and is in agreement with phenolic-rich extracts reportedly mitigating the risk of chronic diseases (Fratta Pasini and Cominacini, 2023). The lower content of phenolics in the aqueous extract can be attributed to the limited solubility of these components in highly polar solvents and this emphasizes the importance of choosing appropriate solvents for the maximal recovery of bioactives with therapeutic applications (Dirar *et al*., 2019).

Ethanol's polarity enabled the extraction of a wider range of flavonoid subclasses, including flavonols and flavones, which agrees with earlier reports (Tzanova *et al*., 2020; Jurinjak Tusek *et al*., 2022). The TFC was found to be lower in an aqueous extract because of the limited solubility of flavonoid aglycones (Hu *et al*., 2025). Flavonoids exhibit antioxidant, anti-inflammatory and antimicrobial properties by scavenging ROS, modulating enzymes and inhibiting lipid peroxidation (Alharbi *et al*., 2025). Therefore, higher flavonoid contents in the ethanolic extract justified the traditional usage of *F. indica* roots and emphasized the choice of solvent for the maximum phytochemical recovery and bioactivity, as seen in other medicinal plants (Nzor *et al*., 2024).

The efficiency of ethanol likely reflects its moderate polarity, which can effectively solubilize both hydrophilic and moderately lipophilic compounds without degradation (Seal *et al*., 2016). The poor yield from methanol and water may arise from limited solubility or oxidative loss. Ascorbic acid a potent antioxidant that acts supportively toward collagen synthesis and immune defense within the therapeutic action of *F. indica* roots acts in synergy with polyphenols and flavonoids, fostering increased overall antioxidant capacity (Agwu *et al*., 2023; Chen *et al*., 2022; Enko *et al*., 2015). These results emphasize the importance of choosing the right solvent to attain maximum recovery of bioactive vitamins and vindicate the folk use of ethanol based preparations.

Ethanol's high efficiency was attributed to its ability to penetrate cell walls, solubilizing flavonoid pigments in agreement with previous findings of Saleba *et al*. (2022) and Fu *et al*. (2021). These anthocyanins exert antioxidant, anti-inflammatory and antimicrobial activities, which improve the therapeutic value of roots of *F. indica* and modulate oxidative stress pathways, as evident from the studies of Molagoda *et al*. (2020) and Alam *et al*. (2021). This variation points toward the dependency on the solvent and the optimization required for extraction.

The superior activity of the aqueous extract is probably due to the high content of polar phenolics that are capable of donating hydrogen atoms to neutralize free radicals. This finding is in agreement with reports by Das *et al*. (2025) and Al Jumayi *et al*. (2022). Flavonoids and phenolic acids, substances believed to stabilize free radicals through hydrogen or electron transfer, contribute to the effect observed for these extracts (Musewu *et al*., 2019). Lower activity in methanolic and ethanolic extracts reflects poor solubility of hydrophilic antioxidants in organic solvents (Timon *et al*., 2024). These findings confirm the antioxidant potential of F. indica roots and support the traditional medicinal use there of (Al Bashera *et al*., 2024).

The FRAP values agreed with TPC, emphasizing phenolics as major electron donors for the reduction of $Fe^{3}z$ to $Fe^{2}z$ (Gulcin and Alwasel, 2025). The higher values observed for the ethanolic extract follow from ethanol's efficacy in extracting phenolic antioxidants, adding to its potency against oxidative stress-related diseases (Mehta *et al*., 2023). Variations among the DPPH and FRAP data point out both the influence of the polarity of solvents and assay mechanisms, thus justifying the use of more than one assay to better understand the antioxidant activity (Al-Tarifi *et al*., 2020; Rana *et al*., 2024).

Quercetin is known to possess antioxidant, anti-inflammatory and anticancer activities (Lofti *et al*., 2023; Kerna *et al*., 2024), and justifies the traditional use of *F. indica* against oxidative stress disorders such as cardiovascular diseases and diabetes (Mustafa *et al*., 2025). Detection of quercetin underlines the higher efficiency of ethanol for flavonoid extraction compared with methanol or water. The high R_f value was in agreement with earlier TLC studies that proved the reliability of the method for the identification of bioactive flavonoids (Kumar *et al*., 2010) and pinpointed the ethanolic extract as a rich source of therapeutically relevant compounds.

Organosiloxanes including derivatives of heptasiloxane and octasiloxane were the most abundant and apart from their cosmetic applications may also exhibit antioxidant and anti-inflammatory activities (Ivanova *et al*., 2023; Owolabi *et al*., 2018). Nitrogenous compounds detected include indole derivatives, benzylamines and triazoles, known for antioxidant, anticancer and antimicrobial properties (Dixit *et al*., 2021). Glaucine is an isoquinoline alkaloid that suggests antitussive and antispasmodic potential (Meyer *et al*., 2015; Bozkurt *et al*., 2022). Gibberellic acid (Gibb-3-ene dicarboxylic acid lactone methyl ester) points toward plant growth and development and also shows antimicrobial action (Alabdeen Khdar *et al*., 2025). Fatty acid esters like cyclopropane carboxylic acid methyl ester are known for their antimicrobial and anti-inflammatory activities (Ronn *et al*., 2016; Okore *et al*., 2021). These observations are consistent with earlier reports on *F. indica* bark extracts (Perera *et al*., 2018) and therefore suggest that the ethanolic root extract is a rich source of bioactive compounds with antimicrobial, antioxidant, anti-inflammatory and neuroactive properties, which justify its traditional use in medicine (Puri *et al*., 2018).

5. Conclusion

The study evidences the ethnopharmacological relevance of *F. indica* roots by investigating their chemical richness and therapeutic potential. Ethanol proved most effective in extracting diverse bioactive compounds including phenolics, flavonoids and anthocyanins known for antioxidant and cellular protective roles. Chromatographic and

GC-MS analyses confirmed compounds with antioxidant, antimicrobial and cardioprotective activities supporting traditional uses against inflammation, infections and metabolic disorders. These findings position the ethanolic root extract as a promising candidate for pharmacological studies with future research needed to isolate active compounds elucidate mechanisms and validate efficacy *in vivo* for potential therapeutic development.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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