

## Ficus palmata and Ficus auriculata Phytochemical Screening in Different Solvents by HPLC and FTIR Spectroscopic Analysis

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### Abstract

Many cultures all over the world have been using medicinal plants for ages to cure a variety of illnesses and medical concerns. *Ficus palmata* is a kind of fig tree that grows in areas like the Himalayas, Afghanistan, and portions of Africa. *Ficus auriculata* is a species of fig tree that grows throughout Asia, mainly in India, Southeast Asia, and southern China. These plants contain bioactive compounds that can have therapeutic effects on the human body. In chemistry and biochemistry, high-performance liquid chromatography (HPLC) is a commonly used analytical method for the separation, identification, and quantification of chemical components in a mixture. A potent analytical method for identifying and characterising chemical substances based on how they interact with infrared light is Fourier-Transform Infrared (FTIR) spectroscopy. The present study's purpose is to use introductory phytochemical analysis, HPLC, and FTIR spectroscopy to determine the chemical components of petroleum ether, acetone, ethanol, methanol, and water extracts of *Ficus auriculata* and *Ficus palmata* leaves. The existence of phenols, alkaloids, flavonoids, tannins glycosides, terpenoids, steroids and saponins was demonstrated by the phytochemical investigation's results. A C18-150 4.6 mm column, 10 $\mu$  injection volume, and methanol: acetonitrile (80:20 v/v) mobile phase at 30°C were used to carry out a reversed-phase HPLC analysis. FTIR analysis of leaf extracts in methanol and petroleum ether indicated the presence of aldehydes, ethers, primary amines, alcohols, amides, aromatics, alkanes, alkyl halides, alkenes, and aliphatic amine compounds—which display substantial peaks. The current study yielded helpful field data, including phytochemical analysis, HPLC spectra, and FTIR spectrum profiles for these medicinally significant plants.

**Keywords:** Phytochemical analysis, FTIR, HPLC, *Ficus auriculata*, *Ficus palmata*, Figs, Phytonutrients

### Introduction

Phytochemicals, short for phytonutrients, are biologically active compounds found in plants. These natural chemicals serve various functions in plants, such as protecting them from pests, diseases, and environmental stressors, as well as contributing to their color, flavor, and other sensory characteristics. When humans consume plant-based foods, phytochemical can have beneficial effects on health. *Ficus palmata* and *Ficus auriculata* are two species of fig trees be the property of the genus *Ficus*. *Ficus* species are known for their high polyphenol content, which includes compounds like flavonoids, phenolic acids, and tannins. Polyphenols have antioxidant properties and can contribute to various health benefits.

Common phytochemicals found in various *Ficus* species, including figs, may include:

1. **Polyphenols:** Ficus species are known for their high polyphenol content, which includes compounds like flavonoids, phenolic acids, and tannins. Polyphenols have antioxidant properties and can contribute to various health benefits.
2. **Carotenoids:** Figs may contain carotenoids like beta-carotene and lutein, which are responsible for their color and have antioxidant and potential health-promoting effects.
3. **Triterpenoids:** Some Ficus species may contain triterpenoid compounds, which can have anti-inflammatory and other bioactive properties.
4. **Sterols:** Plant sterols, including beta-sitosterol, are often found in figs and have been associated with cholesterol-lowering effects.
5. **Fatty Acids:** Figs can contain various fatty acids, including omega-3 and omega-6 fatty acids.
6. **Alkaloids:** Some Ficus species may contain alkaloids, which can have diverse pharmacological properties.
7. **Glycosides:** Glycosides are sugar-bound compounds that may have various bioactive effects.

It's important to note that the specific phytochemical profile of *Ficusauriculata* and *Ficuspalmata* can vary conditional factors such as the plant's age, growing conditions, and geographic location. To obtain detailed information about the phytochemical composition of these particular Ficus species, it would be necessary to refer to scientific studies or resources that have analyzed their chemical constituents. Research studies and chemical analysis of plant species are typically conducted to identify and quantify the specific phytochemical present and to assess their potential health benefits. Consuming foods high in phytochemical is combined to a number of health advantages, which include lowering the possibility of chronic diseases like cancer and heart disease and enhancing general wellbeing. To optimise phytochemical consumption and potential health advantages, it's critical to maintain a balanced diet with a range of fruits, vegetables, and plant-based meals [1]. Glycosides, alkaloids, saponins, flavonoids, tannins, anthraquinones, steroids and terpenoids are the most significant of these bioactive elements that give the plant its medical properties [2]. The basic, or more accurately secondary, metabolism of living things produces these substances. The roles of secondary metabolites are unclear, despite their great diversity in taxonomy and chemistry. They are extensively employed in numerous fields, including scientific research, veterinary medicine, agriculture, and human therapy [3]. Due to the presence of phytochemical elements, medicinal plants are helpful for both treating and curing human ailments [4]. Medicinal plants, leaves, vegetables, and roots all naturally contain phytochemicals that have defensive mechanisms that shield against a variety of ailments. Phytochemicals are both primary and secondary substances. Primary contents include proteins, common sugars, and chlorophyll; secondary constituents include phenolic, alkaloids, and terpenoid chemicals [1]. There are roughly a thousand phytochemicals in all, both identified and unidentified. Although it is commonly known that plants make these compounds to defend themselves, new studies have shown that many phytochemicals can also shield people from illness [5]. Many physiologically active substances, such as steroids, alkaloids, flavonoids, triterpenoids, carotenoids, phenolic compounds, ketones, triterpenoids, and limonoids such as saladin, valassin, meleicin, Nimbin, Nimbicin, geducin, and azadirachtin can be extracted from neem by looking at its chemical constituents [6-8]. Thus, the phytochemical characteristics of the suggested plant species *F. auriculata* and *F. palmata* are the subject of this study paper's investigation. Its therapeutic qualities are thought to be attributed to certain plant species, and because of this plant's versatility, researchers have been able to examine its diversity of phytochemicals [11]. Because the medicinal plants included in this study have bioactive components, they have been carefully selected. Therefore, performing a phytochemical analysis was the aim of the current investigation. The foundation of FTIR spectroscopy is the way molecules interact with infrared light. At particular wavelengths, molecules absorb infrared light, producing distinctive absorption spectra. The absorption band pattern in a spectrum can reveal details about the chemical bonds and functional groups that are present in a sample. HPLC works on the principle of liquid chromatography, where a sample mixture is dissolved in a liquid (the mobile phase) and passed through a column filled with a stationary phase. Compounds in the mixture interact differently with the stationary phase, causing them to separate based on their chemical properties.

## Materials and Methods:

### *Plant Sample collection and Authentication*

The foliage of *F. palmata* and *F. auriculata* were collected from Tehri region of Uttarakhand, India. The BSI (Botanical Survey of India) validated and taxonomically identified the plant samples. A voucher specimen (872) of *F. auriculata* Lour and a voucher specimen (873) of *F. palmata* Forsk. were accessioned at herbarium BSI, Dehradun.

### *Preparation of plant extracts*

The sequential Soxhlet extraction technique was used for the extraction.

### *Soxhlet extraction*

This method was used to get extracts for biological and pharmacological testing as well as phytochemical screening. To obtain a uniformly sized powder, the leaves and stem were ground together in a grinder. A homogenous 25gm of powdered plant substance was placed in a thimble, and 250ml of numerous solvents were extracted from each separately. After that, the thimble was placed inside the Soxhlet apparatus, where extraction was performed using petroleum ether, acetone, ethanol, methanol, as well as water as solvents in a sequential order from non-polar to polar. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor becomes colourless. Murugan R. et al. (2014) [12]. After the petroleum ether extract was collected, acetone was extracted in a further step using powder made from the thimble, and the yield was estimated. The same process was used for drying, and thimbles were filled with powder that was utilised for ethanol, methanol, and water extraction. 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 a vacuum using a rotating evaporator. The dried extract was kept in a refrigerator at 4°C for their future use in different analysis (Manandhar et al., 2019) [10, 21]. All extracts obtained from Tehri regions of Uttarakhand.

**Percentage Yield:** The following formula was used to determine the extract's percentage yield:

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

### *Qualitative phytochemical analyses of leaf extract:*

The extract was examined using accepted techniques to determine whether any bioactive compounds were present [11, 12, 13].

## *Quantitative phytochemical analysis*

### *Detection of Carbohydrates*

#### *Molisch's test*

One milliliter of concentrated H<sub>2</sub>SO<sub>4</sub> was gradually added along the test tube's sides and let to stand after two drops of an alcoholic naphthol solution were expanded to two milliliters of extract and the combination was thoroughly agitated. Indicating the presence of carbohydrates was a violet ring.

#### *Fehling's Test*

Fehling's solution A consisted of dissolving 34.66 gm of CuSO<sub>4</sub> in distilled water and adding enough water to make 500 ml; 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. On a water bath, one milliliter of extract was brought to a boil, and Fehling solutions A and B were then added.

***Benedict's Test***

1 milliliter of Benedict's reagent was expanded to 0.5 milliliter of extract. For two minutes, the mixture was boiled in a bath of boiling water. When sugar was present, a distinct coloured precipitate was formed.

***Detection of alkaloids******Mayer's Test***

A few milliliters of extract were mixed with one or two drops of Mayer's reagent by the test tube's edge. A white, creamy precipitate signified a favourable result for the test.

***Detection of Phenolic Compounds and Tannins******Ferric Chloride Test***

A small amount of neutral 5% ferric chloride solution was added to a few drops of the extract. A dark green hue suggested the presence of phenolic chemicals.

***Test for Glycosides******Legal test***

To make the extract alkaline, a few drops of 10% NaOH were added. The solution was then supplemented with recently synthesised sodium nitroprusside. The extract included glycosides as evidenced by the presence of blue colouring.

Following the addition of a few drops of concentrated sulfuric acid to the extract, thorough stirring, and a period of time, the existence of terpenoids was recommended by the formation of a yellow-colored lower layer, while the existence of steroids was indicated by the red colour of the bottom layer.

***Test for saponins***

In a sample tube, 5ml of distilled water was combined with crude extract, and the mixture was agitated vigorously. It was believed that the production of stable foam recommended the appearance of saponins.

***Test for phenols and tannins***

Crude extract was combined with two milliliters of a 2% FeCl<sub>3</sub> solution. The existence of phenols and tannins was desired by a blue-green or black colouring.

***Test for flavonoids******Alkaline reagent test***

There was a mixture of 2ml of 2% NaOH solution and crude extract. A bit drops of weak acid were added, the bright yellow hue that had formed went colourless, signifying flavonoids presence.

***Test for terpenoids***

After dissolving the crude extract in two milliliters of chloroform, it was dried out. After adding 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub>, this was boiled for approximately 20 seconds. Terpenoids were characterised by a greyish hue.

***Total phenolic content:***

Two milliliters of the 2% Na<sub>2</sub>CO<sub>3</sub> solution, 2.5 milliliters of the 10% Folin-Ciocalteu reagent, and one milliliter of plant extract were combined. The mixture was allowed to rest at room temperature for fifteen minutes, and then its absorbance at 765 nm was measured using gallic acid as the standard (1 mg/ml). An adjustment was made to the

Folin-Ciocalteu reagent method in order to regulate the phenol concentration of the different extracts. Every test was conducted three times for each operation. The results were computed using the standard curve as well as expressed as Gallic acid equivalent (mg/g of extracted material).

#### **Total flavonoid content:**

The flavonoid concentration was determined by modifying the colorimetric technique using aluminium chloride. One milliliter of the extract requires 5.6 milliliters of distilled water, 0.6 milliliters of methanol, 0.2 milliliters of 10% aluminium chloride, and 0.2 milliliters of 1M potassium acetate. Allow it to rest for half an hour at room temperature. The absorbance was measured at 420 nm...To create 1 ml of the extract, 6 ml of distilled water, 0.6 ml of methanol, 0.2 ml of 10% aluminium chloride, and 0.2 ml of 1M potassium acetate are required. Allow it to rest for half an hour at room temperature. The absorbance was measured at 420 nm. A standard quercetin of 1 mg/mL was used. Every test run was conducted in three copies. Using the standard curve, the flavonoid levels were calculated and expressed as mg/g of the isolated component that was comparable to quercetin [14, 20].

#### **HPLC Analysis**

The method was followed using the protocol suggested by Taralkar and Chattopadhyay (2012). Isolated and purified extract was further analysed by HPLC. HPLC- grade water, methanol, and acetonitrile were used for the analysis. Gallic acid was obtained from Sigma and was used as a standard. Gallic acid, which has a concentration of 1 mg/ml, was employed as a standard. Agilent 1220 LC HPLC model C-18 (4.6 x 250 mm, 5 m) column with automated temperature ( $\pm 0.1^\circ\text{C}$ ) controller module was used for the analysis. For identification, an isocratic mobile phase of acetonitrile: water (70:30 v/v) and acetonitrile: methanol (80:20 v/v) with flow rates of 0.5 ml/min and 1 ml/min was chosen [15, 16]. The temperature of the column was held constant at  $350^\circ\text{C}$  and  $300^\circ\text{C}$  ( $\pm 0.10^\circ\text{C}$ ). Detection wavelength was set at 210 and 290 nm. Purified samples and standard were filtered using a Millipore syringe filter (0.2m). 20  $\mu$  liters of a standard and a purified sample were to be injected, with a 15-minute run duration [18].

#### **FTIR Spectroscopic analysis**

The term "Fourier transforms infrared" (FTIR) refers to the most popular kind of infrared spectroscopy. Every infrared spectroscopy works on the assumption that some infrared light is absorbed as it travels through a substance. Which radiation reaches the sample is documented [19, 21].

## **Results and Discussion**

#### **Result of percentage yield:**

**Table 1: Percentage yield *Ficusauriculata* leaves extract.**

S.no	Solvent	Polarity Index	%yield
1	Petroleum ether	0.1	10
2	Acetone	0.3	23.44
3	Ethanol	0.6	29.12
4	Methanol	0.7	28.48
5	Water	1	50.41

**Table 2: Percentage yield *Ficuspalmata* leaves extract.**

S.no	Solvent	Polarity Index	%yield
1	Petroleum ether	0.1	8.4
2	Acetone	0.3	21
3	Ethanol	0.6	24.96
4	Methanol	0.7	27.68
5	Water	1	46.64

**Table 3: The phytochemical analysis of different leaf extracts of *F. auriculata* Leaf**

S.no	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	+	+	-	+	-
	Salkowski test	-	+	+	+	-
5.	Saponins	+	+	+	+	+
6.	Phenol test	-	-	+	+	-
7.	Flavonoids test	-	+	+	+	-
8.	Terpenoids test	-	-	-	+	-

(+) Indicate the presence of phytochemicals and (-) indicate the absence of phytochemicals.

**Table 4: The phytochemical analysis of different leaf extracts of *F. palmata* Leaf**

S.no	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					
	Salkowski test	+	+	+	+	-
5.	Saponins	+	+	+	+	-
6.	Phenol test	-	-	+	+	-
7.	Flavonoids test	+	-	+	+	-
8.	Terpenoids test	-	-	-	+	+

(+) Indicate the presence of phytochemicals and (-) indicate the absence of phytochemicals.

#### Estimation of Total Phenolic content:

The evaluation curve for gallic acid employed as a standard and encompassing the concentration range of 1 g/mL to 5g/ml, was plotted as indicated in Fig. using concentration against absorbance obtained at 765 nm.

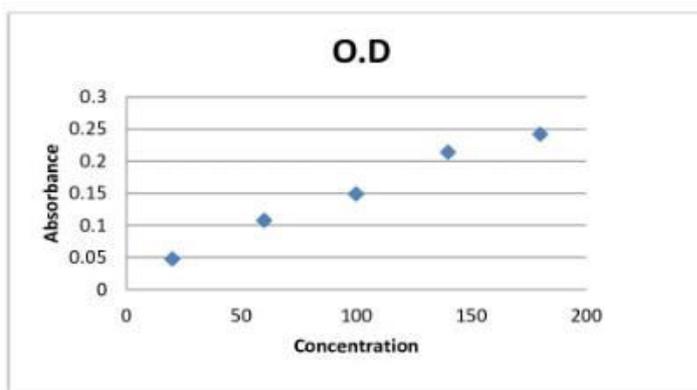


Figure 1: Evaluation curve of standard Gallic acid against absorbance measured at 765 nm

The total phenolic content of each extract of *Ficusauriculata* and *Ficuspalmata* was determined using the standard curve equation,  $y=0.002x+0.013$ , as shown in Tables 5 and 6:

Table 5: Phenolic content of both plants

S.no	Sample	Total phenolic content <i>Ficusauriculata</i> (mg GAE/g of extract)	Total phenolic content <i>Ficuspalmata</i> (mg GAE/g of extract)
1	Petroleum ether	$3.2 \pm 0.023$	$1.5 \pm 0.017$
2	Acetone	$41.95 \pm 0.48$	$33.65 \pm 0.36$
3	Ethanol	$46.75 \pm 0.50$	$46.75 \pm 0.47$
4	Methanol	$47.75 \pm 0.46$	$47.15 \pm 0.50$
5	Water	$37.45 \pm 0.21$	$45.55 \pm 0.32$

Interpreted as Mean  $\pm$  standard error of the mean (S.E.M), n=3.

The amount of gallic acid equivalent (mg GAE/g extract dry weight) used to express total phenolic content (TPC) The TPC of the *Ficusauriculata* methanolic extract was discovered to be  $47.75 \pm 0.46$  mg GAE/g, and the TPC of *Ficuspalmata* was rest to be  $47.15 \pm 0.50$  mg GAE/g according to the evaluation curve of gallic acid ( $y = 0.002x, R^2 = 0.992$ ).

**Estimation of Total flavonoid test:**

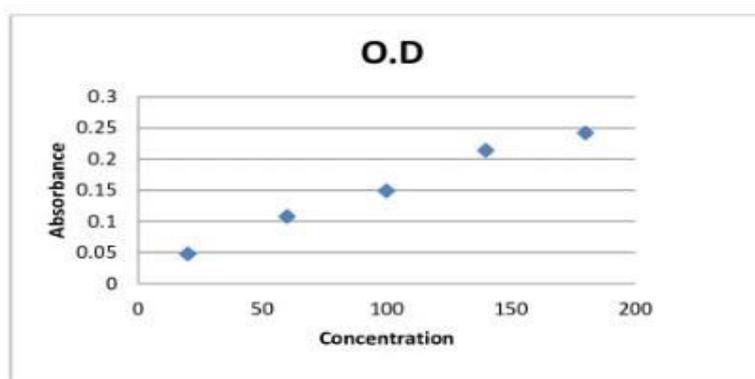


Figure 2: Evaluation curve of standard Gallic acid against absorbance measured at 765 nm

Sr.no	Sample	Total flavonoids content <i>Ficusauriculata</i> ( $\mu\text{g}$ QE/mg of extract)	Total flavonoids content <i>Ficuspalmata</i> ( $\mu\text{g}$ QE/mg of extract)
1	Petroleum ether	$0.7 \pm 0.009$	$0.5 \pm 0.010$
2	Acetone	$7.7 \pm 0.002$	$9.7 \pm 0.012$
3	Ethanol	$7.5 \pm 0.032$	$6.4 \pm 0.040$
4	Methanol	$19.3 \pm 0.004$	$13.8 \pm 0.033$
5	Water	$1.5 \pm 0.14$	$1.3 \pm 0.14$

**Table 6: Flavonoids content of both plants**

For extracts from different solvents, the unit of measurement for TFC is mg QE/g dry weight of extract, or quercetin equivalent. The quercetin assessment curve ( $y = 0.001x$ ,  $R^2 = 0.987$ ) revealed the TFC of the methanolic extract of *Ficusauriculata* to be  $19.3 \pm 0.004$  mg QE/g, whereas that of *Ficuspalmata* was found to be  $13.8 \pm 0.033$  mg QE/g.

#### **HPLC analysis:**

The methods of reverse osmosis (RO) as well as high performance liquid chromatography (HPLC) used to purify gallic acid are showcased in Figure 3. Gallic acid was present in larger concentrations in the retentate stream than in the feed, although it is 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. When reinjected for examination, the fraction recovered from the elute in the HPLC system produced a highly 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 overall. The purity of the gallic acid was verified by HPLC using RT 2.44.

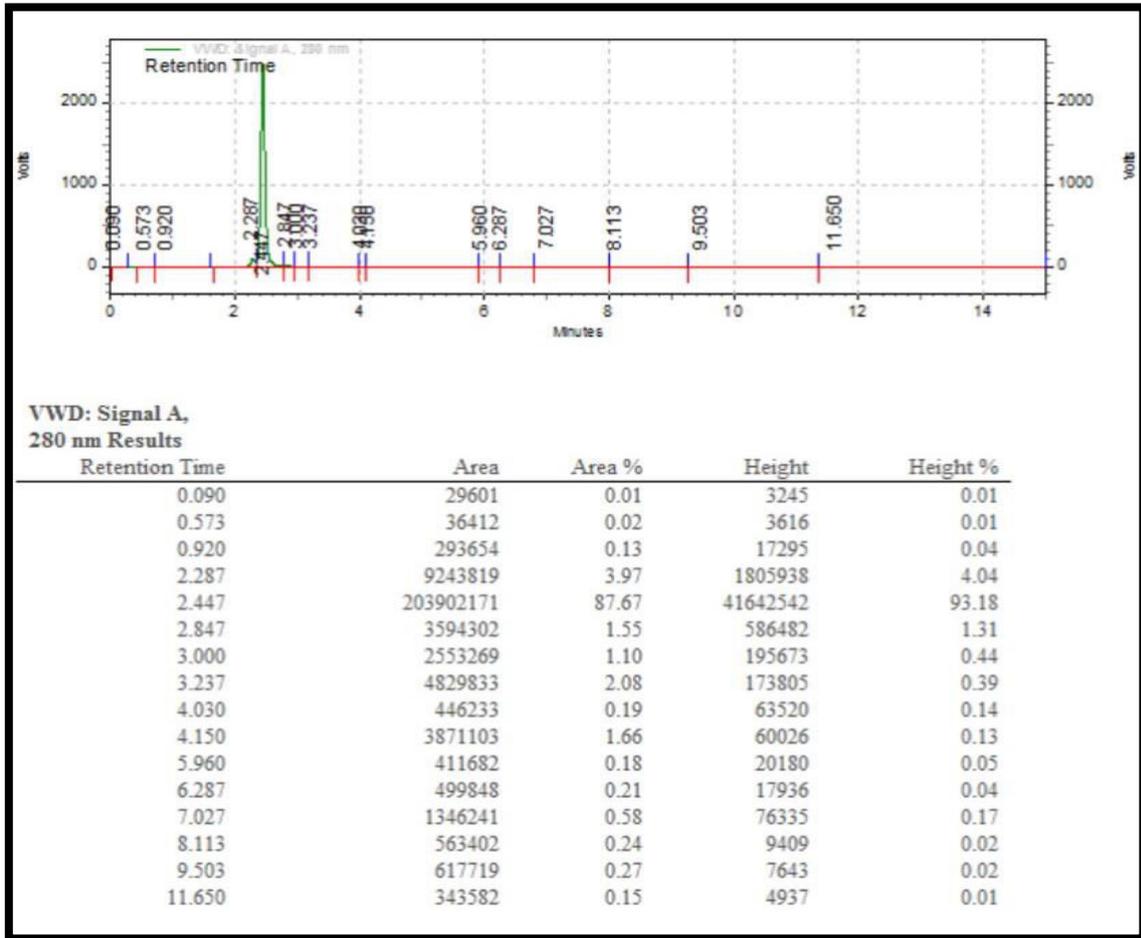
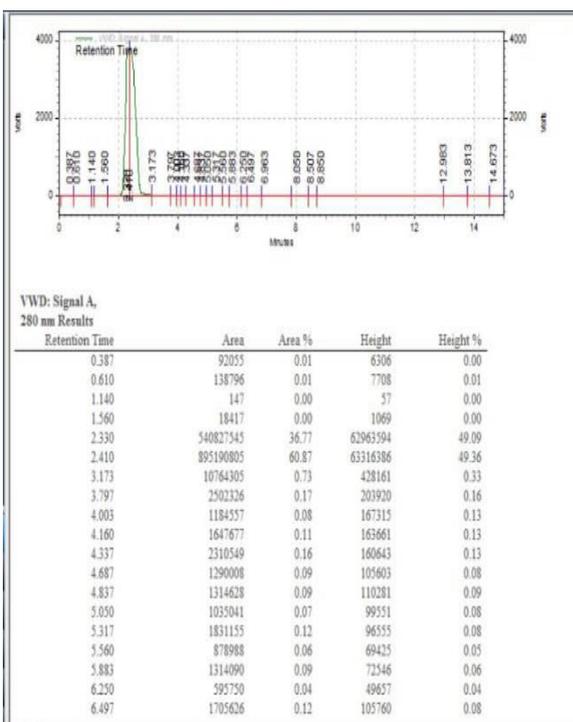


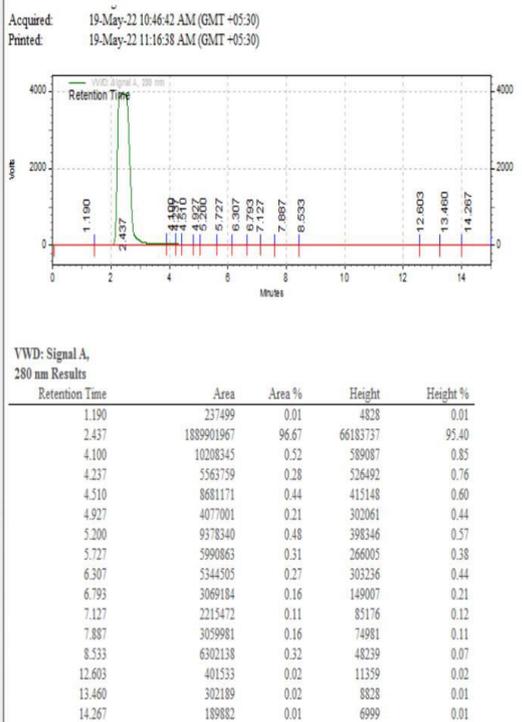
Figure 3: HPLC data of gallic acid

The purity of the methanol extract of *F. auriculata* leaf



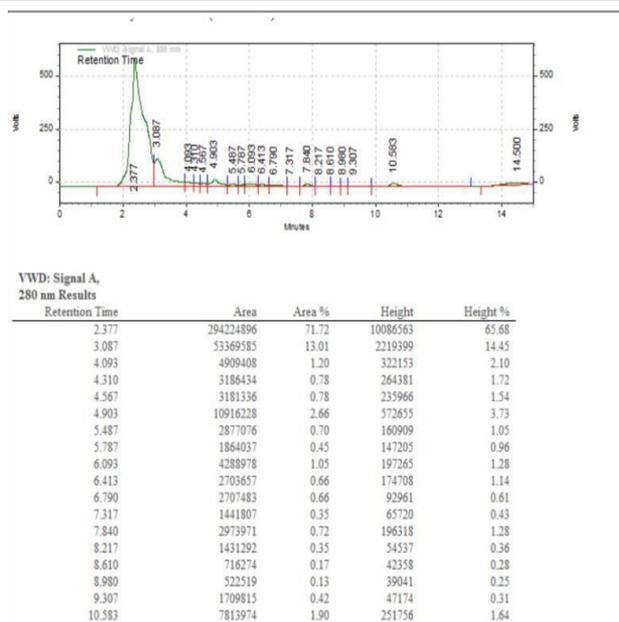
(a)

The purity of the methanol extract of *F. palmata* leaf



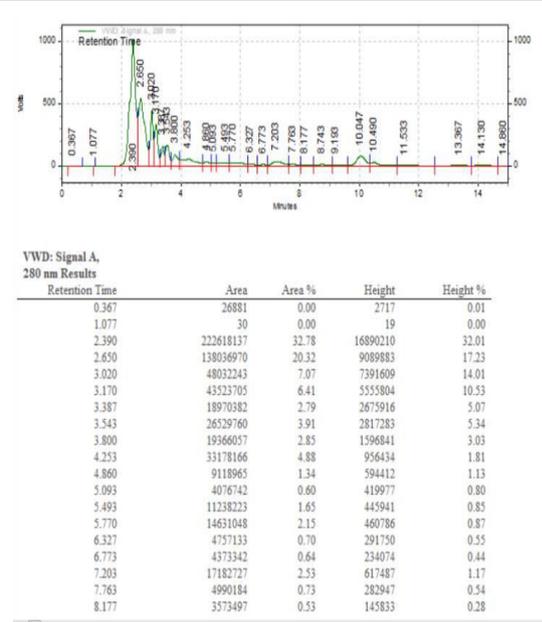
(b)

The purity of P. ether extract of *F. auriculata* leaf



(c)

HPLC data of *F. palmata* leaf

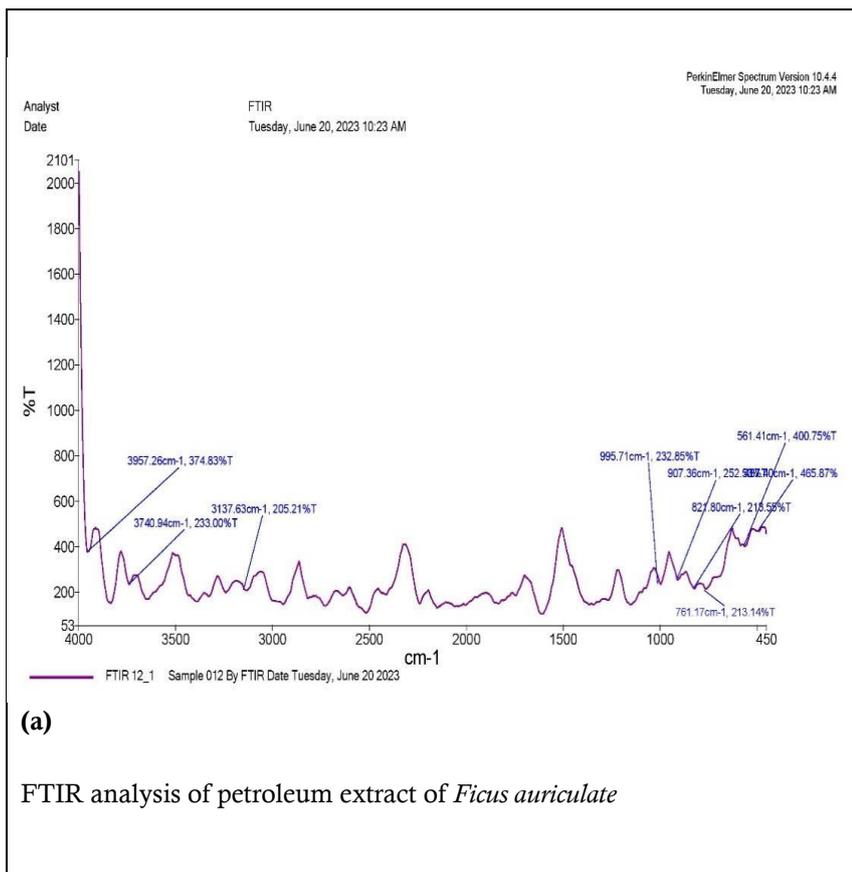


(d)

Figure 4: (a) HPLC data of *F. auriculata* leaf, (b) HPLC data of *F. palmata* leaf (c) HPLC data of *F. auriculata* leaf (P. ether), (d) HPLC data of *F. palmata* leaf (P. ether)

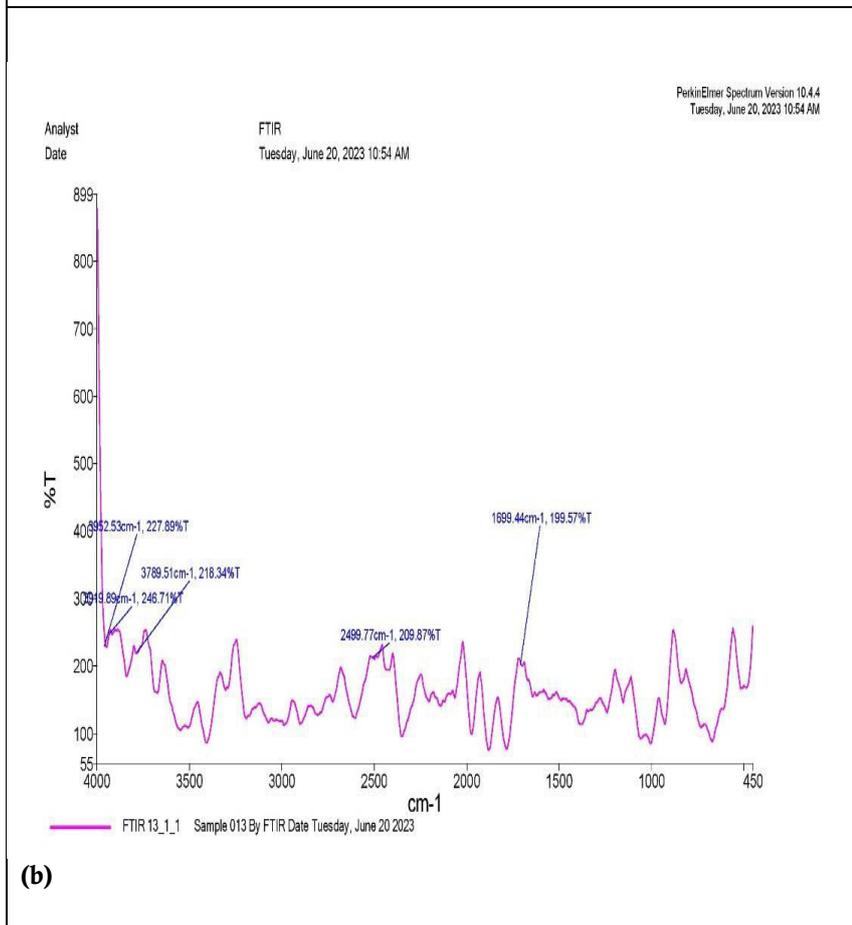
**FTIR Analysis**

Based on the functional group's peak value in the IR region, the petroleum ether and methanol extracts of both plants were subjected to FTIR analysis to determine its presence. Analysis shows that C-Br, C-H, C-O, C-C, N-O, and O-H bands are present (Table)



FTIR Peak Value of petroleum extract of *Ficus auriculata*

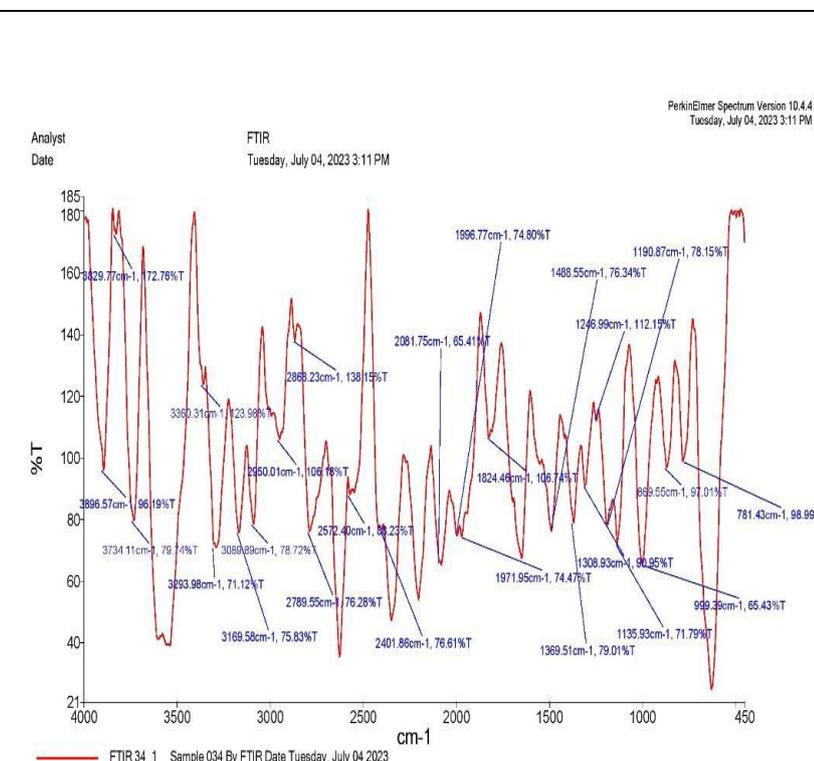
S. no	Peak values	Functional Groups
1	561.41	C-Br (Alkyl halides)
2	995.71	C-H Bend (Benzene & Derivative)
3	1265.50	C=N (Aromatic amine)
4	1364.78	S=O (Sulfonamide)
5	2240.27	C≡N (Nitrile)
6	2639.78	C-H (Aldehyde)
7	3242.74	C-H (Alkyne)
8	3740.94	O-H (Alcohol)



FTIR Peak Value of petroleum extract of *Ficus palmata*

S.n o	Peak values	Functional Group
1	514.77	C-Br (Alkyl halides)
2	729.47	C=C (Alkene)
3	838.79	C-H Bending 1,2 disubstituted
4	1057.22	C-O (Primary amide)
5	1699.44	C=O stretching (conjugated acid)
6	1784.69	C-H (aromatic compound)
7	2203.61	C≡C stretching Alkynes
8	2725.63	C-H (Aldehyde)
9	3306.47	O-H stretching (carboxylic acid)

FTIR analysis of petroleum extract of *Ficus palmata*



FTIR Peak Value of methanol extract of *Ficusauriculata*

S. no	Peak values	Functional Group
1	781.43	C=C (Alkene)
2	1650.66	C-H (Aromatic amine)
3	1824.46	C=O (Acid halide)
4	2203.06	C≡C stretching Alkynes
5	2868.23	C-H (Alkane aldehyde)
6	2950.00	O-H (Alcohol)
7	3360.31	O-H (Alcohol)

(c)

FTIR analysis of methanol extract of *Ficusauriculata*

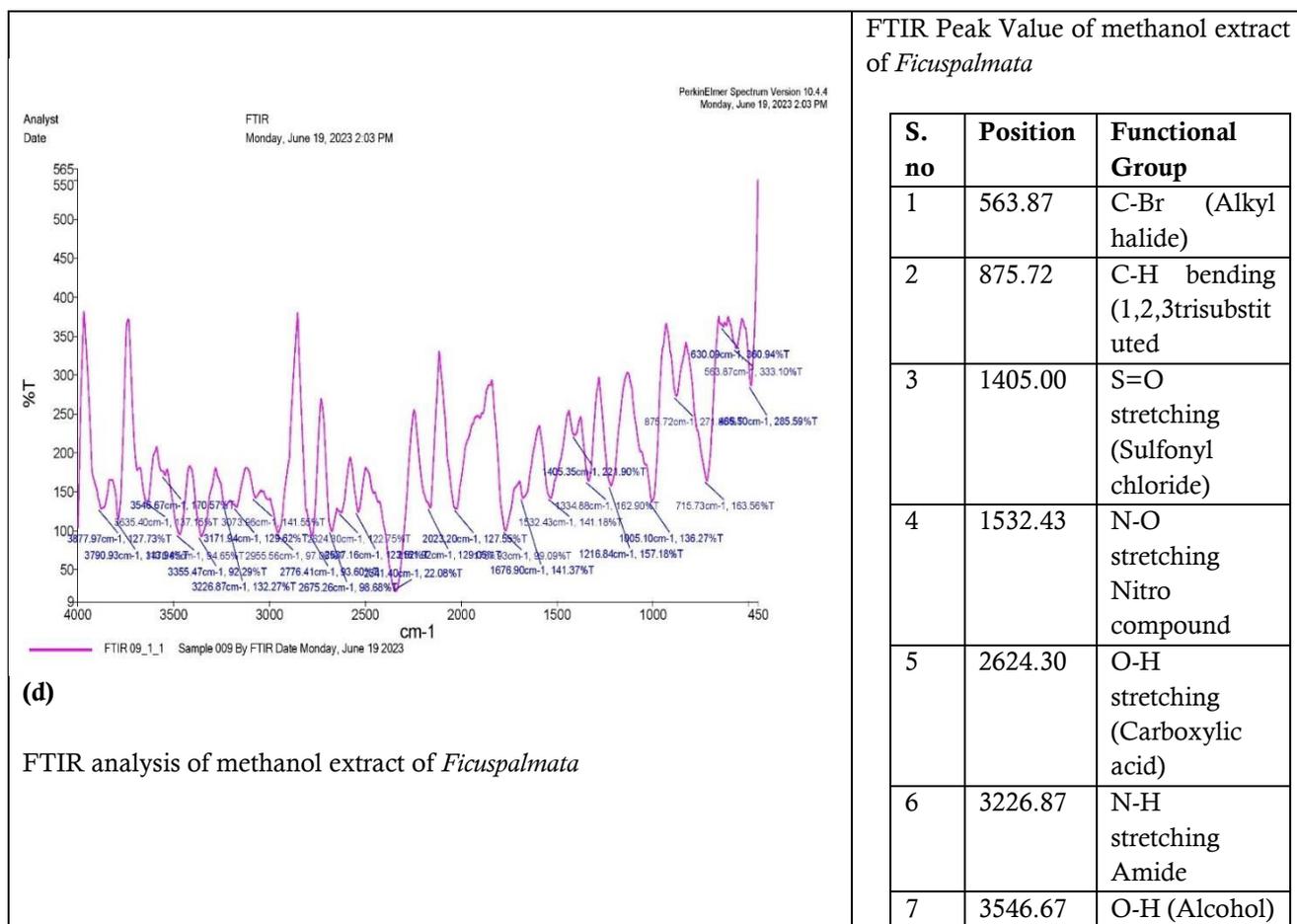


Figure 5: (a) FTIR analysis and peak value of petroleum extract of *Ficus auriculata*, (b) FTIR analysis and peak value of petroleum extract of *Ficus palmata*, (c) FTIR analysis and peak value of methanol extract of *Ficus auriculata*, and (d) FTIR analysis and peak value of methanol extract of *Ficus palmata*

#### Conclusion:

According to the study's aforementioned findings, methanol and petroleum ether leaf extracts from both plants, along with their phytoconstituents, may be useful in the management of illness. The range of plant extracts most likely indicates the existence of phytonutrients such phenol, flavonoids, sugars, alkaloids, glycosides, and saponin. It was discovered that the -CH<sub>3</sub> functional group was the most often identified of the numerous functional groups present in these plant extracts. In order to extract, characterise, and improve these molecules for use as lead compounds in the development of pharmaceuticals with a range of biological effects, more investigation is needed. To evaluate the efficacy of bioactive chemicals through in vivo studies as well as to show their efficacy and clinical trials safety, more research on bioactive substances is required. Remember, traditional uses of plants for medicinal purposes often lack extensive scientific validation. While they may contain compounds with therapeutic potential, it's crucial to consult with healthcare professionals before using any plant-based remedies, especially for medicinal purposes. As with many traditional medicinal plants, scientific research is ongoing to validate the efficacy and safety of using *Ficus auriculata* and *Ficus palmata* for various health purposes. Consulting with healthcare professionals before using any plant-based remedies is always advisable, as proper guidance can help ensure safety and effectiveness.

#### Acknowledgement:

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