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Research Article

Phytochemical Screening and FT-IR Analysis of *Ficus benghalensis* Fruits

Gopukumar S T, Princy Alexander, Jainamboo M, Praseetha P K*

Department of Nanotechnology, Noorul Islam Centre for Higher Education, Noorul Islam University, Kumaracoil, Tamilnadu, India.

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ABSTRACT

Medicinal plants are in use for thousands of years and are renowned for their effectiveness in various treatments. The medicinally usable plants were identified and extracted for biochemical profile and characterized for finding functional groups. *Ficus benghalensis* is a large evergreen tree, belongs to the family Moraceae. It is commonly known as "Indian Banyan Tree". Phytochemical screening of *Ficus benghalensis* fruit were performed in three solvents viz; methanol, chloroform, and aqueous were used to obtain extracts from powdered fruits which extracts were subjected to qualitative and quantitative phytochemical screening using standard procedures. Phytochemicals constituents were abundant in methanolic fruit extracts. The characterization of functional groups were analyzed using FT-IR spectroscopy.

Keywords: Phytochemistry, FT-IR, Fig fruits, Extraction, *Ficus benghalensis.*

INTRODUCTION

Biodiversity is blessed with a perennial source of medicinal plants which provided mankind with a rich source of medicines[1]. In developing nations, more than 80% of the people depend on traditional medicines for health care. Medicinal plants are in use for thousands of years and are renowned for their effectiveness in various treatments[2]. The medicinally usable plants were identified and extracted for biochemical profile and formulated for medical applications. They derive drugs mainly from wild plants and plants under cultivation[3]. Medicinal plants provide raw materials for indigenous health care systems such as Ayurveda, Unani and Siddha and also for modern medicines. Some estimates indicate that, over 25,000 effective plant based formulations are available in Indian medicine, 1.5 million practitioners use medicinal plants in preventive, promotional and curative applications[4]. 7800 medicinal drug manufacturing units in India consume 2000 tons of herbs annually and 119 drugs developed and marketed today, 74% were discovered from a pool of traditional herbal medicines. Medicinal plants provide not only drugs to treat some important diseases like cancer; it is also widely used for extracting herbal products such as cosmetics[5]. Over the years due to deforestation and over exploitation of forests for varied purposes, medicinal plant resources are depleting and this calls for conservation of medicinal plant germplasm resources. Conversation of resources can be done through creating large scale awareness on the importance of medicinal plants[6]. *Ficus benghalensis*, large evergreen tree, belongs to the family Moraceae[7]. It is commonly known as "Indian Banyan Tree". It one among the four sacred trees "Nalpamara" (Ksirivarkas) meant to be planted around the home and temples. Their ever-extending branch symbolizes eternal life, which is why it considered as sacred. The banyan tree also comprises of numerous spiritual and mythological contexts[8]. The banyan tree is considered as India's National Tree and it also symbolizes spiritual knowledge. Banyan tree is aboriginal to South Asia especially India, Sri Lanka and Pakistan. It is often being planted around temples and a place of religious importance. It is considered as sacred tree by both Hindus and Buddhists. Banyan tree is widely cultivated in city parks and botanical gardens throughout the New World and Old World tropics[9]. It grows well in tropical, semi-tropical regions, monsoon and rain forests with moderate to ample rainfall. Humid air and moist soil and is hardy, drought resistance and withstands mild frost is well suited for its growth[10]. The Chinese term for fig, "no flower fruit," gives a hint as to its convoluted form. Similar to pomegranates, figs contain numerous seeds, each technically representing a tiny fruit. However, unlike pomegranate, which issues its tough leathery shell from a bright orange, red, or pink trumpet-shaped flower heralding its emergence, the more modest and inwardly feminine fig hides its flowers within its fruit, while the pomegranate hides its fruit within its flower. Flowers of the fig are not visible to the uninitiated, but they do exist. In fact, the flowers (achenes) are inside the fig fruit; they are out of sight and do not ordinarily have access to the outside. All Ficus species produce figs, that is, fig fruits[11]. These fruits have thin and delicate skins (which toughen a bit on drying) and contain the flowers in an inverted form; that is, the flowers grow into the interior of the

**Author for Correspondence*

Table1: Qualitative Phytochemical Analysis of *Ficus benghalensis* fruits extracts

S. No	Phytochemical constituents	Methanol	Chloroform	Water
1.	Carbohydrate	-	-	-
2.	Reducing sugars	-	-	-
3.	Amino acids	-	-	+
4.	Protein	+	-	-
5.	Steroid	-	+	-
6.	Flavonoids	+	-	+
7.	Saponins	-	-	-
8.	Alkaloids	+	-	+
9.	Tannins	+	-	+
10.	Phenol	+	+	+
11.	Vitamin C	+	-	-
12.	Terpenoids	+	+	+
13.	Glycosides	-	-	-
14.	Phlobatanins	+	-	+
15.	Anthroquinones	-	-	-
16.	Chloride	+	-	+

'+' represents the presence of compounds; '- 'represents the absence of compounds

Table 2: Quantitative Phytochemical Analysis of *Ficus benghalensis* fruits extracts.

S. No	Phytochemical constituents	Amount in 1ml
1.	Total protein	0.90 mg
2.	Total flavonoid content	1.38 mg
3.	Ascorbic acid	0.52 mg

fruits. Fertilization occurs exclusively by true fig wasps that burrow into the fruits from their exteriors, mate, and leave their eggs to hatch. Males in some species also enlarge the entrance holes to be used later as exit holes for their mates, who may go on to find other figs[12]. *Ficus benghalensis* fruits are globose, sessile in axillary pairs, fleshy pericarp and with achenesen trenched in them, they are dark red in colour, 1.5-2.0 cm diameter, red to dark purple when ripe; seeds are tiny. Fruit is not edible for humans but is eaten by birds and monkeys[13].

MATERIALS AND METHODS

Plant Collection and Identification

The plant species were collected from South Western Ghats regions of Kanyakumari District, Tamilnadu, India during the months of August, September and October in the year 2013. The plants were sent for proper identification. The fruits were validated by an eminent botanist. The authenticated fruits were stored and preserved for future extraction.

Preparation of Extract

The *Ficus benghalensis* fruits were separated and cleaned well. Cleaned fruits were then dried under shade. The drying was done until all the water molecules evaporated and fruits became well-dried for grinding. After drying, the fruits were ground well using mechanical blender into fine powder and transferred into air-tight container with proper labeling for further use. The dried and powdered *Ficus benghalensis* fruits were extracted sequentially with methanol, chloroform, and aqueous using soxhlet apparatus.

Extraction of Ficus benghalensis Fruits by Continuous Hot Percolation Method

500 g of dried *Ficus benghalensis* fruit powder was weighed and successively extracted with 2.5 liters of solvents like methanol (60°- 80° C), chloroform, and aqueous by soxhelation for a period of 72 hours. To concentrate the extract, the solvent was subjected to distillation and further concentrated with a rotary evaporator under reduced pressure and dried.

Qualitative Phytochemical Screening

The methanol, chloroform and aqueous fruit extracts were screened for different phytochemical constituents' viz., carbohydrate, reducing sugars, amino acids, protein, steroid, flavonoids, saponins, alkaloids, tannins, phenol, vitamin C, terpenoids, glycosides, phlobatannins, anthroquinones and chloride. The method employed to analyze the phytochemicals are described below.

Tests for Carbohydrates using Benedict's test

2 ml of crude extract was mixed with 2ml of Benedict's reagent and boiled. Formation of reddish brown precipitate indicates the presence of the carbohydrates.

Test for reducing sugar

The extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for minutes. An orange red precipitate indicates the presence of reducing sugars.

Tests for Amino Acids using Ninhydrin test

For the analysis of amino acid 3 ml test solution with 3 drops 5% Ninhydrin solution was heated in water bath for 10 min. The appearance of purple or bluish colour indicates the presence of amino acids.

Tests for Proteins using Biuret test

With 3 ml of test solution, few drops of 4% NaOH and 1% $CuSO_4$ solution were added. The tubes were observed for violet or pink colour formation.

Tests for Vitamin C

1 ml of 2% w/v solution was diluted with 5 ml of water. 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of diluted sodium hydroxide solution were added. Then 0.6 ml of hydrochloric acid was added drop wise and stir, the yellow colour turns blue it's indicates positive results.

Tests for Chloride

3 ml test solution prepared in HNO_3 and few drops 10% $AgNO_3$ solution was added. White precipitate of $AgCl_2$ is observed.

Tests for Tannins

With 2-3 ml test solution, 5% $FeCl_3$ solution was added and observed for deep blue-black colour reactions.

Tests for Alkaloids using Wagner's test

2-3 ml filtrate was taken into separate tubes. To that few drops of Wagner's reagent was added and observed reddish brown precipitate.

Detection of flavonoids using Lead acetate test

Table 3: FT-IR Analysis of *Ficus benghalensis* crude chloroform fruits extracts.

S. No	Peak	Functional group	Type of Vibration	Intensity
1.	720	C–H aromatics	"Oop"	Strong
2.	1174	C–O alcohols, carboxylic acids, esters, ethers	Stretch	Strong
3.	1466	C–H alkanes	Bend	Strong
4.	1710	C=O α, β unsaturated aldehydes, ketones	Stretch	Strong
5.	2858	C–H alkanes	Stretch	Strong
6.	2929	C–H alkanes	Stretch	Strong
7.	1246	C–H (–CH_2 X) alkyl halides	Wag	Medium
8.	1375	C–H alkanes	Rock	Medium
9.	586	C–Br or C–Cl alkyl halides	Stretch	Medium
10.	1102	C–N aliphatic amines	Stretch	Weak
11.	3015	=C–H alkenes	Stretch	Weak

Table 4: FT-IR Analysis of *Ficus benghalensis* methanolic crude fruits extracts

S. No	Peak	Functional group	Type of Vibration	Intensity
1.	1016	C–O alcohols, carboxylic acids, esters, ethers	Stretch	Strong
2.	2944	C–H alkanes	Stretch	Strong
3.	3326	N–H 1°, 2° amines, amides	Stretch	Strong, b
4.	1404	C–C (in–ring) aromatics	Stretch	Medium
5.	1456	C–H alkanes	Bend	Medium
6.	1112	C–N aliphatic amines	Stretch	Weak
7.	567	C–Br or C–Cl alkyl halides	Stretch	Weak

Table 5: FT-IR Analysis of *Ficus benghalensis* aqueous crude fruits extracts.

S. No	Peak	Functional group	Type of Vibration	Intensity
1.	720	C–H aromatics	"oop"	Strong
2.	1069	C–N aliphatic amines	Stretch	Strong
3.	1461	C–H alkanes	Bend	Strong
4.	1619	N–H 1° amines	Bend	Strong
5.	1743	C=O aldehydes, saturated aliphatic	Stretch	Strong
6.	2858	C–H alkanes	Stretch	Strong
7.	2920	C–H alkanes	Stretch	Strong
8.	3349	N–H 1°, 2° amines, amides	Stretch	Strong, b
9.	1370	C–H alkanes	Rock	Weak
10.	3011	=C–H alkenes	Stretch	Weak

The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.

Test for phlobatannins

Formation of red precipitate when plant sample was boiled with 1 % aqueous hydrochloric acid indicates the presences of phlobatannins.

Tests for Steroids

With 2 ml of test solution, 2 ml of acetic anhydride and 2ml of H_2SO_4 was added. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Detection of Terpenoids using Salkowski reaction

To 2 ml of sample, 2 ml chloroform and 2 ml Concentrated H_2SO_4 were added and observed chloroform layer for red color and acid layer for fluorescence. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Test for Phenolic compounds using Ferric chloride test

The extract was diluted to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green color indicates the presences of phenolic compounds.

Test for saponins

The fruits samples were diluted with distilled water and made into 20 ml. The suspension was shaken well in graduated cylinder for 15 minutes; 2cm layer of foam indicates the presences of saponins.

Test for glycosides

The extract (2 ml) was hydrolyzed with HCl solution (0.5ml) and neutralized with NaOH solution (0.5 ml). A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

Test for anthroquinones

About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the

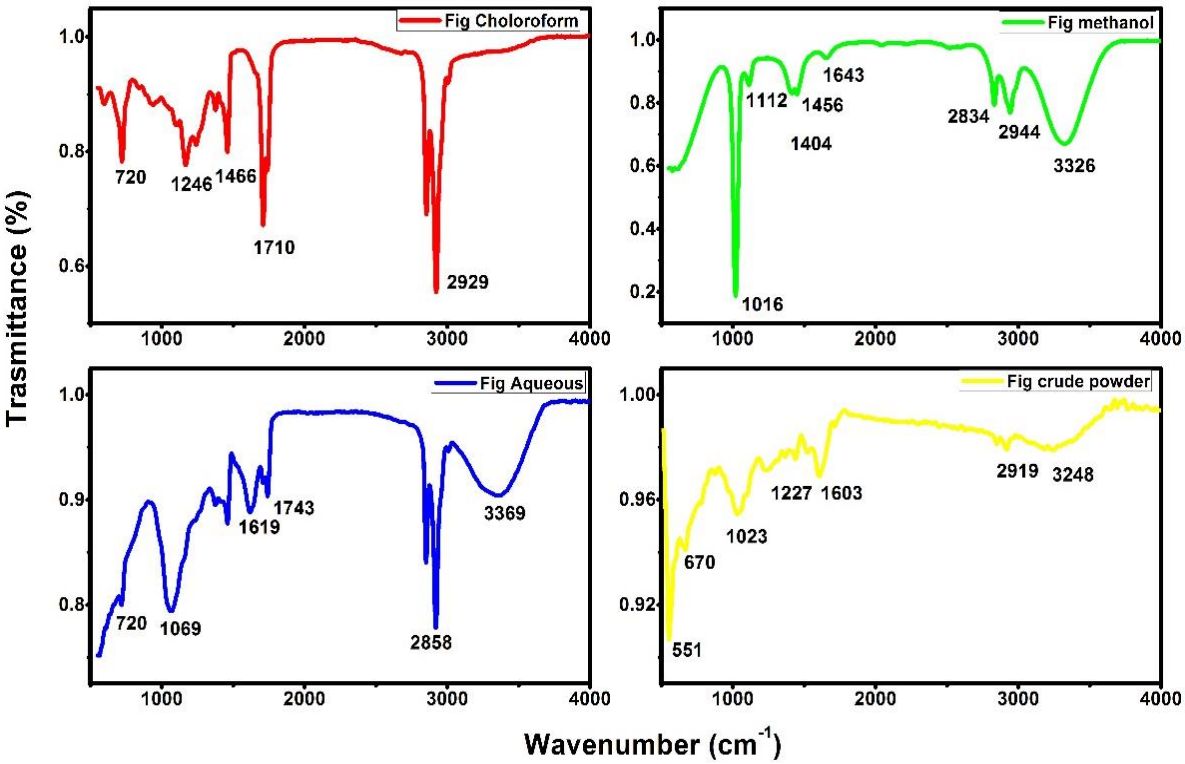

Figure1: FT-IR spectra of *Ficus benghalensis* different fruit extracts.

filtrate. Few drops of 10% ammonia were added to the mixture and heat. Formation of rose-pink colour indicates the presence of anthroquinones.

Quantitative Phytochemical screening

The preliminary qualitative phytochemical screening confirms the presence of maximum number of secondary metabolites in methanolic extracts of *Ficus benghalensis* fruits. The methanolic fruit extracts were screened for major three phytochemical constituents' viz., total protein, total flavonoid content and ascorbic acid. The method employed to analyze the phytochemicals components are described below.

Estimation of Protein

Total protein content was estimated using the protocol of Lowry *et al*., A stock solution (1mg/ml) of bovine serum albumin was prepared in 1 N NaOH; five concentrations (0.2, 0.4, 0.6, 0.8 and 1 ml) from the working standard solution were taken in series of test tubes. In another set of test tubes 0.1 ml and 0.2 ml of the test sample were taken and the volume was raised up to 1 ml in all the test tubes. To each test sample, 5 ml of freshly prepared alkaline solution was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocaltcau reagent was rapidly added and incubated at room temperature for 30 minutes until the blue colour developed. The spectronic colorimeter was adjusted at wavelength of 750 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively. A regression curve was worked out of various concentrations of the standard solutions against their respective absorbances, which followed the Beer's law.

Estimation of Flavonoid

Total flavonoid assay was conducted using aluminium chloride colorimetric method (Marninova *et al*.,). One milliliter of sample was added with 4 ml distilled water in a flask. After that 0.3 ml 5% $NaNO_2$ was added. After 5min, 0.3 ml of 10% $AlCl_3$ was added. After the sixth minute, 2 ml of 1 M NaOH was added. Then the mixture was diluted to 10 ml adding 2.4 ml distilled water. The mixture was mixed and the absorbance was measured at 510 mm. Total flavonoids content was expressed as mg catechin equivalents (CE)/g samples.

Estimation of ascorbic acid

Ascorbic acid content in fruit material was estimated as per the method described by Sadashivam and Manikam (1996). 50 µg sample was homogenized in 10 ml 4% oxalic acid and centrifuged at 5000 rpm for 15 min. The supernatant were collected and bromine water was added drop wise with constant stirring to give a yellow color. The excess bromine was expelled by blowing in air with a pipette. Final volume 25 ml with 4% oxalic acid, 2 ml of brominated extract was adjusted to 3 ml with distilled water. This was allowed to react with 1 ml of 2% DNPH filtered and used, followed by 1-2 drops of Thiourea (10%). Blank was prepared as above with distilled water in the place of Ascorbic acid or extract and incubated at 37◦C for three hours. The orange red Osazone crystals were dissolved by adding 7 ml of 80 % H_2SO_4. The absorbances were measured at 540 nm using UV-Vis spectrophotometer. The results were expressed as milligrams of ascorbic acid equivalent per gram of dry weight.

Fourier Transform Infrared Spectrophotometer (FT-IR) analysis of Ficus benghalensis fruits

Dried powder of crude fig fruits, chloroform, methanolic and aqueous extracts of *Ficus benghalensis* fruits was considered for instrumental analysis. For the FTIR study, dried powder of methanolic extract (10 mg) of *Ficus benghalensis* was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered samples were treated for FT-IR spectroscopy.

RESULTS AND DISCUSSION

Qualitative phytochemical screening was used to determine the presence of some secondary metabolites. The results of screening test revealed the presence of medically active compounds in *Ficus benghalensis* fruits. Results obtained for qualitative screening of phytochemical fruit extracts of *Ficus benghalensis* in three different solvents are shown in Table 1. The phytochemical compounds such as terpenoids and phenol were effectively present in all the three solvents of fruit extracts. Carbohydrate, reducing sugars, saponins, glycosides and anthroquinones were completely absent in all the three solvents. Flavonoids, alkaloids, tannins, phlobatannins and chloride were present in aqueous and methanolic extracts. Vitamin C and protein were present in methanolic extracts. Steroids were completely present only in chloroform extracts. Amino acids were present in aqueous extracts. From the Table 1, it could be seen that most of the compounds were present in the methanolic extract of *Ficus benghalensis* fruits. Deewwe[14,15]. From the preliminary qualitative phytochemical screening of *Ficus benghalensis* fruit extracts it was confirmed that, the methanolic extract possessed maximum amount of secondary metabolites. The quantitative phytochemical screening of three important secondary metabolites such us total protein, total flavonoid content and ascorbic acid present in the *Ficus benghalensis* methanolic fruit extract were analyzed and tabulated in Table.2. xSXwqs[16,17]. FT-IR spectra of *Ficus benghalensis* chloroform fruit extract is shown in Figure 1. The functional groups, intensity and type of vibration were tabulated in Table. 3. The weak band at 586 cm^{-1} corresponds to C–Cl stretching due to alkyl halides of the chloroform. The bands at 586 cm^{-1} and 720 cm^{-1} corresponds to C–H aromatic compound which is found in the crude extract. The weak bands at 1174 and 1246 cm^{-1} can be ascribed to C–O stretching ether compounds available from the crude which was extracted using chloroform. The band at 1466 cm^{-1} corresponds to C–H bends due to alkane compounds. The absorption appearing at 1710 cm-1 can be assigned to C=O stretch of the crude extract. The intense peak at 2858 cm^{-1} and 2929 cm^{-1} corresponds to C–H stretching of alkane compounds[14,18]. FT-IR spectra of *Ficus benghalensis* methanolic fruit extract is shown in Figure 1. The functional groups, intensity and type of vibration were tabulated in Table. 4. The peak at 1016 cm^{-1} because of C–O stretching due to alcohols can be seen in the below graph. The weak bands at 1112 cm^{-1} and 1404 cm^{-1} are due to the C–N stretching of aliphatic amines in the crude extract. C–H bending of alkanes are viewed as weak bands at 2834 cm^{-1} and 2944 cm^{-1}. The stretching at 3226 cm^{-1} indicates the presence of N–H group in the crude extract[19]. FT-IR spectra of *Ficus benghalensis* aqueous fruit extract is shown in Figure 1. The functional groups, intensity and type of vibration were tabulated in Table. 5. A weak band at 720 cm^{-1} can be observed in the below figure. This is due to C–H aromatic bond of the extract. A broad peak at 1069 cm^{-1} indicates the presence of C–N stretching of aliphatic amines. At 1619 cm^{-1}, a weak band is shown which ascribes the presence of N–H bend due to primary amines. A band at 1743 cm^{-1} indicates the presence of C=O stretching of aldehydes. Strong peaks at 2858 cm^{-1} and 2920 cm^{-1} can be observed which shows the presence of C–H stretch due to alkanes found in the crude extract. The absorption appearing at 3369 cm^{-1} can be assigned to the N–H stretch of primary amine compounds[20].

CONCLUSION

It has been estimated that 80% of the populations of developing countries rely on traditional medicines i.e., mostly plant drugs[21,22]. The experimental material selected for the study was *Ficus benghalensis* fruits. The present study was carried out to determine the qualitative and quantitative phytochemical constituents and the functional groups present in the *Ficus benghalensis* fruit extracts. The result reveals that the methanolic extract of *Ficus benghalensis* fruits showed maximum phytochemical constituents. The same extract could be utilized for the isolation of further bioactive metabolites. The FT-IR analysis results revealed that the C-H stretching in the range 2800 - 3000 cm^{-1} shows a strong peak and in the range of 1600 -1750 cm^{-1} shows the presence of carboxyl (C=O) groups. These indicate the strong presence of flavonoid group in the crude extract. The study also provide a strong evidence for the use of *Ficus benghalensis* fruit extract to treat various pharmacological activities.

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