ORIGINAL ARTICLE

Phytochemical investigation, gas chromatography–mass spectrometry, and Fourier transform infrared analysis in adventitious roots of *Ficus benghalensis L.*

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Abstract

Aim: The aim of the study was to analyze the phytochemical components of adventitious roots of *Ficus benghalensis* by biochemical reactions, Fourier transform infrared (FTIR), and gas chromatography–mass spectrometry (GC-MS) analysis. **Materials and Methods:** Phytochemical screening was performed using shade-dried powder of the roots. FTIR and GC-MS as analysis were performed using the organic solvent extracts of the root powder. **Results:** The phytochemical analysis revealed the presence of bioactive compounds such as phenols, saponins, tannins, terpenoids, amino acids, and proteins. FTIR has revealed four major peaks. The first major peak has the value of 2916 with aromatic group (C-H), the second peak with the value of 2848 indicated the presence of amines and amides (N-H), followed by the peak values of 1735 and 1242 which belongs to esters, saturated aliphatic group (C=O), and aromatic amines (C-N), respectively. Chromatogram of GC-MS analysis revealed the presence of two compounds, i.e., silicic acid, diethyl bis(trimethylsilyl) ester at 24.28 min R_t and cyclotrisiloxane, hexamethyl- at 29.90 min R_t in the root extract, suggesting that these two are only volatile molecules present in the methanolic extract. The other phytochemicals present in the extract might be non-volatile and/or heavy weight molecules. Further analysis of the extract of *Ficus benghalensis* would possibly yield novel bioactive phytochemicals. **Conclusion:** Results of this study give a summary of various phytochemicals present in the adventitious roots of *F. benghalensis*.

Key words: Adventitious root, *Ficus benghalensis,* Fourier transform infrared, gas chromatography–mass spectrometry, phytochemical analysis

INTRODUCTION

Medicinal plants are being used for thousands of years and well known for their effectiveness in multiple treatments.[1] For medical applications, plants' extracts were used to know their compound profile and formulated as drugs for medicinal uses.[2] Medicinal plants were used to treat various diseases and extensively used for extracting herbal by-products such as cosmetics.[3] They medicinal plants are mostly wild plants and they are not cultivated often. Certain estimates specify that over twenty thousands of actual plant-based formulations are available in Indian medicine; about 1.5 million practitioners use medicinal plants in preventive, persuasive, and healing applications.[4] *Ficus benghalensis* grows as a huge evergreen tree, usually called as "Indian Banyan Tree." Mostly Banyan trees are grown in city parks and botanical gardens throughout the old world tropics and new world.[5] It is one among the four sacred trees meant to be planted everywhere around temple and homes. It contains various spiritual and mythological circumstances.[6] It grows well in semi-tropical, tropical, and areas with medium

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rainfall with forest cover.[7] The taxonomical classification of *Ficus benghalensis* L. is Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: Magnoliophyta, Class: Magnoliopsida, Subclass: Hamamelidae, Order: Urticales, Family: Moraceae, Genus: Ficus, and Species: *F. benghalensis* (L). Markets for medicinal plants and herbal medicine are productive and important for economic growth of India. Several pharmacopeia have included a number of imperative herb and herbal products. For instance, the Ayurvedic Pharmacopoeia of India included monographs for 258 Ayurvedic drugs, the Indian Pharmacopoeia 2010 incorporated 89 monographs for herb and herbal products, whereas the Indian Herbal Pharmacopoeia 2002, published by the Indian Drug Manufacturers Association, included 52 monographs on widely used medicinal plants of India. *F. benghalensis* is also used in Indian traditional medicine systems such as Siddha, Unani, Ayurveda, and Homeopathy. Various portions of the tree were found to have medicinal properties; the powder of bark is used in treating dysentery, and in seminal weakness, leukorrhea, menorrhagia, nervous disorders, erysipelas, and in conditions of burning sensation. Milky sap and seeds can be applied topically to sores, ulcers, cracked soles of the feet, and to treat rheumatic soreness. Buds can be given as a decoction along with milk to prevent hemorrhages. Adventitious root extracts are antiemetic and can be topically applied to pimples. The leaves paste can be applied externally to abscesses and wounds for promoting suppuration. Gonorrhea can be treated using adventitious roots, consume of leaves good for ulcers, and seeds and fruits can be used to keep our body cool. Diabetes can be treated using adventitious roots of *F. benghalensis* and bark juice was used as tonic.[8,9] In Ayurveda, bark of banyan tree is used to treat inflammatory diseases and rheumatism.[10-12] Leaf bud and adventitious roots were used in the treatment of hemorrhages and piles bleedings.[13] Healthy leaves of plants were identified to encourage conception, which turns as natural plasma disinfectant and to treat ulcers and skin diseases.[14,15] Bark extract can also be used in wound healing, antistress, and antiallergic.[16,17] Immunomodulatory activity is found in adventitious root extracts of banyan tree. Adventitious root extracts are used for enhancing growth and reducing hair loss.[18] Considering the above facts, the aim of this work was to characterize the compounds present in the plant extract by performing phytochemical screening, Fourier transform infrared (FTIR), and gas chromatography–mass spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

Materials

Adventitious roots of *F. benghalensis* were collected from Foundation for Revitalization of Local Health Traditions (FRLHT) Bangalore, India. Then, the plants were taxonomically authenticated by the botanist NM Ganesh Babu PhD, FRLHT, Bangalore.

Preparation of Extracts from Adventitious Root

The collected adventitious roots were washed with distilled water to remove unwanted material. Then, the roots were cut into small pieces and allow dried at room temperature. Dried samples were powdered using mixer grinder. Then, 5 g of the powdered sample was mixed in 300 mL of solvent was eluted sequentially based on the polarity index of the solvents. Extract was filtered using Whatman filter paper and used to preserve the sample for future use.

Qualitative Phytochemical Analysis

The preliminary phytochemical analysis was tested the presence of bioactive compounds according to the standard procedure.[19-21] The plant material was extracted using methanol, which was subjected to phytochemical analysis. The tests were performed to check the presence or absence of bioactive compounds such as carbohydrate, protein, amino acids, phenol, tannins, saponins, and terpenoids.

FTIR Spectroscopy

FTIR analysis was performed (Perkin Elmer Spectrophotometer system, UK) for the *F. benghalensis* adventitious root extract, which was used to perceive the typical peaks and their functional groups. FTIR spectrophotometry is plausibly the most controlling tools for recognizing the kinds of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is unique to the chemical bond can be seen in the annotated spectrum. The chemical bonds of a molecule can be determined using the infrared absorption spectrum. The plant constituents of dried powder sample of methanol extract were used for FTIR investigation. For instrumentation analysis, dried powered was used. To make translucent sample, 8 mg of methanol extract dried sample was condensed in 100 mg of KBr pellet.

GC-MS Analysis

Preparation of sample for GC-MS

One milligram of adventitious root sample was dissolved in 1 mL of high-performance liquid chromatography grade methanol. Then, 0.2 µl of sample was filtered using syringe filter. In automatic programmed syringe injector, 1 µL of sample was injected for GC-MS analysis.

Chromatographic circumstances used in GC-MS

Analysis of GC-MS was supported on thermal desorption TD-20 system, GC-MSQP-2010 Plus (Shimadzu, Japan) made up of autosampler. Mass spectrometer instrument was interfaced with RT_X-5MS column (30 mm × 0.25 mm × 0.25 µm) working in electron impression mode at 70eV. In this instrument, 99.99% of helium gas was used as carrier gas

with the movement frequency of 1.2 ml/min. Early column oven temperature was 80°C (isothermal for 4 min) with steady increase of 5°C/min to 310°C, flow rate 1.21 ml/min, and column pressure of 81.7 kPa. At the scan interval of 0.50 s, mass spectrum was prepared with mass scan from 40 to 650 m/z.

RESULTS

Phytochemical Screening

The study was designed to evaluate phytochemical, FTIR, and GC-MS. The preliminary qualitative screening revealed phytochemical constituents which are recognized to exhibit physiological as well as medicinal activities.[22] Plant extract analysis revealed that the presence of carbohydrate, proteins, amino acids, phenol, tannin, saponins, and terpenoids mentioned in Table 1.

FR-IR Analysis

FTIR was used to analyze the functional group of the compound present in Figure 1. It has four major peaks. The first major peak has the value of 2916 belongs to the functional group of aromatic (C-H). The second major peak has the value of 2848, which belongs to the primary, secondary amines and amides (N-H) group. The third major peak of 1735 belongs to the esters and saturated aliphatic group (C=O) and the last peak 1242 belongs to the group of aromatic amines (C-N).

GC-MS Analysis

GC-MS analysis indicates the retention time, chemical structure, and its pharmacological activities of the plant *F. benghalensis* [Table 2]. The study on the active principles of adventitious root of *F. benghalensis* exhibited two major bioactive compounds [Figures 2 and 3], namely silicic acid, diethyl bis(trimethylsilyl) ester (with R_t 24.287) and the second compound cyclotrisiloxane, hexamethyl (29.904) reported already for their antibacterial activity.[23]

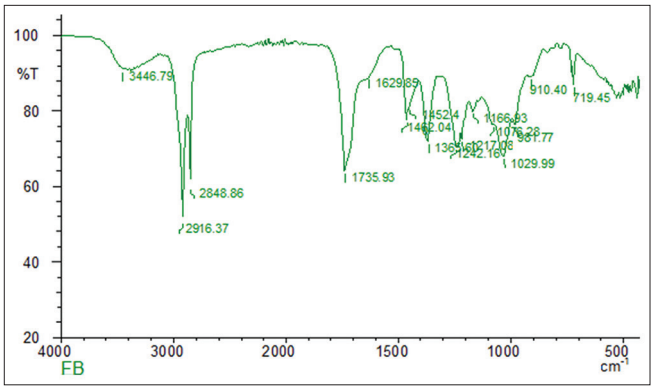


Figure 1: Fourier transform infrared spectrum of *Ficus benghalensis* adventitious root

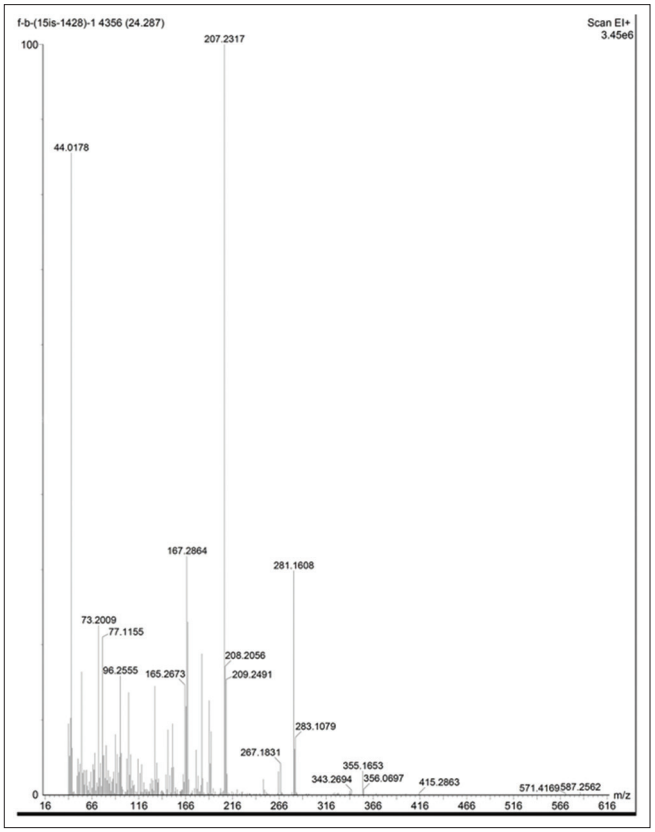
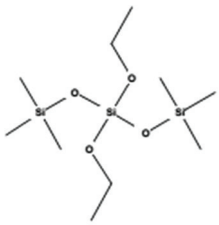
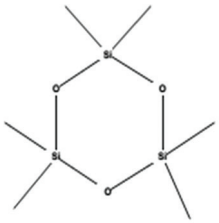


Figure 2: Spectrum of gas chromatography–mass spectrometry at retention time of 24.287

Table 1: Qualitative phytochemical screening of *Ficus benghalensis* adventitious root extract

Phytochemicals tests	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Carbohydrate	++	++	++	++
Proteins	++	--	++	++
Amino acids	++	++	++	--
Phenol	++	--	++	++
Tannins	++	++	++	++
Saponins	--	++	--	++
Terpenoids	++	++	++	++

Table 2: Chemical constituents of GC-MS analysis *Ficus benghalensis* adventitious root

R_T	Peak name	Chemical formula	Structure	Pharmacological activity
24.28	Silicic acid, diethyl bis (trimethylsilyl) ester	$C_{10}H_{28}O_4Si3$		Activity not reported
29.90	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si3$		Antibacterial activity[23]

GC-MS: Gas chromatography–mass spectrometry

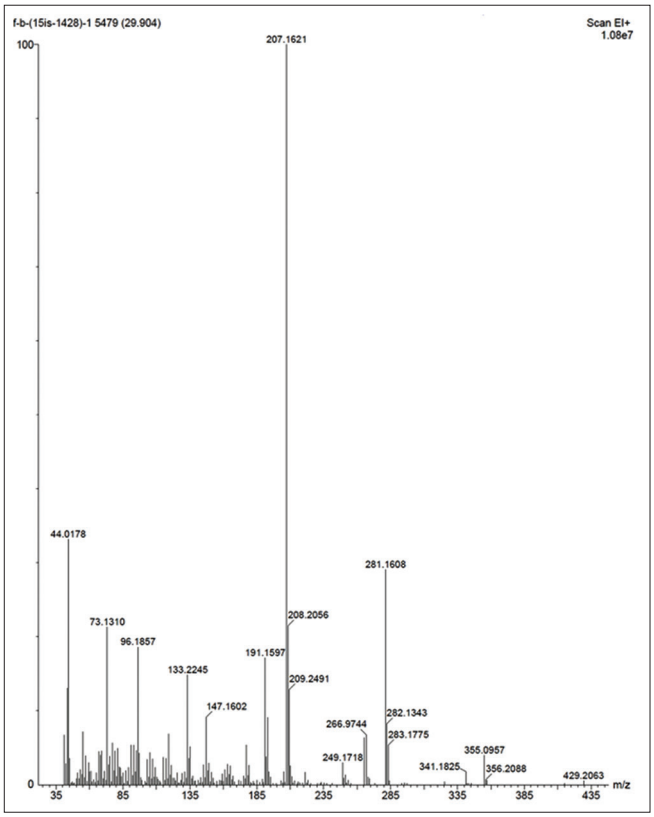


Figure 3: Spectrum of gas chromatography–mass spectrometry at retention time of 29.904

CONCULSION

The results of this study revealed the presence of medicinally significant phytochemicals in *F. benghalensis* adventitious root. Crude methanolic extract of *F. benghalensis* adventitious root was subjected to GC-MS and qualitative phytochemical analysis. Among the two volatile compounds present in the crude extract, cyclotrisiloxane, hexamethyl was found to be an antibacterial agent, based on previous reports. Phytochemical screening results show that crude extract of different solvents shows the presence of bioactive molecules. Furthermore, the FTIR analysis of the crude extract showed the presence of key functional groups, identical to the GC-MS observed compounds. This result strongly suggests that *F. benghalensis* root extract could possess antibacterial activity, due to the presence of cyclotrisiloxane, hexamethyl derivative. This approach helps to identify the possible bioactivity of plant extracts and phytochemical components, without the need for performing various experiments. *In vitro* and *In vivo* studies for biological activities are expensive, laborious, and time consuming. Using the phytochemical analysis and library match of molecules in GC-MS analysis, it is possible to predict the biological activities of various plants and its components, and also it is a cost-effective approach.

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