

Phytochemical screening and Characterization using HPLC and FTIR Techniques and analysis of Antioxidant activity of *Ficus Palmata* (Bedu)

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Abstract

From ancient times, traditional medicines have been used in Ayurveda, Siddha and Unani. Numerous illnesses are amenable to *Ficus palmata* treatment belonging to the Moraceae family. The goal of this work is to identify the chemical components through water, petroleum ether, acetone, ethanol and methanol extracts of *Ficus palmata* stems using basic phytochemical analysis, HPLC and FTIR spectroscopy. The existence of phenols, tannins, flavonoids, alkaloids, glycosides, steroids, saponins and terpenoids was demonstrated by the results of the phytochemical study. A C18-150 4.6 mm column, 10 μ injection volume and 80:20 v/v (methanol: acetonitrile) mobile phase at 30°C were used to conduct a reversed-phase HPLC study.

The UV detector was used to record the detection at 210 and 290 nm. FTIR analysis of stem extracts in methanol and petroleum ether indicated the presence of aliphatic amine compounds with significant peaks along with peaks of primary amines, alcohols, amides, aldehydes, alkanes, alkyl halides, ethers, aromatics and alkenes. The assay for 1,1-diphenyl-2-pycryl-hydrayl "DPPH" and the test for hydrogen peroxide were used to quantify the free radical scavenging activity.

Keywords: Phytochemical analysis, HPLC, FTIR, *Ficus Palmata*, DPPH, Diseases.

Introduction

Ficus species exhibit a wide range of growth habits, leaf shapes and sizes. They often have a fascinating ecological role, providing food and habitat for various animals including certain species of birds and insects. Additionally, many *Ficus* species have cultural or religious significance in different parts of the world¹⁰. *Ficus palmata*, also known as the Indian rock fig or wild fig, is a type of fig tree found in parts of India and Southeast Asia. While it might not be as extensively studied for medicinal purposes compared to some other plants, various parts of the *Ficus palmata* have been traditionally used in folk medicine²².

In Ayurveda, the traditional medicinal system of India, various parts of plants were utilized for their therapeutic properties. *Ficus palmata* holds significance in Ayurvedic

practices, particularly its stem for its potential medicinal benefits: The stem's latex or sap is believed to have properties that aid in wound healing. It has been used topically as a poultice for cuts, wounds and skin infections due to its reported antimicrobial and healing properties. In Ayurvedic traditions, various parts of the *Ficus palmata*, including the stem, are believed to possess anti-inflammatory properties. While the stem might not be commonly used for digestive issues, some Ayurvedic practices suggest that certain parts of the *Ficus palmata* including the fruits, might aid in digestion.



Fig. 1: Image showing the stem of *Ficus palmata*

Antioxidants in plant extracts may scavenge free radicals, reducing oxidative damage to cells and tissues. Some antioxidants possess anti-inflammatory properties which can help to mitigate inflammation-related conditions internally and externally. Antioxidants are associated with reducing the risk of certain chronic diseases like cardiovascular diseases, neurodegenerative disorders and cancer.

Further research involving *in vitro* and *in vivo* studies specific to *Ficus palmata* stem extracts would provide more concrete information on their antioxidant potential¹². Techniques such as assays measuring the scavenging activity of free radicals like DPPH or ABTS assays are commonly used to assess the antioxidant capacity of plant extracts. FTIR is a powerful analytical technique used to identify and analyze the chemical composition of substances based on their molecular structure. It helps in identifying

various compounds present in the extract such as phenols, flavonoids, alkaloids and other functional groups. This analysis can aid in understanding the potential bioactive components of the plant material and their potential applications in medicine or other industries.

Material and Methods

Plant sample collection and authentication: The foliage of *F. palmata* was collected from the Tehri region of Uttarakhand, India. The BSI (Botanical Survey of India) validated and taxonomically identified the plant samples. A voucher specimen (873) of *F. palmata* Forsk. was deposited at herbarium of BSI, Dehradun.

Preparation of plant extracts: The sequential Soxhlet extraction technique was used for the extraction. The leaves and stem were ground together in a grinder. A homogenous 25 gm of powdered plant substance was placed in a thimble and 250 ml of numerous solvents were used for extraction. After that, the thimble was placed inside the Soxhlet apparatus, where extraction was performed using petroleum ether, acetone, ethanol, methanol and water as solvents in sequential order from non-polar to polar. The extraction process lasts for a whole day, or until the solvent in the extractor's siphon tube turns colorless¹⁷.

Finally, soluble fractions in water, methanol, ethanol, acetone and petroleum ether were obtained. Crude extracts were then left behind as the extract was allowed to concentrate in vacuum using a rotating evaporator. The dried extract was kept in refrigerator at 4°C for future use in different analyses^{14,25}. All extracts were obtained from the Tehri regions of Uttarakhand. The following formula was used to determine the extract's percentage yield:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Extract (g)}}{\text{Weight of leaf powder (g)}} \times 100$$

Phytochemical Screening: Phytochemical screening of petroleum ether, acetone, ethanol, methanol and water extracts of *Ficus palmata* stem extract was carried out to detect the presence of various phytochemicals such as phenols, flavonoids, alkaloids, coumarins, tannins, saponins, terpenoids, steroids, alkaloids etc. according to the standard protocols^{14,25}. Changes in color and/or formation of precipitate with the addition of specified detecting reagents to the test solutions of various fractions were observed and the results were recorded as present (+) or absent (-) based on the observations. All the qualitative phytochemical tests were replicated thrice for confirmation^{2,5}.

Molisch's Test: In 2000 μ l of extract, add α - naphthol and shake well, slowly add 1ml of concentrated H₂SO₄. A blue-violet ring indicates the test as positive for carbohydrates.

Fehling's Test: Fehling's solution A consisted of dissolving 34.66 gm of CuSO₄ in distilled water. Fehling's solution B consisted of dissolving 173 gm of potassium sodium tartrate

and 50 gm of NaOH in water to make 500 ml. The existence of sugar was recommended by a crimson precipitate after mixing A and B with the extract.

Meyer's Test: In 1ml of extract, add drops of Meyer's reagent, the test will be positive for alkaloids if the precipitate is white and creamy.

Test for Glycosides: To 1ml of extract, 10% NaOH was added in two to three drops. Add 500 μ l of sodium nitroprusside. A change in color to blue will indicate the test as positive. For the Keller-Kiliani test, to 1.2ml of sample, add 1-2 ml of CH₃COOH acid, 2-3 drops of 5% ferric chloride and 4-5 drops of concentrated H₂SO₄. A ring of reddish-brown color indicates the existence of glycosides⁴.

Salkowski Test: After adding a couple of drops of concentrated sulfuric acid to the extract, the existence of terpenoids was recommended by the establishment of a yellow-colored lower layer, while the existence of steroids was indicated by the red color of the bottom layer.

Test for Saponins: 5 ml of distilled water was combined with crude extract and the mixture was agitated vigorously. It was believed that the production of steady foam recommended the appearance of saponins.

Alkaline reagent Test: To 1000 μ l of crude extract, add 4-5 drops of NaOH solution. If a bright yellow color forms and then turns colorless when a few drops of acid are added, flavonoids are present.

Liebermann-Burchard's Test: To 1ml of extract, add two to three drops of acetic anhydride solution and boil in a water bath. After cooling it, add concentrated sulphuric acid. Two different types of rings will be shown, the lower layer will be red-brown which indicates the presence of triterpenoids and the upper layer will show a green color²⁴.

Fourier Transform Infrared Spectroscopy: FTIR spectroscopy was used to recognize the chemical bonds formed in the extract of *Ficus palmata* stem. 10mg of dried extract powder was encapsulated in 100mg of analytical grade KBr pellet to prepare the sample disc^{15,25}. Plate was loaded in a spectroscopy machine and all data was obtained by collecting the full scan range spectra from 400-4000 cm⁻¹.

DPPH Scavenging for Free Radicals: The activity of the antioxidant was tested with some minor modifications of the method of Jothi and Jebamalar¹¹. Various sample concentrations (20%, 40%, 60%, 80% and 100%) were combined with 0.1mM of working DPPH reagent. Keep the mixture incubated at ambient temperature for 2 hours. Discoloration of the sample was predicted by a spectrophotometer at 517 nm. Utilizing the standard ascorbic acid, the results were stated as mg ASC/g of extract¹¹.

Hydrogen Peroxide Scavenging Activity: 0.1ml of the sample (20%, 40%, 60%, 80% and 100%) was added in 0.4ml of 50mM of phosphate buffer to which 2mM of 0.6ml of H_2O_2 was added, vortex the sample for 10 minutes, absorbance was taken through spectrophotometer at 230nm. The results were articulated as mg ASC/gm extract, using ascorbic acid as the benchmark²⁴.

Results and Discussion

Extraction yield refers to the amount or percentage of a desired substance obtained from a raw material or source during a process of extraction. A higher extraction yield is generally desirable. Factors influencing extraction yield include the extraction technique, solvent used, extraction time, temperature and the characteristics of the raw material. Furthermore, phenolic and flavonoid component recovery is enhanced by the extractant's polarity⁸. Table 1 displays the acquired data, which showed that polar solvents produced better extraction yields and that the extracted yield was significantly higher than that of any other solvent utilized^{1,26}.

According to table 2, the initial screening of *Ficus palmata* revealed that the stem's methanolic extract contained the highest concentration of bioactive substances including protein, alkaloids, flavonoids, tannin, phenol and polysaccharides compared to petroleum ether extracts. The highest phenolic and flavonoid contents were observed at

Ficus palmata methanol extract 100% as 49.2 ± 0.48 mg/g (R²=0.992) and 16.87 ± 0.43 mg/g (w/w) (R²=0.987) (Table 3) respectively in the methanolic extract of *F. Palmata* stem as shown in figures 2 and 3^{8,29}.

Studies have demonstrated that phenolic moiety components such as flavonoids, carotenoids and tannins are commonly found in natural extracts having antioxidant activity. As a result, phenolic substances are important in lowering the DPPH radical. The evaluation curve for gallic acid, employed as a standard and encompassing the concentration assortment of 1 g/mL to 5 g/ml, was plotted using concentration against absorbance obtained at 765 nm²⁸ [Figures 2 and 3] [Table 3].

The methods of reverse osmosis (RO) as well as HPLC used to purify gallic acid are showcased in figure 4. Gallic acid was present in larger concentrations in the retentate stream than in the feed, although it was barely detectable in the permeate.

The RO membrane also disqualifies a few additional substances. According to the results, gallic acid is not a selective target for RO. On the other hand, as a result of some other substances passing through the membrane, the content of gallic acid in the retentate extended by 35%. It is possible that RO, which is easy to use and beneficial to the environment, is a useful technology for purifying gallic acid.

Table 1
Percentage yield *Ficus palmata* stem extract.

S.N.	Solvent	Polarity index	% yield
1.	Petroleum ether	0.1	16.72%
2.	Acetone	0.3	28.12%
3.	Ethanol	0.6	46.93%
4.	Methanol	0.7	48.23%
5.	Water	1	55.15%

Table 2
The phytochemical analysis of different stem extracts of *F. palmata*

S.N.	Phytochemical Test	Pet. Ether	Acetone	Ethanol	Methanol	Water
1	Carbohydrate					
	Molisch's test	+	+	+	+	-
	Fehling's test	-	+	+	+	-
	Benedict's test	+	-	-	+	-
2	Alkaloid					
	Mayer's test	-	+	+	+	-
3	Phenol and Tannins	+	-	-	+	-
4	Glycosides					
	Legal test	+	-	+	+	-
5	Salkowski test	-	+	-	+	-
	Saponins	+	-	+	+	+
6	Phenol test	-	-	+	+	-
7	Flavonoids test	-	+	+	+	-
8	Terpenoids test	-	-	-	+	-

Table 3
Phenol and Flavonoid content of both plants

S.N.	Sample	Total phenolic content of <i>Ficus palmata</i> (mg GAE/g of extract)	Total flavonoid content of <i>Ficus palmata</i> (µg QE/mg of extract)
1	Petroleum ether	23.1 ± 0.30	3.46 ± 0.08
2	Acetone	29.6 ± 0.31	7 ± 0.16
3	Ethanol	43.5 ± 0.45	9.2 ± 0.21
4	Methanol	49.2 ± 0.48	16.87 ± 0.43
5	Water	35.7 ± 0.19	20.14 ± 0.51

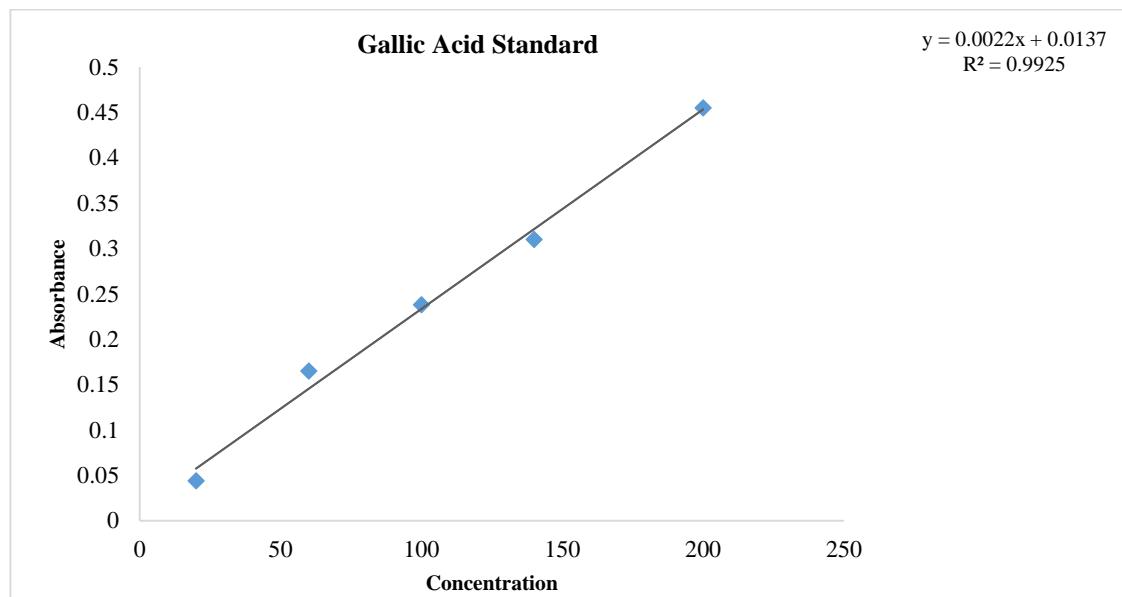


Fig. 2: Evaluation curve of standard Gallic acid against absorbance measured at 765 nm

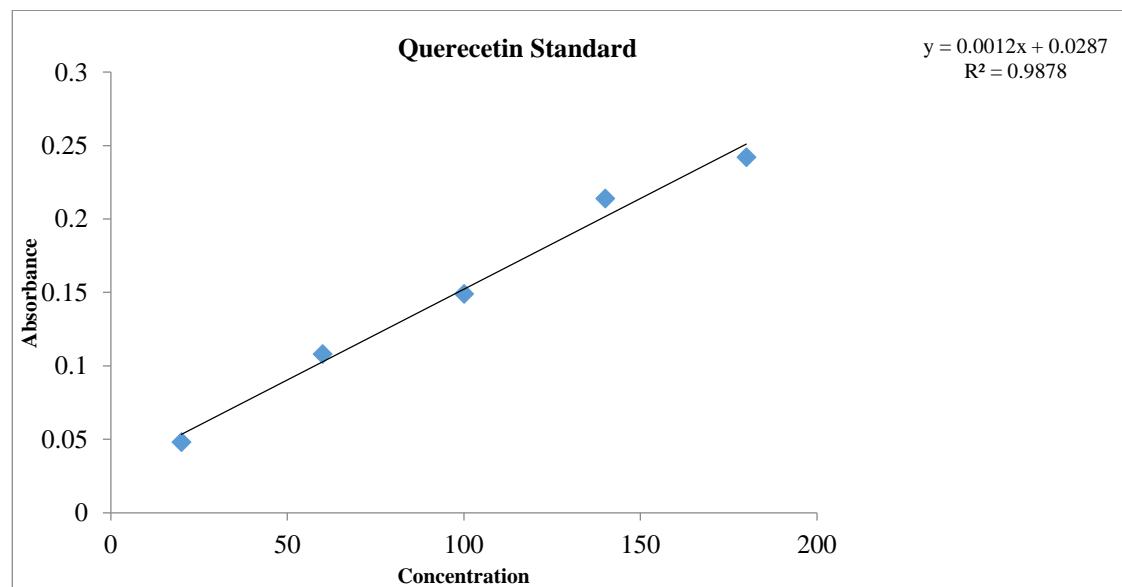


Fig. 3: Evaluation curve of standard Quercetin against absorbance measured at 765 nm

When reinjected for examination, the fraction recovered from the elute in the HPLC system produced a clear gallic acid peak. The percentage area covered in the chromatogram increased dramatically from 17% in the feed to 95% in the collected fraction. This demonstrates that gallic acid may be effectively purified by HPLC without any additional

processing such as adsorption, which would increase complexity and expense overall²⁶ [Figures 4, 5 and 6].

To estimate the components in the extract, petroleum ether and methanol extracts were subjected to HPLC analysis. *Ficus palmata* stem extracts extracted in methanol and

petroleum ether were subjected to HPLC analysis which revealed the presence of several glycoside chemicals in variable quantities including quercetin, flavonoids and anthraquinone. Both phytochemical and HPLC analyses, which exhibited different peaks and retention durations, verified the existence of secondary metabolites.

FTIR Analysis: The FTIR analysis was utilized to measure the functional group present in petroleum ether and methanol extract of *Ficus palmata* (stem) based on its peak value in the IR region. The analysis confirms the presence of C-Br, C-H, C-O, C-C, N-H and O-H bands [Tables 4 and 5] [Figures 7 and 8].

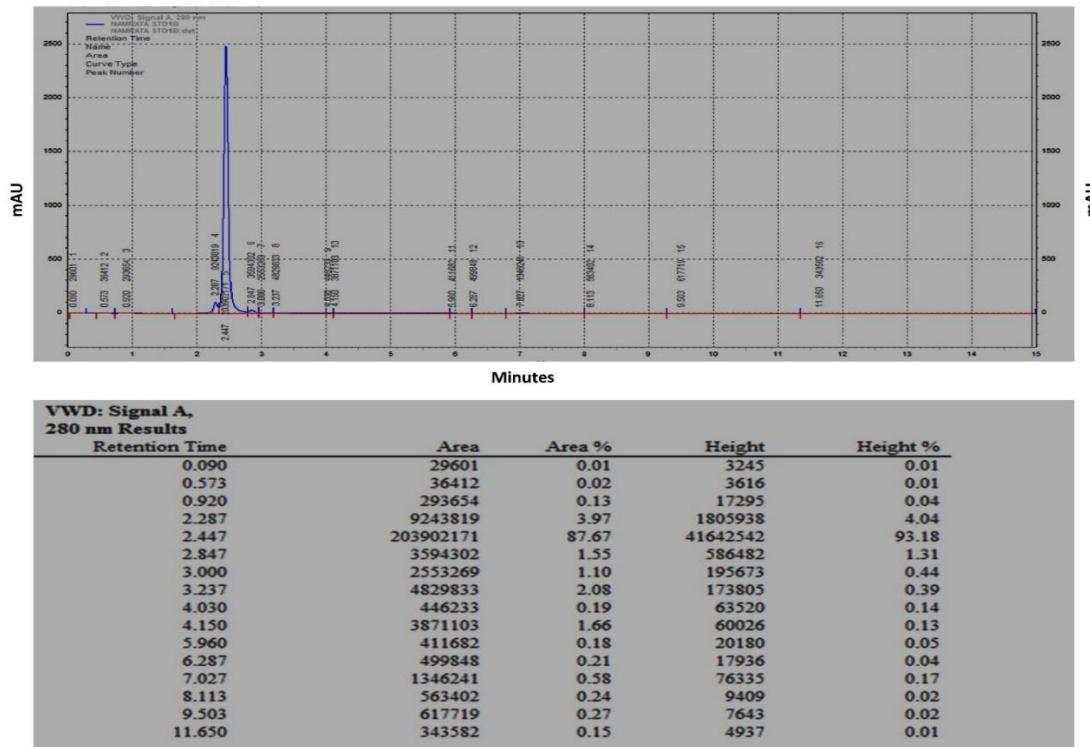


Fig. 4: The purity of the gallic acid was verified by HPLC using RT 2.44

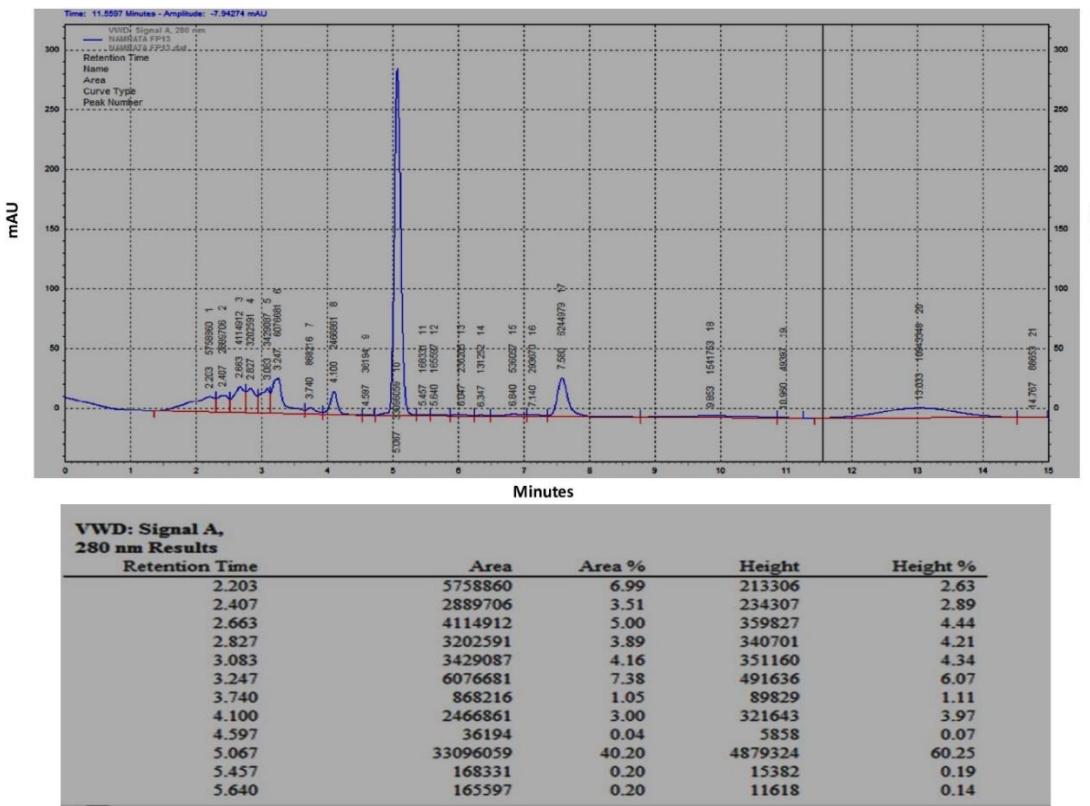


Fig. 5: HPLC data of *F. palmata* methanol extract

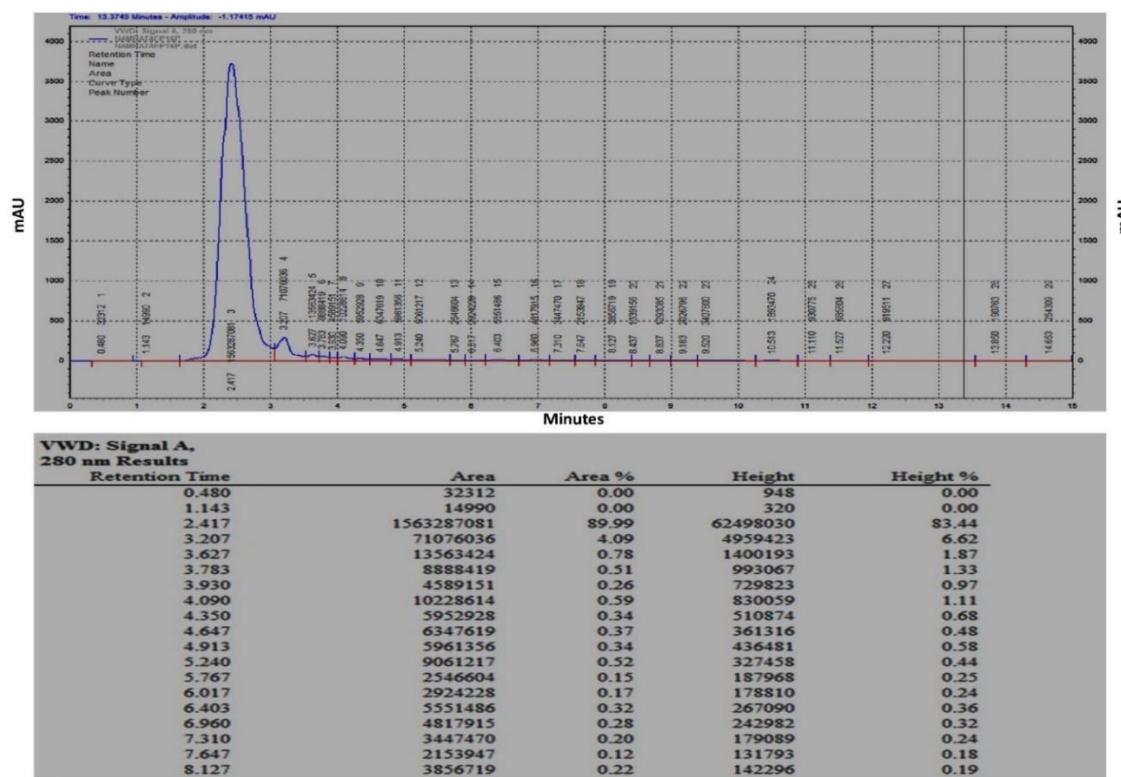
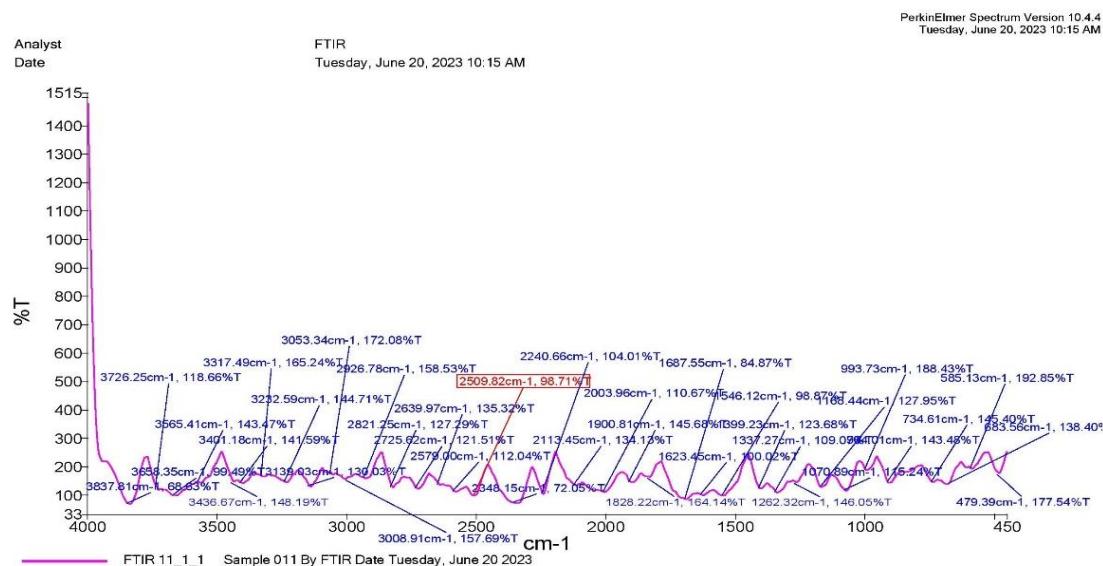
Fig. 6: HPLC data of *F. palmata* petroleum ether extractFig. 7: FTIR analysis and peak value of methanol extract of *Ficus palmata*

Table 4
FTIR spectral peak values and functional groups of methanol extract of *F. palmata* (stem)

Spectrum No.	Wave Number cm^{-1}	Functional Group Assignment	Predicted Compound
1	3317.49	N-H stretch	Amines
2	3053.34	C-H stretch	Alkenes
3	2926.78	C-H stretch	Alkanes
4	2113.45	C=C stretch	Alkynes
5	1828.22	C=O stretch	Anhydrides
6	1262.32	C-F stretch	Alkyl & Aryl halides
7	993.73	C-H stretch	Aromatic compounds
8	585.13	C-Br stretch	Alkyl halides

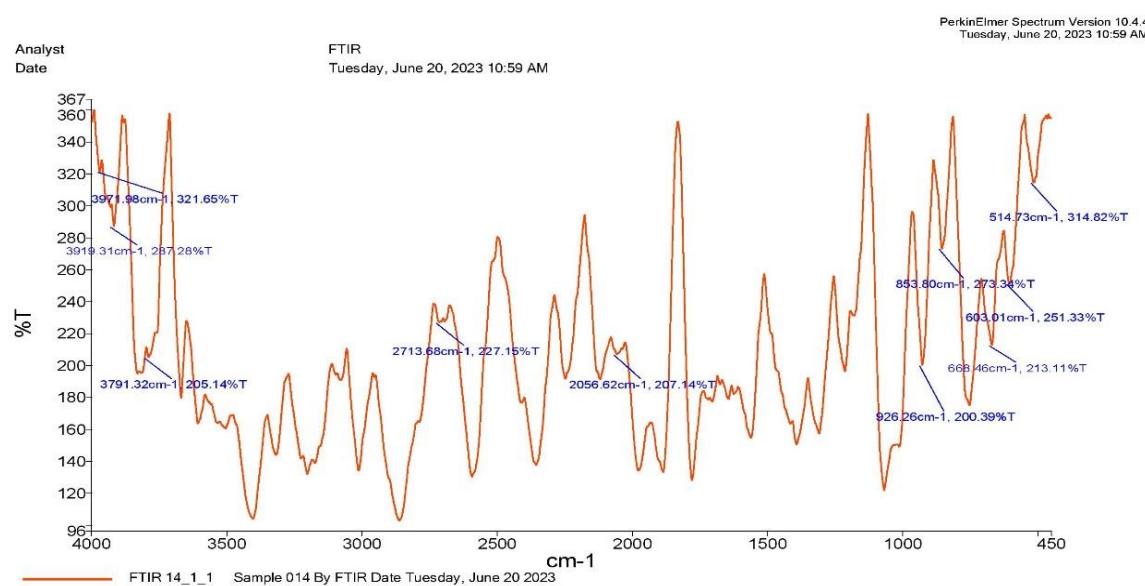
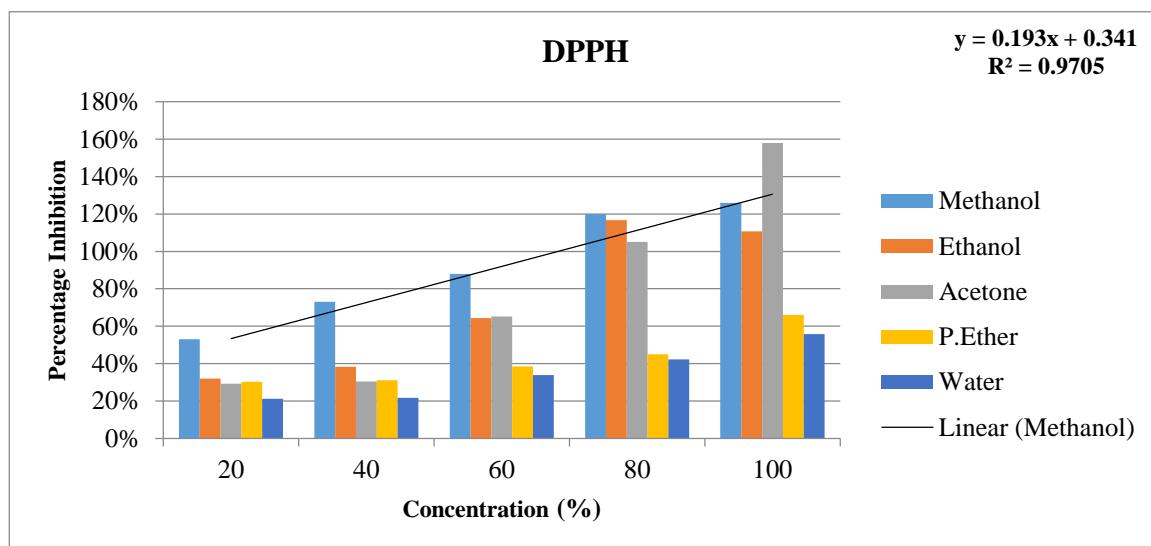
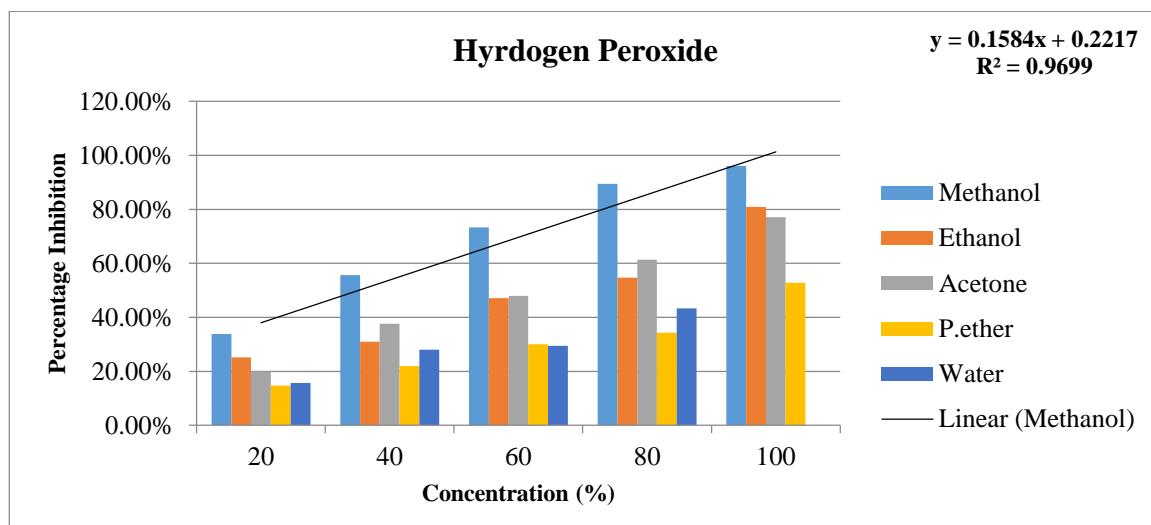
Fig. 8: FTIR analysis and peak value of petroleum ether extract of *Ficus palmata*Fig. 9: Percentage inhibition of DPPH for different solvents of *Ficus palmata*Fig. 10: Percentage inhibition of Hydrogen peroxide for the different solvents of *Ficus palmata*

Table 5

FTIR spectral peak values and functional groups of dried petroleum ether of *F. palmata* (stem)

Spectrum No.	Wave Number cm^{-1}	Functional Group Assignment	Predicted Compound
1	3974.94	O-H stretch	Alcohol
2	3337.89	N-H stretch	Amines
3	2773.42	C-H stretch	Carboxylic acid
4	2117.77	C=C stretch	Alkynes
5	1645.78	C=C stretch	Aromatic compounds
6	1162.12	C-F stretch	Alkyl and Aryl halides
7	587.64	C-Br stretch	Alkyl halides

Table 6

Ficus palmata (stem) DPPH scavenging activity in various solvent extracts⁶

Concentration ($\mu\text{g/ml}$)	Methanol	Ethanol	Acetone	Petroleum ether	Water
20	53.2 \pm 0.30	32 \pm 0.19	29.28 \pm 0.16	30.2 \pm 0.05	21.19 \pm 0.12
40	73.8 \pm 0.34	38.3 \pm 0.22	30.4 \pm 0.20	31.1 \pm 0.21	21.66 \pm 0.15
60	88.12 \pm 0.28	64.28 \pm 0.24	65.2 \pm 0.23	38.5 \pm 0.14	33.8 \pm 0.20
80	120.1 \pm 0.35	116.6 \pm 0.30	105 \pm 0.29	45 \pm 0.12	42.3 \pm 0.22
100	126.5 \pm 0.27	110.71 \pm 0.31	158 \pm 0.34	66.1 \pm 0.09	55.7 \pm 0.27

Table 7

Ficus palmata (stem) H_2O_2 activity in various solvent extracts⁶

Concentration ($\mu\text{g/ml}$)	Methanol	Ethanol	Acetone	Petroleum ether	Water
20	33.80 \pm 0.021	25.23 \pm 0.10	20 \pm 0.12	14.7 \pm 0.007	15.71 \pm 0.11
40	55.71 \pm 0.001	30.95 \pm 0.10	37.61 \pm 0.12	22 \pm 0.04	28.09 \pm 0.15
60	73.33 \pm 0.04	47.14 \pm 0.12	48 \pm 0.13	30 \pm 0.10	29.52 \pm 0.16
80	89.5 \pm 0.12	54.7 \pm 0.14	61.4 \pm 0.15	34.28 \pm 0.21	43.3 \pm 0.18
100	96.1 \pm 0.13	80.9 \pm 0.15	77.1 \pm 0.16	52.8 \pm 0.19	48.5 \pm 0.19

Free Radical Scavenging Activity: The primary cause of plant's antioxidant activity is the presence of bioactive compounds within them. The hydrogen peroxide assay and the DPPH assay were used to measure antioxidant activity. Methanolic extract of *Ficus palmata* shows a strong free radical activity shown in figure 9. DPPH assay and H_2O_2 assay show strong activity at FP100% whereas FP80% shows strong activity for hydrogen peroxide assay^{8,26} [Tables 6 and 7] [Figures 9 and 10].

Conclusion

The current investigation validated the antioxidative properties of *Ficus palmata* (stem) methanol and petroleum ether extract. The range of plant extracts most likely indicates the existence of phytonutrients such as phenol, flavonoids, sugars, alkaloids, glycosides and saponin. To evaluate the efficacy of bioactive chemicals through *in vivo* studies as well as to show their efficacy and clinical trial safety, more research on bioactive substances is required.

Traditional uses of plants for medicinal purposes often lack extensive scientific validation. While they may contain compounds with therapeutic potential, it is crucial to consult

with healthcare professionals before using any plant-based remedies, especially for medicinal purposes. Numerous studies have verified that the existence of these phytochemicals provides the plants physiological and therapeutic qualities, which can be used to cure a variety of illnesses. As a result, extracts from these plants may be valuable source of medications.

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