

Phytochemical Profiling of *Ficus religiosa* Using Solvent Extractions with Antimicrobial Assay and Antioxidant Analysis

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ABSTRACT

Ficus religiosa (L.) Roxb., the Peepal tree, a large and evergreen medicinal plant of the sacred fig family, has many therapeutic uses in traditional medicine. In the present investigation, we conducted a qualitative and quantitative phytochemical profile of *F. religiosa* leaf extracts and determined the in vitro antibacterial, antifungal, and antioxidant activities of the extracts, thus providing a scientific basis for its ethnomedicinal use. Sequential extractions of both the Leaf and Stem were performed with polar solvents in following order: distilled water, followed by methanol, chloroform and ethyl acetate for 4 h each. Results: Qualitative screening detected high amounts of tannins and phenols, especially in methanolic and aqueous extracts, whereas saponins, steroids, cardiac glycosides, terpenoids, and coumarins were also present. A marker for all solvents but concentrated, alkaloids, anthraquinones, and anthocyanins were also absent across the board. The total phenolic, tannin, and flavonoid contents, as determined by quantitative spectrophotometric analysis, were 8.68 mg/g ($R^2 = 0.9443$), 506 mg/g ($R^2 = 0.9817$), and 47.8 mg/g, respectively ($R^2 = 0.9462$). Antibacterial activity tested using the agar well diffusion method showed low inhibition zones (0.1–0.3 mm) only for the methanolic extracts of four bacterial strains, with *Serratia marcescens* being the most sensitive, followed by *Pseudomonas putida*, and *Enterobacter cloacae*. DMSO and methanol also showed antifungal activity against *Aspergillus niger* at 4 mm and 1 mm, respectively. Antioxidant activity (FRAP method) showed the highest absorbance at 60 μ l concentration (O.D. = 0.604). Together, these findings highlight the potential of *F. religiosa* as an excellent source of bioactive compounds for pharmaceutical standardization and drug development.

Keywords: *Ficus religiosa*, Phytochemical profiling, Tannins, Flavonoids, Phenols, Antibacterial activity, Antifungal activity, Antioxidant activity, FRAP.

1. INTRODUCTION

For thousands of years, medicinal plants have formed the basis of traditional handbooks of medicine and remain an essential source for modern drug discovery. Globally, approximately 80% of the population depends on plant-based medicines as primary health care (WHO, 2019). The rising burden of chronic and degenerative diseases in the last decades, alongside the drawbacks and secondary effects associated with synthetic pharmaceutical drugs and the emergence of multidrug microbial resistance, have increased the attention on phytochemicals as safer, cheaper, biodegradable, and sustainable therapeutic alternatives (Rates 2001; Fabricant & Farnsworth 2001).

Plant secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, saponins, terpenoids, and glycosides, have been shown to have multiple biological effects. They are important candidates for drug development because these bioactive molecules interact with cellular targets to exert antimicrobial, antioxidant, anti-inflammatory, anticancer, and neuroprotective activities (Cowan 1999; Koehn & Carter 2005). Free radical scavenging characteristics and oxidative stress modulation pathways are the topics of research investigating phenolics and flavonoids, particularly the primary plant secondary metabolites that have been widely studied (Scalbert et al., 2005; Kumar & Pandey, 2013).

Ficus religiosa L. (Family: Moraceae); Peepal tree; Bo tree; Sacred Fig is a large semi-evergreen widespread along the Indian subcontinent, tropical and southeastern Asia. It is of great cultural and religious importance in Hinduism, Buddhism, and Jainism. In fact, leaves, bark, fruit, seeds, and latex of *F. religiosa* have been used to treat infections, diabetes mellitus (DM), epilepsy, asthma, skin disorders, etc. in traditional systems of medicine. Ayurveda and Unani systems (Aqil et al., 2006; Mousa & Mackay 2015; Kapile et al., 2022).

Phytochemical investigations of *F. religiosa* have identified a diverse array of compounds, including flavonoids (quercetin, rutin, kaempferol), tannins, phenolic acids, β -sitosterol, lanosterol, α -amyrin, lupeol, and various glycosides (Nayak et al., 2010; Kapile et al., 2022). Methanolic leaf extracts have been demonstrated to contain substantial total flavonoid content (23.37 mg QE/g) and total phenolic content (37.35 mg GAE/g), indicative of significant antioxidant capacity comparable to ascorbic acid. Sequential solvent extraction using hexane, chloroform, ethyl acetate, and methanol has enabled the isolation of bioactive metabolites with arginase inhibitory and cardiovascular-protective activities, including berberine and edoxudine (Shaikh et al., 2020). Additionally, bark extracts rich in tannins and anthraquinones have demonstrated functional utility in natural textile dye applications (Bhandari et al., 2025).

The selection of appropriate extraction solvents is crucial for phytochemical profiling, as the solubility and yield of certain classes of secondary metabolites are driven by polarity. More polar solvents are useful for extracting phenolics, tannins, and flavonoids, which are the most numerous class of phytochemicals with recognized biological activity in *Ficus religiosa*. Despite the relatively long ethnomedicinal use of this plant, integrative studies that quantitatively systemize all aspects seen qualitatively, combining biological evidence (in vitro), remain an essential requirement for its pharmaceutical standardization (Singh et al., 2011).

This study was therefore initiated: (i) to carry out qualitative phytochemical screening of leaf extracts of *F. religiosa* in four polarities; (ii) to investigate the quantitative determination of total phenolic, tannin, and flavonoid content by spectrophotometric methods; (iii) to test in vitro antibacterial, antifungal, and antioxidant activity; and (iv) experimentally classify a chemical and biological basis for the possible pharmaceutical development of natural product-based therapeutic agents from *Calotropis procera* with special reference to neurodegenerative effects as well as inflammatory disorders.

2. MATERIALS AND METHODS

2.1 Study Site and Plant Collection

Freshly grown mature leaves of *Ficus religiosa* were collected from a particular site in the Mehsana district of North Gujarat, India (Latitude N 23.5803666°, Longitude E 72.8047783°; Table 1). It was botanically identified and authenticated using standard morphological keys and cross-checked with herbarium specimens. Morning-harvested leaves were washed with running tap water to remove surface dust, debris, and microbial contaminants, followed by a distilled water rinse. The geographical coordinates of the collection sites are listed in

Table1.

Table 1: Coordinates of Collection Site

Sr.No.	District	Latitude (N)	Longitude (E)
1	Mehsana	23.5803666°	72.8047783°

2.2 Plant Material Preparation and Preservation

The leaves were dried at ambient temperature (25–30°C) in the shade for 10–14 days until free from moisture to avoid microbial degradation, making it safer for long-term storage of the materials (Harborne, 1998, Karande and Thakur 2026). Dried leaves were first coarsely crushed in a mortar and pestle and then powdered mechanically using a mixer-grinder. The resultant powder was subjected to a thorough screening process using a 40 mesh sieve to obtain a uniform, fine, and homogeneous powder with an increased surface area for solvent extraction. Until use, the powders were stored in sealed glass containers in a cool, dry, and dark environment.

2.3 Solvent Extraction

Phytochemical extraction was performed using the maceration or cold extraction method, which is applied to maintain heat-labile bioactive compounds that affect biological activity (Azwanida, 2015; Karande et. al. 2020). In this method, known quantities of fine leaf powder were immersed in the solvents and stirred intermittently over a fixed period until all soluble constituents were transferred to the solvent phase.

Four solvents of increasing polarity were selected to extract phytochemicals: (I) distilled water (polar protic), (II) methanol (polar protic), (III) chloroform (semi-polar aprotic), and (IV) ethyl acetate (semi-polar aprotic). This polarity-based strategy is commonly used for the fractional isolation of phytochemicals with different solubility profiles (Moyo et al., 2012). All extracts were filtered through Whatman No. 1 filter paper, evaporated, and stored at 4°C until tested.

2.4 Qualitative Phytochemical Screening

Standard qualitative phytochemical tests for the detection of secondary metabolite classes were performed using the methods described by Harborne (1998) and Trease and Evans (2002) (Table 2) to identify the constituents in all four solvent extracts.

Table 2: Qualitative Phytochemical tests

Phytochemical	Reagent/Test	Positive Indication
Anthraquinones	H ₂ SO ₄ + dilute ammonia	Rose-pink colouration
Quinones	Alcoholic KOH	Red to blue colouration
Alkaloids (Mayer's test)	Mayer's reagent	Creamy/white precipitate
Glycosides	Glacial acetic acid + FeCl ₃ + H ₂ SO ₄	Blue colouration
Cardiac Glycosides	Glacial acetic acid + FeCl ₃ + H ₂ SO ₄	Brown/purple/green rings at interface
Phenols (Lead Acetate test)	10% lead acetate solution	Bulky white precipitate
Polyphenols	5% lead acetate solution	Yellow precipitate
Tannins	5% FeCl ₃ neutral solution	Dark green colouration
Flavonoids	10% lead acetate solution	Yellow colouration
Saponins	Vigorous shaking with distilled water	Stable froth for ≥1 min
Steroids	Chloroform + conc. H ₂ SO ₄	Red colour and yellowish-green fluorescence
Terpenoids	Chloroform + H ₂ SO ₄	Reddish-brown at interface
Anthocyanins	2N HCl + ammonia	Pink-red to blue-violet shift
Coumarins	10% NaOH	Yellow colouration

2.5 Quantitative Phytochemical Analysis

2.5.1 Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Malik and Singh, 1980; Blainski et al., 2013; Patil et al., 2023.; Madhavan, 2015) with some modifications. Briefly, diluted extracts were prepared at different concentrations in 10 ml test tubes, and the volume was adjusted with distilled water (3 ml). Then, 0.5 ml of Folin-Ciocalteu reagent (1:1 v/v) in water and 20% Na₂CO₃ solution were added (2 ml). An alkaline medium is necessary for the redox reaction between phenolic compounds and phosphomolybdic acid to produce a blue molybdenum complex in the reaction mixture (Jain et al., 2025a; Uzma et al., 2022). The absorbance at 650 nm against a reagent blank was obtained after incubating the tubes for 1 min at 70°C and cooling, as per the manufacturer's instructions. Catechol was used as a standard for TPC, and the results were expressed as mg catechol equivalent/g dry weight (mg CAE/g DW) [23–25].

2.5.2 Total Tannin Content (TTC)

Tannin content was estimated using the Folin-Denis method (Galvão et al., 2018; Makkar 2003; Kardel et al., 2013). The sample (0.5 g) was placed in a 250 ml conical flask with 75 ml distilled water and boiled for 30 min (on gentle heat). The amount of autologous serum was extracted and centrifuged at 2,000 rpm for 20 min, and the supernatant was reconstituted to 100 ml. It was prepared by diluting 1 ml of the extract sample in a volumetric flask containing (75 ml of water) followed by the addition (5 ml of Folin-Denis reagent and 10 ml Na₂CO₃ solution) up to a total volume of 100 ml, and then stirred. The

absorbance was measured at 760 nm for 30 min against a reagent blank using tannic acid as a standard (Shetty & Kale, 2020; Das et al., 2020).

2.5.3 Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method (Jain et al., 2025b; Kamtekar & Patil 2014; Dhianawaty et al., 2022) was used for the quantitative analysis of total flavonoid content. Method Standard (pure quercetin) 25 mg/100 ml in methanol. Aliquots of 0.2–1.0 ml were distributed into test tubes, the volume was made up to 2 ml with methanol, and AlCl₃ (0.1 ml) and sodium/potassium acetate solutions (0.1 ml) were successively added. Distilled water was used to make the final volume equal to 5 ml and incubation was performed at room temperature for 45 min. UV–Vis spectrophotometer — absorption at 415 nm versus blank. The quercetin equivalent (QE) data of TFC were expressed in terms of mg QE/g dry weight (mg QE/g DW) (Uzma et.al. 2022)

2.6 In Vitro Biological Activity Assays

2.6.1 Antibacterial Activity

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Malik and Singh, 1980; Blainski et al., 2013; Patil et al., 2023.; Madhavan, 2015) with some modifications. Briefly, diluted extracts were prepared at different concentrations in 10 ml test tubes, and the volume was adjusted with distilled water (3 ml). Then, 0.5 ml of Folin-Ciocalteu reagent (1:1 v/v) in water and 20% Na₂CO₃ solution were added (2 ml). An alkaline medium is necessary for the redox reaction between phenolic compounds and phosphomolybdic acid to produce a blue molybdenum complex in the reaction mixture (Jain et al., 2025a; Uzma et al., 2022). The absorbance at 650 nm against a reagent blank was obtained after incubating the tubes for 1 min at 70°C and cooling, as per the manufacturer's instructions. Catechol was used as a standard for TPC, and the results were expressed as mg catechol equivalent/g dry weight (mg CAE/g DW) [23–25].

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Tannin content was estimated using the Folin-Denis method (Galvão et al., 2018; Makkar 2003; Kardel et al., 2013). The sample (0.5 g) was placed in a 250 ml conical flask with 75 ml distilled water and boiled for 30 min (on gentle heat). The amount of autologous serum was extracted and centrifuged at 2,000 rpm for 20 min, and the supernatant was reconstituted to 100 ml. It was prepared by diluting 1 ml of the extract sample in a volumetric flask containing (75 ml of water) followed by the addition (5 ml of Folin-Denis reagent and 10 ml Na₂CO₃ solution) up to a total volume of 100 ml, and then stirred. The absorbance was measured at 760 nm for 30 min against a

reagent blank using tannic acid as a standard (Shetty & Kale, 2020; Das et al., 2020).

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2.6 In Vitro Biological Activity Assays

2.6.1 Antibacterial Activity

Agar well diffusion method was used for screening antibacterial activity in NA (Nutrient Agar) medium (Bauer et al. 1966). In these experiments, four bacterial strains were prepared and utilized as: *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Bacillus subtilis* (Grampositive), and *Serratia marcescens* (gramnegative). Liquid cultures of active bacteria were kept in nutrient broth. The corresponding bacterial suspensions were seeded onto nutrient agar plates, and wells (6 mm diameter) were bored using sterile cup borer. The following plant extract from each well was loaded: one for distilled water extract and one for methanolic extract. Distilled water was used as a negative control, while penicillin was the positive control antibiotic. Plates set up in triplicate and incubated at 37 °C for 24 h. A Vernier caliper was used to measure zones of inhibition (ZOI) in mm.

2.6.2 Antifungal Activity

The agar well diffusion method was used to determine the antifungal activity of the medium of potato dextrose agar (PDA) against *Aspergillus niger*. PDA Plates: Active cultures of *A. niger* were prepared and spread on PDA plates. For each plate, four wells were drilled: (I) a negative control with DMSO, (II) distilled water extract, (III) methanolic extract, and (IV) extract dissolved in DMSO. Plates were incubated at 28°C for 72 h, and the zones of inhibition were measured in millimeters.

2.6.3 Hydroxyl Radical Scavenging Activity Assay

The FRAP assay assesses the ability of an extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) and is defined as antioxidant activity (Benzie & Strain, 1996). Leaf extract was

prepared in methanol, and aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml were taken. Methanol was used to equilibrate the volume of each aliquot to 1 ml. Then, 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml of a solution containing 1% potassium ferricyanide were added in that order. Each tube was sealed with aluminum foil and incubated for 20 min in a water bath at 50 °C. The indicated volume of 10% trichloroacetic acid (TCA) was added in the dark for a total working volume of the reaction mixture of 2.5 ml and shaken properly. Subsequently, 2.5 ml distilled water and 0.5 ml of a solution containing 0.1% ferric chloride were added. A blue-green color developed in the presence of ferrous reducing capacity, as described by a UV-Vis spectrophotometer was used to measure the optical density (OD) at 700 nm using a blank.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Screening

Qualitative phytochemical analysis of *F. religiosa* leaf extracts in the four solvents (Table 3) revealed the presence of various secondary metabolites. Tannins showed the highest (+++) observations in both aqueous and methanolic extracts. The methanolic extract showed a high (+ +)content of polyphenols, and the ethyl acetate extract showed a moderate content of phenols (+ +). Cardiac glycosides were identified in aqueous, methanolic, and chloroform extracts but not in ethyl acetate. Saponin was uniformly distributed in all four solvents tested. Stirrings were detected in the aqueous extract (abundant ++). In contrast, alkaloids, anthraquinone, anthocyanins, and free glycosides were not detected in any of the solvents.

These observations are largely consistent with the previously described qualitative profiles of *Ficus religiosa*. Polar solvents were characterized by their high occurrence of tannins and phenolics, indicating that these classes of compounds are hydrophilic in nature, making them most likely to be extracted with water and methanol (Kapile et al., 2022). This lack of alkaloids is consistent with reports that while some *Ficus* species contain alkaloids, these occur in very small amounts or are absent from leaf tissue altogether (Nayak et al., 2010). The presence of saponins in all solvents indicates the presence of structural variants with both hydrophilic and lipophilic moieties (Singh et al., 2011). The results confirmed that *F. religiosa* leaves were a good source of phenolic compounds, tannins, and saponins, supporting its ethnomedicinal uses.

Table 3: Qualitative Phytochemical Analysis of *Ficus religiosa* Leaf Extracts

(+++ = **Highly abundant**; ++ = **Moderately abundant**; + = **Present**; - = **Absent**)

Sr.	Phytochemical	Distilled Water	Methanol	Chloroform	Ethyl Acetate
1	Anthraquinones	-	-	-	-
2	Alkaloids	-	-	-	-
3	Glycosides	-	-	-	-
4	Cardiac Glycosides	+	+	+	-
5	Phenols	+	-	+	++
6	Polyphenols	+	++	-	+
7	Tannins	+++	+++	+	+
8	Flavonoids	+	+	-	-
9	Saponins	+	+	+	+
10	Steroids	++	+	-	+(trace)
11	Terpenoids	+	+	-	-
12	Anthocyanins	-	-	-	-
13	Coumarins	+	+	+	+(trace)

3.2 Quantitative Phytochemical Analysis

Spectrophotometric quantification of total phenolic, tannin, and flavonoid contents in *F. religiosa* leaf extracts yielded strong calibration curves and measurable phytochemical concentrations, as summarized in Table 4.

Table 4: Quantitative Phytochemical Content of *Ficus religiosa* Leaf Extract

Phytochemical	Standard Used	Wavelength (nm)	R ² Value	Content (mg/g)
Total Phenolic Content	Catechol	650 nm	0.9443	8.68
Total Tannin Content	Tannic Acid	760 nm	0.9817	506.00
Total Flavonoid Content	Quercetin	415 nm	0.9462	47.80

F. religiosa leaves have very high total phenolic content (8.68 mg CAE/g DW based on calibration R² = 0.9443 of phenolic compounds). The data obtained is consistent with an earlier study by Jain et al(2025a), who reported substantial methanolic extract of *Ficus religiosa*, gives 37.35 mg GAE/g also showed that levels of phenolics were high in some of the *Ficus* species. The remarkably high total tannin content of 506 mg/g DW (R² = 0.9817) . They have a high level of tannins with strong antimicrobial and antioxidant activities by binding the metal ions, precipitation of proteins and damage to the outer structures membranes (Dhianawaty et al., 2022; Kardel et al., 2013). *F. religiosa* had a total flavonoid concentration of quercetin and rutin (47.8 mg QE/g DW, R² = 0.9462) as the main components

in specific profiles that are well studied for their antioxidant and anti-inflammation activity (Kamtekar et al., 2014; Sharma et al., 2022).

3.3 In Vitro Antibacterial Activity



Figure 1: Agar plates showing zone of inhibition (*S. marcescens* top; *E. coli* bottom)



Figure 2: *E. coli* / *Serratia marcescens* plate – close-up (EC/23/12/25)



Figure 3: Additional antibacterial plate – *E. coli* (EC/23/12/25)

Antimicrobial activity of *F. religiosa* leaf extracts against four bacterial pathogens by disc diffusion assay. The results were presented in Table 5. Only the methanolic extracts showed inhibition zones, while all wells containing aqueous extracts were devoid of any inhibitory activity. *Serratia marcescens* showed an inhibition zone size of 0.3 mm, while the methanolic extract inhibited *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* with zone sizes of mm. These small regions illuminate, suggesting successful anti-bacterial activity; however, no intended and important antimicrobial action has been observed, such as methanol better than antibacterial components dissolve in it than with water.

Table 5: Antibacterial Activity of *Ficus religiosa* Leaf Extracts

Sr.	Bacterial Strain	ZOI- Methanolic Extract (mm)	ZOI- Aqueous Extract (mm)	Gram Status
1	<i>Escherichia coli</i>	0.1	No zone	Negative
2	<i>Bacillus subtilis</i>	0.1	No zone	Positive
3	<i>Staphylococcus aureus</i>	0.1	No zone	Positive
4	<i>Serratia marcescens</i>	0.3	No zone	Negative

The basic reason for a greater ZOI against *S. marcescens* (gram-negative) than against other bacteria may be due to the specific susceptibility of its outer membrane lipopolysaccharide to the components present in higher concentrations in the methanolic extract. These components can disturb bacterial membrane integrity and more complex phenol ring flavonoid glycosides, whose aglycones also inhibit efflux pump activity (Cowan,1999). The absence of activity in aqueous extracts is consistent with the dilution effect and lower concentration of lipophilic antibacterial compounds within that fraction. The moderate inhibitory activities observed in this study are consistent with the findings of Kapile et al. (2022) and Nayak et al. (2010), who reported low-to-moderate antibacterial activity in *F. religiosa* leaf extracts, suggesting that isolated or semi-purified fractions may exhibit more potent activity.

3.4 In Vitro Antifungal Activity

The in vitro antifungal activity against *Aspergillus niger*, as shown in Table 6, showed that the inhibition depended on the solvent used for extraction. The highest (4 mm) inhibition zone was obtained with the DMSO extract, while the methanolic extract produced a 1 mm zone with no antifungal activity. The control (DMSO only) recorded a 5 mm zone, revealing that the solvent itself possesses an intrinsically weak antifungal property, thus complicating the interpretation of the extract dissolved in DMSO.

Table 6: Antifungal Activity of *Ficus religiosa* Leaf Extracts Against *Aspergillus niger*

Solvent / Treatment	ZOI- Extract (mm)	ZOI- Control (mm)
DMSO	4	5
Methanol	1	1
Distilled Water	No zone	No zone

The moderate antifungal activity of the methanolic extract (1 mm zone) against *A. niger* could be ascribed to the presence of tannins, terpenoids, and saponins, which inhibit fungal ergosterol biosynthesis and cell membrane integrity [Singh et al., 2011]. However, the total lack of activity in the aqueous extract reinforces that the polar-aqueous fraction of *F. religiosa* does not contain strong antifungal constituents at the evaluated concentrations. In summary, these findings corroborate the mild but real antifungal effect of *F. religiosa* extracts, where both methanol and DMSO served as extraction solvents.

3.5 Antioxidant Activity (FRAP Assay)

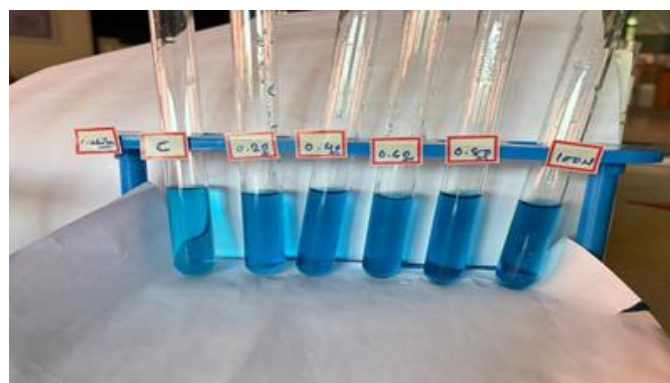


Figure 4: FRAP assay test tubes showing blue colour development at increasing concentrations (Inhibitor, C, 0.20, 0.40, 0.60, 0.80, 100 µl)

Table 7 shows the antioxidant activity of methanolic *F. religiosa* leaf extract in five concentration aliquots as FRAP (µg AAE) g-1 FW. After the addition of ferric chloride, characteristic blue colour developed in test tubes verifying ferric-reducing capacity as shown by Fig. 4. The optical density data showed a non-linear pattern of dose-responsiveness, as maximum absorbance was observed at 60 µl (0.604), with an apparent decline between the doses tested (80 µl: 0.507; 100 µl: 0.404), probably due to pro-oxidant activity or aggregation mechanisms when higher extract concentrations are used.

Table 7: FRAP Antioxidant Activity – Optical Density Readings at 700 nm

Concentration (μl)	20	40	60	80	100
Absorbance (OD)	0.335	0.499	0.604	0.507	0.404

The high levels of tannins (506 mg/g) and flavonoids (47.8 mg/g) identified in the quantitative analysis may account for the observed antioxidant activity. Similar to other powerful natural antioxidants of plant origin, including tannins and flavonoids, they are capable of donating hydrogen (Scalbert et al., 2005; Benzie & Strain, 1996) to free radicals and chelating transition metal ions. Phytochemical analysis and antioxidant studies have confirmed that some compounds derived from *F. religiosa* exhibit significant antioxidant capacity comparable to ascorbic acid when present in methanolic leaf extracts. The FRAP results obtained in this study support the qualitative and quantitative findings and reaffirm *F. religiosa* as a potentially important natural source of antioxidants for use in nutraceuticals and pharmaceutical formulations.

4. CONCLUSION

The current study offers exhaustive phytochemical, antimicrobial, and antioxidant characterization of *Ficus religiosa* leaf extracts. Qualitative screening revealed that tannins (in very large quantities), polyphenols, phenols, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, and coumarins were present in each solvent, with the abundance of identified metabolites being greatest in polar solvents (water and methanol). Quantitative analysis confirmed the total contents of tannin (506 mg/g), flavonoid (47.8 mg/g), and phenolic (8.68 mg/g) with strong linear calibration models, confirming that this plant has a high content of pharmacologically active secondary metabolites. Only the methanolic extracts exhibited in vitro antibacterial activity, with *Serratia marcescens* showing the highest susceptibility. DMSO and methanolic fractions exhibited antifungal activity against *Aspergillus niger*. A concentration-dependent ferric-reducing activity with a peak at 60 μL was confirmed using the FRAP antioxidant assay.

These findings support the traditional medicinal uses of *F. religiosa* and highlight the crucial role of solvent polarity in the selective extraction of bioactive compounds. Exploiting methanol as a polar solvent, extracts with enhanced biological activity and phytochemical richness were obtained in all but one instance. The isolation and structural elucidation, mechanism of action studies, and preclinical and clinical evaluations for the safety and efficacy of the minor actives should be a future focus

to leverage their potential roles in treating infections or diseases resulting from oxidative stress, such as neurodegenerative disorders like Parkinson's disease. Therefore, this investigation provides a strong chemical-biological basis for *F. religiosa* as a potential pharmaceutical reference standard, nutraceutical source, or drug discovery matrix.

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