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Research Article

A comparative study on GC-MS analysis and antimicrobial activity of bioactive compounds present in aerial parts (leaf and fruit) of *Ficus benghalensis* L.

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Abstract
Plants are being looked upon for medications derived mainly from different plant parts. The majority of the population worldwide, especially in underdeveloped nations, relies on herbal formulations for basic medical requirements. *Ficus benghalensis* L., member of moraceae family is renowned for its ethano-medicinal applications. In this study, polar (aqueous, methanolic, and acetone) and non polar (petroleum ether) extracts of leaves and fruits of *F. benghalensis* L. were investigated for their antimicrobial activity and phytochemical constituency. Antimicrobial activity was estimated by investigating Zone of Inhibition (ZOI) and Minimum Inhibitory Concentration (MIC) against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus)* and gram-negative (*Salmonella typhi* and *Escherichia coli*) bacteria; and fungal strains (*Aspergillus niger, Fusarium oxysporum*, and *Rhizopus oryzae*). The diameter of ZOI ranged from 18.8 ± 1.2mm to 6.2 ± .88mm for various bacterial strains, whereas from 10.2 ± 1.3mm to 6.2 ± 1.6mm for fungal strains. Aqueous and petroleum ether extracts exhibited comparatively lesser or no activity in some cases whereas methanol and acetone extracts exhibited moderate to good activity. MIC values ranged between 50µg/µl to 0.024µg/µl against both bacterial and fungal strains. Methanolic extracts were further analyzed using Gas Chromatography -Mass Spectrometry (GC-MS) for their phytochemical profile since they showed higher antimicrobial activity. The major compounds detected in leaf extracts were Lup-20(29)-en-3-one (20.45%), Lupeol (17.40%), Beta amyrone (9.07%), Squalene (5.17), Stigmasta-5-en-3-ol (5.62%), Vitamin-E (3.89%), and n-Hexadecanoic acid (1.32%); and in fruit extract were Octadecatrienoic acid (15.24%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (14.89%), 5-Hydroxymethylfurfural (15.32%), 24-Norursa-3,12-diene (2.79%), and 9,12-Octadecadienoic acid (z, z)-2-hydroxy-1- (hydroxymethyl) ethyl (2.07%). This study supports using *F. benghalensis* L. in microbial infection therapy.

Keywords: Antimicrobial activity, GC-MS analysis, *Ficus benghalensis* L., Phytochemical estimation

INTRODUCTION

The production of the primary metabolites (sugars, amino acids and fatty acids) is crucial to the plant's survival. However, secondary metabolites are necessary for their defense. Secondary metabolism provides protection from different stresses, which initiates secondary metabolite synthesis, which further activates the plant's mechanism of defense (Kliebenstein and Osbourn, 2012; Isah, 2019). Alkaloids, terpenes, and polyphenols are plants' most diverse secondary metabolites (Rai *et al*., 2017). Polyphenols, having higher antioxi-

dant capabilities are composed of a phenyl ring with an attached hydroxyl group. Due to their fundamental structure, polyphenols can be divided into several subfamilies including tannins and flavonoids. Flavonoids are produced in almost all the plant parts and cause flower pigmentation (Mierziak *et al*., 2014; Gutiérrez-del-Río *et al*., 2018; Durazzo *et al*., 2019; Platzer *et al*., 2021) whereas tannins are the high molecular weight compounds formed in the roots, seeds, bark, leaves, and wood. They have an astringent flavor that makes them resistant to predators, including insects, birds, and herbivores (Pizzi, 2019; Platzer *et al*., 2021). Reactive oxygen species, sulfur species, and nitrogen species are examples of free radicals that tannins can scavenge. These radical species influence cells through a variety of processes, including homeostasis disturbances, interfering with signaling pathways, modification of DNA integrity, and structural destabilization in cell membranes. Chronic degenerative diseases, cardiovascular illnesses, and various types of cancers can all be caused by these pathways (Pizzi, 2019, Flieger *et al*., 2021).

Plants are a great source of chemical compounds that are renewable and have a variety of advantages. Since they have been around for thousands of years, they are used in a variety of ways for sustaining life, such as to cure a variety of ailments, to produce herbal products like cosmetics, medicines, etc. (Gupta *et al*., 2007). Approximately 70,000 plant species have reportedly been shown to be able to treat various diseases (Kuruppu *et al*., 2019). According to the World Health Organization (WHO), 21,000 medicinal plants are currently in use for a variety of medical applications. It has always been challenging to pinpoint individual chemicals linked to a given trait since plants create a vast range of molecules. To overcome this difficulty, a variety of chromatographic techniques have been created so far to separate and identify the components of a complex sample, such as gas chromatography coupled to mass spectrometry (GC-MS), which is used in numerous studies to identify and characterize various compounds having different activities like antimicrobial, antioxidant, antiinflammatory, etc. obtained from the extraction of a variety of plant parts with different types of solvents (Ara *et al*., 2013; Maree *et al*., 2014; Valle *et al*., 2016; Wagner *et al*., 2019; Safdar *et al*., 2019). On the other hand, few studies on *F. benghalensis* L. reveal its phytochemical profiling (Tharini *et al*., 2018; Karthikeyan *et al*., 2019).

The world is passing through a phase that could be critical since antibiotics are losing their efficacy and the previously treatable common microbial diseases are becoming more challenging to treat. Due to the incorrect and excessive use of microbes, they have acquired multidrug resistance, which poses a serious hazard and a greater challenge to the medical community (Rudramurthy *et al*., 2016). Thus, the hunt for novel active compounds with antimicrobial potential has become crucial, and the key source is plants. Furthermore, given that synthetic pharmaceuticals have a variety of negative side effects on people, current researchers are prioritizing the development of plant-based medicines.

F. benghalensis L., frequently called the "Indian Banyan Tree" grows huge up to several meters in length and breadth. It is an evergreen tree that predominates in parks and botanical gardens all over the world's tropics. Being an Indian indigenous plant, it is considered sacred and can be found planted around homes and religious places everywhere. It thrives well in semi-tropical, tropical, and regions with moderate amounts of precipitation and forest cover (Lansky *et al*., 2008; Joseph and Raj, 2010).

The aqueous and the other organic extracts of *Ficus benghalensis* L. were discovered to exhibit a variety of pharmacological effects, including anti-diabetic, antitumor, hypolipidemic, hypocholesterolemic, anthelmintic, antiinflammatory, and antimicrobial (Ahmad *et al*., 2011). Its various parts are reported to be used in the treatment of diseases like dysentery, seminal weakness, menorrhagia, leukorrhea, erysipelas, nervous disorders, rheumatism, inflammatory illnesses, ulcers, sores, cracked soles, rheumatic soreness, piles bleeding, hemorrhages, burning sensations, gonorrhea, diabetes, acne, etc. It has also been found to promote pregnancy and acts as a natural plasma disinfectant. It has immunomodulatory action and is good for wound healing, stress, and allergy relief (Papitha *et al*., 2017). Thus, the present work was carried out to study the differential phytochemical activity of the aerial parts, i.e., fruit and leaf of *F. benghalensis* L. by comparing their antimicrobial activity and GC-MS analysis.

MATERIALS AND METHODS

Collection and authentication of plant material

Plant samples (leaf and fruit) were collected in November 2021 from district Rohtak (28.8955° N, 76.6066° E), Haryana, India. Samples were authenticated and submitted to CSIR - National Institute of Science Communication and Policy Research, New Delhi, under the voucher Id - NIScPR/RHMD/Consult/2022/4122-23.

Preparation of extracts

Samples were washed thoroughly with tap water followed by distilled water, shade-dried for 10-12 days and ground to a coarse powder. Extraction using the soxhlet method was done for methanol, acetone, and petroleum ether extracts. 50gm sample of each of plant part was put into the thimble and 280ml of respective HPLC grade solvent for each sample was added to the round bottom flask. The extraction cycle was run for 16-

18 hours or until the solvent in the extraction chamber reached almost a colorless state. For aqueous extraction, Maceration method was adopted. Since the boiling point of water is very high, extraction with Soxhlet method is not advisable as it can readily destroy thermolabile/heat-sensitive phytoconstituents present in the sample. Plant sample was soaked with water in a ratio of 1:5 for 48-72 hours and filtered. In both the methods, i.e., Soxhlet (for Organic extract) and Maceration (for Aqueous extract), the crude extract was filtered and the solvent was totally evaporated using a rotary evaporator. Extracts were reconstituted using Dimethyl sulfoxide (DMSO) for further analysis.

Antimicrobial activity

Microorganisms

Four strains of bacteria, gram-positive (*Baciillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 96)), gram-negative (*Salmonella typhi* (MTCC 98) and *Escherichia coli* (MTCC 443)), and three strains, namely *Rhizopus oryzae* (MTCC 262), *Aspergillus niger* (MTCC 514), and *Fusarium oxysporum* (MTCC 7392) of fungi procured from IMTECH, Chandigarh, India, were used in the present study. The ZOI (Zone of Inhibition) and MIC (Minimum Inhibitory Concentration) were ascertained by Disc diffusion and Broth microdilution methods, respectively.

Estimation of ZOI by disc diffusion assay

The Disc diffusion method prescribed by Berghe and Vlietinck (1991) was used to assess the antimicrobial activity of the plant extracts. Petri plates filled with 25ml of solidified Muller Hinton agar media in the case of bacteria, whereas Czapek dox agar (for *R. oryzae* and *A. niger*) and potato dextrose agar (for *F. oxysporum*) in the case of fungi were spread with 25µl of 10^8 spores/ml of the culture/inoculum of the respective strains (Saharan *et al*., 2023). Stock solution of the extract was prepared by dissolving 100mg of each of the plant extracts into 1ml of DMSO, and further dilutions were performed as per requirement. Following this, the 6 mm-sized discs were placed equidistant in the plates and poured with 10µl of each of the respective concentrations (100, 50, and 25mg/ml for bacteria and 100 and 50mg/ml for fungi) of plant extracts against bacterial and fungal cultures. Discs of the standard drug Chloramphenicol and miconazole (10µl of 1mg/ml each) were also placed alongside in the plates. Further, the plates were incubated at 37ºC and 30ºC for at least 24 hours, respectively in the case of bacteria and 72 hours for fungi. Further, the diameter of ZOI was measured for three trials and an average value was derived. Chlorempheniсol (10µl of 1mg/ml), a standard drug, and DMSO (in pure form) were used as positive and negative controls, respectively.

Estimation of MIC by broth microdilution assay

MIC represents the lowest plant extract concentration that inhibits microbial growth. Method of serial two-fold dilutions prescribed by Eloff (1998) was used to determine MIC. 100µl (from the stock of 100mg/ml) of each plant extract was poured into the first well of the microtiter plate, followed by further dilutions up to 12^{th} well (i.e., 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.390, 0.195, 0.097, 0.048, and 0.024µg/µl). Further added to the each well are 50µl of growth media and 25µl of freshly prepared standardized inoculum (10^6cfu/ml). Plates were incubated overnight at 30ºC. Further, 25µl of *p*-iodonitrotetrazolium violet diluted with water was added to wells and incubated for 1 hour. The tetrazolium gets reduced to formazan crystals of red color by biologically active organisms indicating no inhibition (Eloff, 1998). The concentration of extract at which clear solution (no change in color from purple to pink) was considered as the MIC value for that particular strain. Values were recorded in triplicates.

GC-MS analysis

Methanolic extracts of the plant parts were analyzed for the presence and identification of volatile and semi-volatile compounds through GC-MS (Gas chromatography-mass spectrometry) analysis as prescribed by Zothanpuia *et al*. (2017) using GCMS-QP-2010 Plus ultra-equipment (Shimadzu corporation) at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. The concentration of the plant sample solution used was 1mg/ml. Helium (with more than 99.99% purity) was used as a carrier gas. The plant sample solution (1mg/ml) of each extract was injected into the column with a flow rate of 1.21ml/min, a total flow of 16.3ml/min, a linear velocity of 40.5 cm/sec, a split ratio of 10.0, and purge flow 3.0ml/min. The ion source temperature was set at 220ºC whereas the interface temperature was 270ºC. Solvent cut time was kept at 4.50 minutes with relative detector gain mode. The injection temperature was 260ºC with split injection mode, and the pressure was 90.5kPa. Initially, the column oven temperature was 100ºC with a hold time of 3 minutes and gradually increased up to 300ºC at a rate of 10ºC/min, with a hold time of 17 minutes. Phytoconstituents were machine identified based on their relative retention time and peak area. The MS spectrum of the unknown constituents was compared with those of known constituents stored in the NIST (National Institute of Standards and Technology) database and compared with the available literature. The compound name, retention time, and the peak area percentage were ascertained.

Data analysis

The findings of each experiment were presented as the

mean ± standard deviations of three parallel trials. ANOVA (Analysis of Variance) was used, and the differences at P≤0.05 were deemed significant.

RESULTS AND DISCUSSION

Antimicrobial activity

The evaluation of antibacterial and antifungal activity of leaf and fruit extracts of *F. benghalensis* L. against MTCC cultures of bacteria (*Bacillus subtilis*, *Staphylococcus aureus, Salmonella typhi*, and *Escherichia coli*) and fungi (*Aspergillus niger, Fusarium oxysporum*, and *Rhizopus oryzae*) showed that the findings were resoundingly conclusive. Nearly all the tested solvents showed successful inhibition of tested microorganisms.

Antibacterial activity

The antibacterial activity in terms of the diameter of ZOI is represented in Table 1 and 2, whereas Table 4 demonstrates the MIC values of leaf and fruit extracts of *F. benghalensis* L. against bacterial strains. Methanol and acetone extracts showed the most promising activity, whereas aqueous and petroleum ether extracts failed to inhibit bacterial growth in a few cases. The gram positive *B. subtilis* and *S. aureus* were reported to be relatively more susceptible than the gram-negative *E. coli* and *S. typhi.* Methanol and aectone extracts showed a relatively wide range of activity that significantly affected all the bacterial strains. Leaf methanolic extract at 100mg/ml showed the highest diameter of ZOI (18.8 ± 1.2mm) against *B. subtilis* followed by the leaf acetone extract (17.6 ± .75mm) (Table 1 & 2). Against *S. aureus,* the highest ZOI (16.5 ± .90) was shown by leaf acetonic extract followed by leaf methanolic extract (14.6 ± .75). Similarly, for gram-negative bacteria *E. coli* and *S. typhi,* leaf methanolic and acetonic extracts exhibited maximum inhibitory activity (Table 1 and 2). A dose-dependent effect on the growth and multiplication of bacteria was observed. Gram-positive bacteria, i.e., *B. subtilis* and *S. aureus* were more strongly affected by the plant extracts than Gram-negative bacteria (*E. coli* and *S. typhi*). These results are in accordance with the outcomes obtained by Tkachenko *et al*. (2017) for *F. benghalensis* L. leaf extracts, where more promising antibacterial activity was obtained against Gram-positive bacteria *S. aureus* than the Gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa*.

The morphological distinctions between the Gram-positive and the Gram-negative bacteria (Gram-negative bacteria having an outer phospholipidic membrane bearing the structural lipopolysaccharide components) may cause the sensitivity variation between these microorganisms (Bland *et al*., 2001). Yet, despite these permeability discrepancies, many of the extracts have shown some degree of inhibition of Gram-negative microorganisms.

The present study's results agree with a study conducted by Uma *et al*. (2009) where methanolic extract of *F. benghalensis* L. bark exhibited the most efficient activity against *E. coli* compared to aqueous, hexane, chloroform, and petroleum ether extracts. However, the present investigation slightly deviated from the results of the study conducted by Gaherwal (2013), where petroleum ether and aqueous extracts of *F. benghalensis* L. fruit showed efficient activity against *Lactobacillus acidophilus, E.coli,* and *S. typhi* which is in contrast to the present investigation.

The efficacy of the plant extracts against bacterial strains was assessed by calculating the MIC (Table 4). A range of 1.56µg/µl to 50µg/µl of MIC values was observed. The lowest MIC of 1.56µg/µl was observed in the case of *B. subtilis* by methanolic leaf extract, which again suggested the high susceptibility of *B. subtilis*. Methanol and acetone extracts of leaf and fruit exhibited lowest MICs against all the bacterial strains. From these results, it can be concluded that *F. benghalensis* L. extracts, especially the methanol and the acetone extracts, could be explored for further studies to find natural antimicrobial compounds. Considering their antimicrobial potential, they may also be considered natural preservatives against various microorganisms causing food contamination.

Antifungal activity

The antifungal activity is shown in Tables 3 and 5. Table 3 shows the diameters of ZOI of different extracts, whereas Table 5 represents the MIC values. Two concentrations (100 and 50mg/ml) of extracts for disc diffusion assay were observed and compared with the positive control (standard drug) miconazole and the negative control DMSO. The methanolic and acetonic extracts exhibited significant activity compared to aqueous and petroleum ether extracts. At a concentration of 50mg/ml, negligible inhibition took place. Methanolic extracts showed high susceptibility for the fungal strains compared to the rest of the solvents. The highest ZOI (10.2 ± 1.3mm) was shown by methanolic extract of the leaf against *R. oryzae* followed by acetone leaf extract, which exhibited slightly lesser (10.0 ± .90 mm) ZOI against *A. niger*. Leaf aqueous extract exhibited 8.2 ± .90mm ZOI against *A. niger*. For the rest of the strains, aqueous extracts of leaf or fruit either showed ZOI below 6mm or no inhibition was observed. The studies conducted by Bhakuni *et al*. (1974) and Muniyan and Anandhan (2015) supported the less efficient activity of the aqueous extracts of certain medicinal plants against fungal pathogens. Further in the present study, petroleum ether (non-polar) extracts also showed negligible or no antifungal activity against any of the strains. These outcomes also agree with the studies conducted by Muniyan and Anandhan (2015), where

Table 1. Antimicrobial activity of leaf and fruit extracts of *Ficus benghalensis* L. against gram-positive bacteria

Extract	Diameter of Zone of Inhibition (mm)							
	Bacillus. subtilis				*Staphylococcus aureus*			
	100mg/ml	50mg/ml	25mg/ml	Positive Control	100mg/ml	50mg/ml	25mg/ml	Positive Control
$Leaf_{Aq}$	10.0 ± .90	7.2 ± .90	+/-	20.6 ± 1.2	11.4 ± .60	9.4 ± .55	7.4 ± .70	19.9 ± .90
$Leaf_{M}$	18.8 ± 1.2	12.4 ± .75	8.5 ± .9	21.0 ± .60	14.6 ± .75	9.0 ± 1.2	7.2 ± .90	18.5 ± .55
$Leaf_{Ac}$	17.6 ± .75	9.7 ± 1.2	7.5 ± .65	20.8 ± 1.2	16.5 ± .90	9.8 ± .75	6.6 ± .66	18.9 ± 1.2
$Leaf_{PE}$	8.6 ± .75	6.4 ± .70	+/-	19.6 ± 14	7.8 ± 1.2	+/-	-	19.2 ± .90
$Fruit_{Aq}$	9.8 ± 1.2	+/-	+/-	16.0 ± .70	8.2 ± .90	6.4 ± .77	+/-	16.8 ± .55
$Fruit_{M}$	13.2 ± 1.2	8.8 ± .80	6.2 ± 1.2	17.9 ± 1.4	14.30 ± 1.2	7.7 ± .90	6.2 ± 1.4	18.8 ± .90
$Fruit_{Ac}$	14.2 ± 1.4	9.8 ± 1.2	6.8 ± 1.4	18.9 ± .55	11.4 ± 1.2	8.6 ± .90	+/-	15.5 ± .75
$Fruit_{PE}$	8.4 ± .77	6.2 ± .90	-	16.0 ± 1.2	+/-	+/-	-	17.5 ± 1.2

Positive control: Chloremphenicol, - indicates No Inhibition, +/- indicates ZOI below 6mm, Aq: Aqueous, M: Methanol, Ac: Acetone, PE: Petroleum Ether

Table 2. Antimicrobial activity of leaf and fruit extracts of *Ficus benghalensis* L. against gram-negative bacteria

Extract	Diameter of Zone of Inhibition (mm)							
	Escherichia coli				*Salmonella typhi*			
	100mg/ml	50mg/ml	25mg/ml	Positive Control	100mg/ml	50mg/ml	25mg/ml	Positive Control
$Leaf_{Aq}$	10.0 ± .7	7.6 ± .90	+/-	18.6 ±.75	7.2 ± 1.2	6.2 ± .75	+/-	16.4 ± .65
$Leaf_{M}$	15.3 ± 1.3	10.5 ± .77	7.4 ± 1.4	16.5 ± 1.2	11.0 ± .75	8.5 ± .60	+/-	17.4 ± 1.2
$Leaf_{Ac}$	12.6 ± .90	8.4 ± .75	6.8 ± .66	19.2 ± .75	12.2 ± .60	7.5 ± .77	+/-	18.6 ± 1.2
$Leaf_{PE}$	7.4 ± .75	+/-	-	16.8 ± 1.4	+/-	+/-	-	18.5 ± .55
$Fruit_{Aq}$	7.8 ± .70	+/-	-	17.5 ± .90	6.6 ± 1.2	+/-	+/-	19.5 ± 1.2
$Fruit_{M}$	11.0 ± 1.2	7.7 ± .88	+/-	18.9 ± 1.4	8.8 ± .75	6.9 ± 1.4	+/-	19.2 ± .90
$Fruit_{Ac}$	9.3 ± 1.2	6.8 ± .90	+/-	18.7 ± .66	9.0 ± 1.2	6.2 ± .88	+/-	19.5 ± 1.4
$Fruit_{PE}$	-	-	-	16.8 ± .80	-	-	-	18.0 ± .90

Positive control: Chloremphenicol, - indicates No Inhibition, +/- indicates ZOI below 6mm, Aq: Aqueous, M: Methanol, Ac: Acetone, PE: Petroleum Ether

Table 3. Antimicrobial activity of leaf and fruit extracts of *Ficus benghalensis* L. against fungal strains

Extract	Diameter of Zone of Inhibition (mm)								
	Aspergillus niger			*Rhizopus oryzae*			*Fusarium oxysporum*		
	100mg/ml	50mg/ml	Positive Control	100 mg/ml	50 mg/ml	Positive-Control	100 mg/ml	50 mg/ml	Positive Control
$Leaf_{Aq}$	8.2 ± .90	+/-	16.6 ± .20	+/-	+/-	16.8±.75	+/-	+/-	18.4 ± .65
$Leaf_{M}$	9.2 ± .66	6.2 ± .75	17.2 ± 1.2	10.2± 1.3	7.4 ± .75	18.5±.60	9.4±1.2	+/-	17.4 ± 1.2
$Leaf_{Ac}$	10.0 ± .90	6.8 ± 1.4	19.2 ± .75	9.8±.77	-	19.4±.77	8.2±.90	+/-	16.6 ± .77
$Leaf_{PE}$	7.2 ± .77	+/-	18.8 ± 1.4	+/-	+/-	19.5±.60	+/-	-	17.2 ± .77
$Fruit_{Aq}$	-	-	17.5 ± .90	+/-	+/-	18.5±.60	+/-	+/-	17.5 ± 1.2
$Fruit_{M}$	8.0 ± 1.2	+/-	17.9 ± 1.4	+/-	+/-	18.4±1.4	+/-	+/-	18.2 ± .1.2
$Fruit_{Ac}$	8.2 ± 1.2	+/-	19.7 ± 1.2	6.2±.88	+/-	18.2±.88	7.0 ± 1.4	+/-	19.0 ± 1.4
$Fruit_{PE}$	-	-	18.6 ± .80	+/-	+/-	16.4±.77	+/-	+/-	18.0 ± 1.2

Positive control: Miconazole, - indicates No Inhibition, +/- indicates ZOI below 6mm, Aq: Aqueous, M: Methanol, Ac: Acetone, PE: Petroleum Ether

the non-polar extract (chloroform) of *F. benghalensis* L. leaf failed to inhibit growth of *A. niger*, *A. flavus* and *A. fumigates*. In contrast, methanol and acetone extract exhibited remarkable activity against all three. However, in the present study, the standard drug miconazole (positive control) exhibited prominent activity against all the strains, whereas DMSO (negative control) did not show any inhibition.

The MIC values ranged between 50µg/µl to 0.048µg/µl (Table 5). The least MIC among plant extracts was reported to be 12.5µg/µl, exhibited by methanolic leaf extract against *A. niger* and *F. oxysporum*, leaf acetonic extract against *A. niger* and *R. oryzae*, and leaf aqueous extract against *A. niger*. In the case of petroleum ether extracts, MIC either remained undetected or was reported to be highest, i.e. 50µg/µl. For fruit extracts, MIC did not vary much and oscillated between 25µg/µl to 50µg/µl. Miconazole possessed lowest MICs i.e., 0.78µg/µl to 0.048µg/µl. These outcomes suggested the high susceptibility of *A. niger* against plant extracts. The antimicrobial activity of these extracts is quite encouraging for the use of *F. benghalensis* L. as

an antimicrobial agent; however, none of the extracts could completely inhibit the fungal growth of any of the strains.

Two factors may have contributed to the antimicrobial effects of the methanolic extracts of *F. benghalensis* L.: (a) the increased extraction capacity of methanol may have created a significant number of active ingredients, which may be the cause of the antimicrobial activity, (b) the presence of physiologically active substances such as tannin, alkaloids, terpenoids, flavonoids, saponins, phenolic compounds, essential oils, etc. which are present in significant amounts is what gives these substances their antibacterial characteristics (Bhawana *et al*., 2018).

GC-MS Analysis

GC-MS is one of the most reliable techniques for the quantitative analysis of volatile and semi-volatile chemicals since it combines GC (separation approach) with MS (identification technique) (Grover and Patni, 2013). The methanolic extracts (since they exhibited the most efficient activity) were analyzed through GC-MS and the chromatographs are represented in Fig. 1 and Fig. 2; and Table 6 and 7 illustrating the compounds, their percentage peak area, and retention time. The components were identified by comparing the recorded spectra of the components with the mass spectra of the NIST library V11 provided by the instrument's software. The retention index was used in a similarity search with the GC/MS metabolomics database. The compounds discovered fall in terpenes, triterpenoids, saturated fatty acids, vitamins, tocopherols, sitosterols, stigmasterols, flavonoids, and polyphenols and their biological properties include antifungal, antibacterial, antioxidant, anticancer, insecticidal, antiinflammatory, anti-diabetic etc. Numerous GC-MS - identified compounds are utilized as medicines, flavors, propellants, antiseptics, disinfectants, and pesticides in the fragrance, food, pharmaceutical, beverage, perfume, and detergent sectors

Table 4. Minimum Inhibitory Concentration of leaf and fruit extracts of *Ficus benghalensis* L. and positive control against gram-positive and gram-negative bacteri**a**

Extracts/Positive control	***Bacillus subtilis* (µg/µl)**	***Staphylococcus aureus* (µg/µl)**	***Escherichia coli*(µg/µl)**	***Salmonella typhi* (µg/µl)**
$Leaf_{Aq}$	12.5	6.25	12.5	12.5
$Leaf_{M}$	1.56	3.12	3.12	6.25
$Leaf_{Ac}$	3.12	3.12	6.25	6.25
$Leaf_{PE}$	6.25	12.5	12.5	25
Positive control	0.097	0.097	0.024	0.048
$Fruit_{Aq}$	25	12.5	12.5	12.5
$Fruit_{M}$	3.12	6.25	3.12	6.25
$Fruit_{Ac}$	6.25	12.5	12.5	6.25
$Fruit_{PE}$	25	12.5	25	50
Positive control	0.78	0.024	0.024	0.048

Positive control: Chloremphenicol, Aq: Aqueous, M: Methanol, Ac: Acetone, PE: Petroleum Ether

Table 5. Minimum Inhibitory Concentration of leaf and fruit extracts of *Ficus benghalensis* L. and positive control against fungal strains

Extracts/ Positive control	***Aspergillus niger* (µg/µl)**	***Fusarium oxysporum* (µg/µl)**	***Rhizopus oryzae* (µg/µl)**
$Leaf_{Aq}$	12.5	25	25
$Leaf_{M}$	12.5	12.5	25
$Leaf_{Ac}$	12.5	50	12.5
$Leaf_{PE}$	ND	ND	ND
Control	0.19	0.19	0.048
$Fruit_{Aq}$	50	ND	ND
$Fruit_{M}$	25	25	50
$Fruit_{Ac}$	25	50	25
$Fruit_{PE}$	ND	ND	50
Positive control	0.78	0.78	0.39

Positive control: Miconazole, ND: Not detected, Aq: Aqueous, M: Methanol, Ac: Acetone, PE: Petroleum Ether

Table 6. Compounds identified in methanolic extract of *Ficus benghalensis* L. leaf by GC-MS

S. No.	RT (min)	Comp. Name	Comp. Formula	Peak area (%)
1.	10.43	Nonanoic acid	$C_9H_{18}O_2$	0.74
2.	11.98	Quinic acid	$C_7H_{12}O_6$	0.99
3.	13.35	Mintlactone/Menthalactone	$C_{10}H_{14}O_2$	0.23
4.	13.69	Neophytadiene	$C_{20}H_{38}$	1.99
5.	14.64	Octadecanoic acid, methyl ester	$C_{17}H_{35}CO_2H$	0.19
6.	15.08	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.32
7.	16.42	3,7,11,15-Tetramethyl-2-hexadecene-1-ol	$C_{20}H_{40}$	0.84
8.	16.79	(Z,Z)-6,9-Cis-3,4-epoxy-nonadecadiene	$C_{19}H_{34}O$	0.61
9.	19.72	Hexatriacontyl pentafluoro propionate	$C_{39}H_{73}F_5O_2$	0.19
10.	22.06	Squalene	$C_{30}H_{50}$	5.17
11.	22.64	Pentadecafluoro octanoic acid, undecyl ester	$C_{19}H_{23}F_{15}O_2$	0.12
12.	22.81	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexane	$C_{30}H_{50}O$	0.23
13.	22.83	Gamma tocopherol	$C_{28}H_{48}O_2$	0.75
14.	24.44	Vitamine-E	$C_{29}H_{50}O_2$	3.89
15.	25.63	Ursa-9(11),12-dien-3-one	$C_{30}H46O$	0.26
16.	25.76	Stigmasterol	$C_{29}H_{48}O$	0.45
17.	26.43	Stigmasta-5-en-3-ol	$C_{29}H_{50}O$	5.62
18.	26.64	Beta-amyron	$C_{30}H_{48}O$	16.21
19.	27.23	Lup-20(29)-en-3-ol	$C_{30}H_{48}O$	20.45
20.	27.44	9,19-Cyclolanost-24-en- 3-ol	$C_{30}H_{50}O$	1.50
21.	27.61	Lupeol	$C_{30}H_{50}O$	17.40
22.	27.93	24-Methylenecycloartan	C_31H_52O	1.80
23.	28.21	9,19-Cyclolanost-23-ene-3,25-diol	$C_{30}H_{50}O2$	2.55
24.	28.31	Oleanane-12-en-3-ol	$C_{32}H_{52}O_3$	12.7
25.	28.76	Methyl commate-B	$C_{31}H_{50}O_3$	4.58

RT: Retention Time, Comp.: Compound

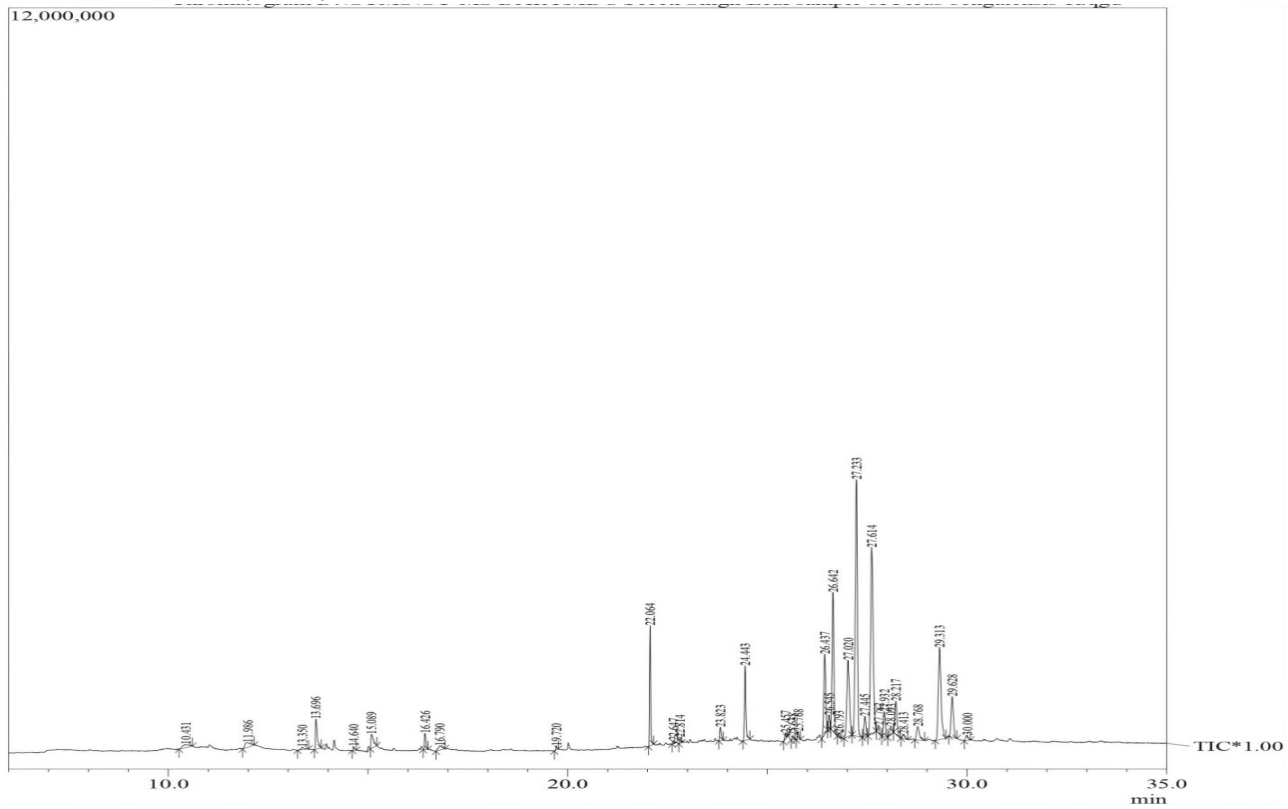

Fig. 1. *A typical GC-MS chromatogram of constituents of methanolic extract of Ficus benghalensis L. leaf*

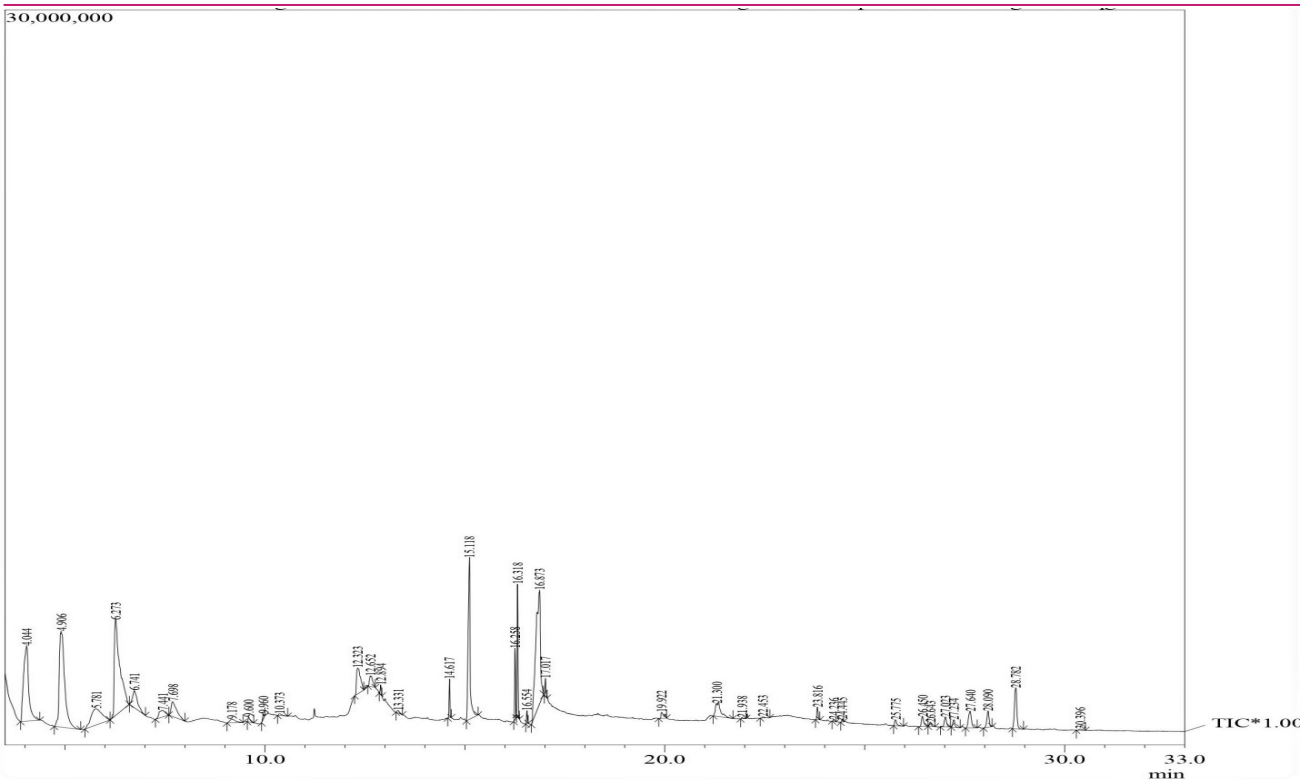

Fig. 2. *A typical GC-MS chromatogram of constituents of methanolic extract of Ficus benghalensi L. fruit*

(Swamy and Sinniah, 2015; Semwal and Painuli, 2019). These are likewise regarded as a crucial component of the plant's defensive mechanism and are known as phytoanticipins (Salehi *et al*., 2019).

Out of 22 peaks from the leaf extract Fig. 1, Table 6, the major compounds detected with higher percentage peak area are Lup-20(29)-en-3-one (20.45%), Lupeol (17.40%), Beta amyrone (16.21%), Squalene (5.17), Stigmasta-5-en-3-ol (5.62%), Vitamine-E (3.89%), n-Hexadecanoic acid (1.32%), etc. The rest of the compounds are present in comparatively lesser quantities. Lup-20(29)-en-3-one is a triterpenoid occurring naturally in different Ficus genera species. It exhibits antimicrobial, antiinflammatory, anti-tumor, anti-leishmanial, anti-protozoal, and chemopreventive activities as reported by Hata *et al*. (2002). Lupeol is also a triterpenoid that has been reported to possess antiinflammatory and anticancerous activities (Saleem, 2009). Many studies have reported the inhibition of tumorigenesis by lupeol, which interferes with the molecular growth signaling involved in cell death and proliferation (Saleem *et al*., 2008; Saleem *et al*., 2009). Many *in vitro* and *in vivo* studies have also proven the anti-mutagenic potential of lupeol (You *et al*., 2003; Lee *et al*., 2007; Lirawde *et al*., 2008). Vitamin-E possesses antioxidant, antimicrobial, and antiinflammatory activity (Archana *et al*., 2014). It also prevents platelet aggregation and lipid peroxidation, and improves the immunological response to a particular antigen (Radhakrishnan *et al*., 2014; Rizvi *et al*., 2014). Triterpenes are the phenolic substances present in some plants' latex and serve as secondary metabolites in the body's defense against pathogens that cause diseases in both humans and animals (Singh and Sharma, 2015). Squalene, a triterpene reported to have antitumor, antioxidant, hepatoprotective, gastroprotective, and many other pharmacological properties (Ryszard, 2009; Gomathi *et al*., 2015). Neophytadiene, another compound detected from *F. benghalensis* L. leaf extract is a good antipyretic, analgesic, antimicrobial, antiinflammatory, and antioxidant compound (Raman *et al*., 2012), whereas n-hexadecanoic acid is a well-known compound exhibiting strong antiinflammatory, antioxidant, hypocholesterolemic, pesticidal, nematicidal, hemolytic, 5-Alpha reductase inhibitory, potent mosquito larvicidal, and antimicrobial activity (Ryszard, 2009; Belakhdar *et al*., 2015; Swamy and Sinniah, 2015). Quinic acid is an astringent with antioxidant and anti-carcinogenic properties (Wang *et al*., 2009). 9, 12-Octadecadienoic acid, methyl ester and n-hexadecanoic acid are unsaturated fatty acids (also known as omega-6 fatty acids) that act as key elements to reduce blood cholesterol levels for normal cell growth and functioning (Igwe and Okwu, 2013) and in sustaining properly lubricated skin (Okwu and Morah, 2006). They possess bioactivities like antioxidant, antifungal, pesticide, nematicide, antimicrobial, hypocholesterolemic, hemolytic, anti-androgenic, 5-alpha reductase inhibitory, anticancerous, antiarthritic, antiinflammatory, etc. (Sudha and Mohan, 2013; Parimalakrishnan *et al*., 2015; Parthipan *et al*., 2015).

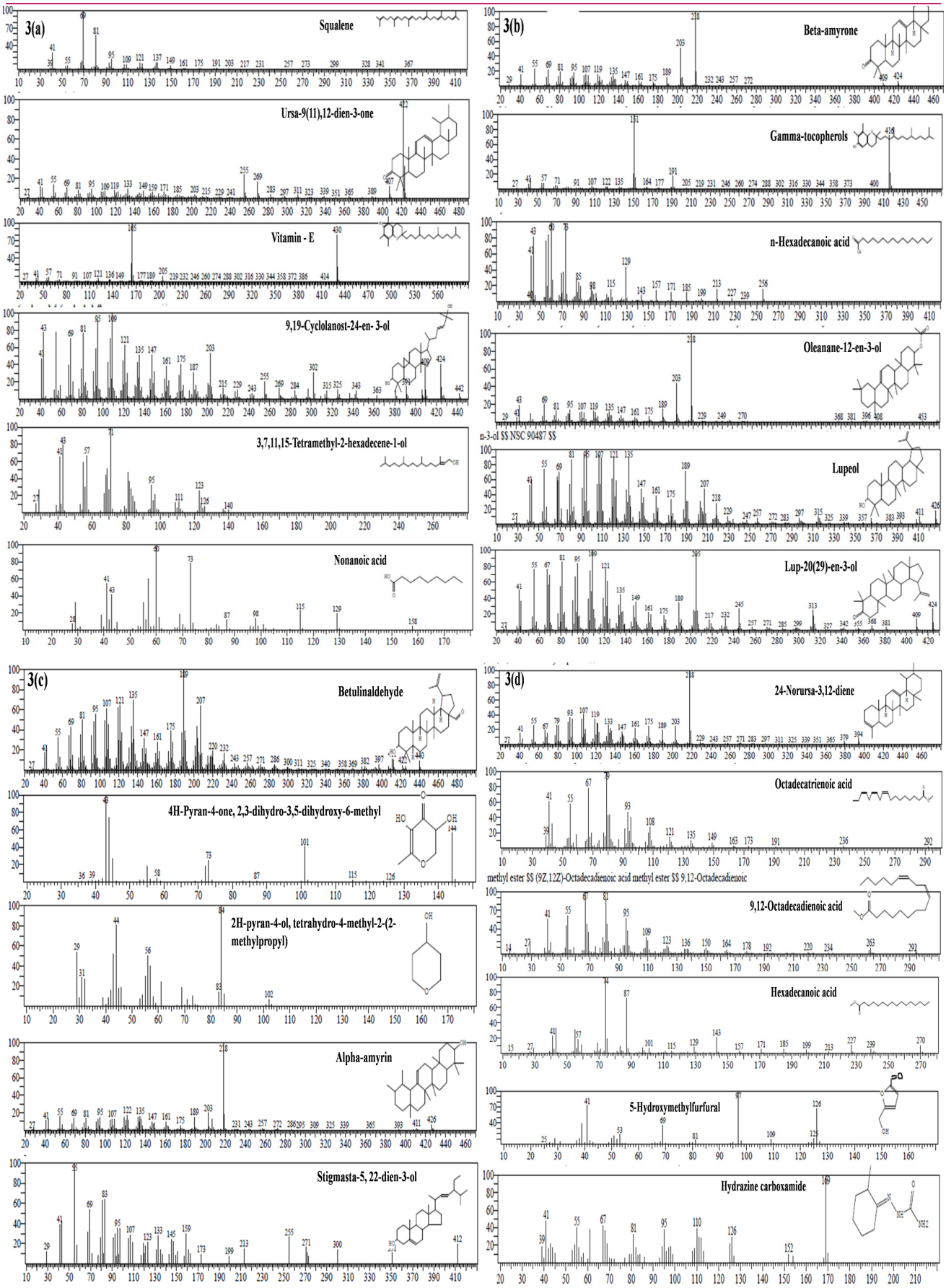

Fig. 3. *Individual fragmentation pattern of some of the important compounds detected in leaf (3a, b) and fruit (3c, d) extract of Ficus benghalensis L.*

Table 7. Compounds identified in methanolic extract of *Ficus benghalensis* L. fruit by GC-MS

S. No.	RT (min)	Comp. Name	Comp. Formula	Peak area (%)
1.	4.04	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	10.92
2.	4.90	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	14.89
3.	5.78	2H-pyran-4-ol, tetrahydro-4-methyl-2-(2-methylpropyl)	$C_{10}H_{20}O_2$	5.12
4.	6.27	5-Hydroxymethylfurfural	$C_6H_6O_3$	15.32
5.	6.74	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	2.42
6.	7.44	Butyl 2-acetoxyacetate	$C_8H_{14}O_4$	1.18
7.	7.69	4-pentanoic acid, 3-hydroxy-, ethyl ester	-	2.39
8.	12.32	Hydrazine carboxamide	CH_5N_3O	3.6
9.	15.11	n-Hexadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	9.35
10.	16.31	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	5.95
11.	16.87	Octadecatrienoic acid	$C_{17}H_{35}CO_2H$	15.24
12.	19.92	Hexadecanoic acid	$C_{16}H_{32}O_2$	2.48
13.	21.30	9,12-Octadecadienoic acid (z,z)-methyl ester	$C_{21}H_{38}O_4$	2.07
14.	23.81	Gamma.-Tocopherol	$C_{28}H_{48}O_2$	1.42
15.	25.77	Stigmasta-5, 22-dien-3-ol	$C_{29}H_{48}O_2$	0.37
16.	26.45	Gamma-Sitosterol	$C_{29}H_{52}O_2$	1.65
17.	27.02	Beta-amyrin	$C_{30}H_{50}O$	0.64
18.	27.23	Lup-20(29)-en-3-one (betulone)	$C_{30}H_{48}O$	0.43
19.	27.64	Alpha-amyrin	$C_{30}H_{50}O$	1.43
20.	28.09	Olean-12-en-3-ol	$C_{32}H_{52}O_3$	1.05
21.	28.78	24-Norursa-3,12-diene	$C_{29}H_{46}$	2.79
22.	30.39	Betulinaldehyde	$C_{35}H_{60}O_7$	1.10

RT: Retention Time, Comp.: Compound

Gamma tocopherols prevent cell and DNA damage and have anticancerous (Shin, 2017), antiinflammatory, and wound healing (Bharathkumar *et al*., 2011) activity. Stigmasterol is a steroidal compound exhibiting anti-osteoarthritic potential, probably due to its aqueous solubility and functional group property (Gabay *et al*., 2010). Other compounds detected in leaf extract are nonanoic acid, beta-amyron, 19-Cyclolanost-24-en-3-ol, Oleanane-12-en-3-ol, 24-methylene cycloartan-3-one, methyl commate B, Stigmasta-5-en-3-ol, (Z,Z)-6,9-Cis-3,4-epoxy-nonadecadiene, Hexatriacontyl pentafluoropropionate, Ursa-9(11),12-dien-3-one, etc. Nonanoic acid possesses antimicrobial and antifungal activities (Sahin *et al*., 2006; Jang *et al*., 2012). Stigmasta-5-en-3-ol (3-beta) exhibit antimicrobial activity (Achika *et al*., 2016) whereas Ursa-9(11),12-dien-3-one has antioxidant and antiinflammatory activities (Sushma *et al*., 2017). Beta-Amyrone is reported to have activities like antifungal, antiinflammatory, anti-α-glucosidase inhibitory, etc. It has also been reported to inhibit the formation of ear edema in a dose-related manner in an *in-vivo* study (Bourjot *et al*., 2012; Patrícia *et al*., 2015). 19-Cyclolanost-24-en-3-ol, also called cycloartenol, is antibacterial, promotes cell regeneration, and inhibits oxidation (Matsuda *et al*., 2000). 24-methylene cycloartenol-3-one has anti-HIV activity (Verotta *et al*., 1998). Compounds like n-Hexadecanoic acid, gamma-tocopherol, Olean-12-en-3-ol, and Lup-20(29)-en-3-one are commonly found in leaf and fruit extracts.

Beta-amyrin and alpha-amyrin, detected in fruit extract; Fig. 2, Table 7, exhibit antiinflammatory, analgesic, gastroprotective, anticonvulsant, anti-depressive, anti-pancreatitis, antihyperglycemic, hepatoprotective, anti-arthritic, anticancer, hypolipidemic, and anticholytic activity (Okoye *et al*., 2014; Ghosh *et al*., 2015). 5-Hydroxymethylfurfural, a major compound exclusive to fruit exhibits effects against hypoxic injury (Li *et al*., 2011). When foods containing amino acids and reducing sugars are heated in an acidic environment, hexoses degrade and the Maillard reaction occurs, producing 5- Hydroxymethylfurfural, which is extensively present in food. It has also been reported to have antioxidant, and antiproliferative effects on human cell lines, including melanoma A375 cells (Zhao *et al*., 2013). Betulinaldehyde has anti-inflammatory, anti-malarial, and antifungal properties (Alakurtti *et al*.,

2006). Another compound present in fruit extract was a flavonoid, 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, which also forms during Maillard reaction possess strong antioxidant and antifungal activity (Teoh *et al*., 2015; Chen *et al*., 2021). Fig. 3 demonstrates the individual fragmentation pattern of some of the important compounds detected in leaf (Fig. 3a, b) and fruit (3c, d) extracts.

Thus, the present study indicates the health-beneficial potential of *F. benghalensis* L., however, further studies are required to determine the toxicological profile, biological activity, and isolation of the specific phytochemical elements in pure form.

Conclusion

The present study concluded that antimicrobial activity and the therapeutic use of *F. benghalensis* L. were significantly aided and supported by diverse bioactive components possessing a range of biological activities. These findings unequivocally back up the usage of *F. benghalensis* L. in conventional medical procedures to treat a range of illnesses. Thus, it is conclusive that this plant has the potential to become a natural and affordable source of a variety of therapeutically valuable metabolites. The research found that the leaf and fruit methanolic extracts contained significant bioactive components that form the foundation for additional biological and pharmacological research on the plant's potential health benefits in future.

Conflict of interest

The authors declare that they have no conflict of interest.

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