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CHARACTERIZATION AND SCREENING OF BIOACTIVE COMPOUNDS IN THE EXTRACT PREPARED FROM AERIAL ROOTS OF *FICUS BENGHALENSIS*

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ABSTRACT: *Ficus benghalensis* is has been used in traditional medicinal practices such as Ayurveda, Unani, Siddha and homoeopathy. All parts of the tree such as fruits, aerial roots, bark and leaves are known to be traditionally associated with diarrhea, dysentery, menorrhagia, nervous disorders and tonic properties. However, in Ayurveda it is mainly known for the diabetes treatment.There are no reports giving a detailed profiling of phytochemicals present in the aerial roots of *F. benghalensis*. The study aims to evaluate its bioactive compounds and comprehend its biological activities with traditional knowledge.Methanol and ethanol extracts from the aerial roots healthy tree of *F. benghalensis* were prepared and their antibacterial activity against various pathogenic gram's positive and gram's negative bacteria were evaluated using disc diffusion assay. DPPH & FRAP bioassays were performed to establish its traditional use as antioxidants. Total phenol and total flavonoids were estimated to correlate its antioxidant potential. The chemical composition of methanol extract was accomplished by GC-MS and bioactivity of these compounds was determined. Lupenyl acetate (35.4 %), α-Amyrenyl acetate (16.34%), γ-Sitosterol (5.45%), Palmitic acid (5.17%) and Lupeol (4.44%) were found to dominate the phytochemical composition. These compounds are known to exhibit various important pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, anti-diabetic and anti-cancerous etc. Various phytochemicals in aerial root can be screened to evaluate its potential role in treatment of various diseases

INTRODUCTION: Secondary metabolites like alkaloids, polyphenols, flavonoids, glycosides, terpenes and few other pigments present in plants provide them protection from disease, stressful environment and help in maintaining the their health status. Active constitutes present in the plants are also known to be important for animals as well.

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These help in to improve digestive, nervous, respiratory, excretory, circulatory and immune system of humans as well as other animals [1, 2]. Active constituents and their quantity present in plants vary in different parts. Majority of present drugs are derived from ancient herbal therapeutics for animals and human. According to WHO it is estimated that around 80% population of Asian and African countries rely on use of herbal therapies because of their effectiveness, low cost and rare side effects compared to synthetic chemicals. So it is important to discover plants which are a rich source of these active components exhibiting various beneficial activities so that can be exploited for further treatments through research processes.

Since long time, plants have been used for antibacterial, antiviral, antifungal, antihelminthic, antioxidant, antiprotozoal, wound healing, anti-inflammatory, anti-tumor, anti-cancerous and various organ specific benefits. The plants have the potential to enhance both humoral and cell mediated immunity because of metabolites like alkaloids, flavonoids, glycosides, polyphenols, terpenes, tannins etc. [1, 3, 4]. Ayurveda, Unani and other local health practices promote the use of herbal medicines. Use of plants, algae, yeast, bacteria, lichens as well as many freshwater and marine water flora and fauna has increased worldwide because of their health benefits.

India is very close to the nations with highest forest cover (26.19%), having a total forest area of about 23.7%. More than 12% of flowering plants were reported having medicinal importance worldwide while in India it was recorded above 43% out of total flowering plants [5- 7]. *Ficus benghalensis* is a member of Moraceae family, having seasonal fruity figs, propagating aerial roots, big trunk with a thick bark and leaves and is found mainly in tropical and subtropical regions of Asia. Tree can grow upto 30m, contains latex, rubber producing laticifer and milky sap [8]. Numbers of plants from same genus are considered sacred by many religions in Southeast Asia and also exhibit ecological and remedial pharmacological importance.

Various plant parts and their extracts have different medicinal properties. Bark, leaves and fruits of *F. benghalensis* has been used because of their anti-bacterial, anti-oxidant, anti-inflammatory, anticancer, anti-diabetic properties. It is also used in the treatment dysentery, diarrhoea, asthama, ulcers or other skin diseases [9, 10]. Plant sap from aerial root and leaf bud is known to be useful in treatment of haemorrhages and bleeding piles[11]. Leaves of healthy plant are known to promote conception, act as a natural blood purifier and keep a check on diarrhoea. Studies suggests anti-inflammatory, anti-diabetic, hypolipidaemia, anti-helminthic, anti-allergic, wound healing and anti-stress properties of bark extracts by ethanolic, methanolic, aqueous and petroleum extraction. Aerial roots of *F. benghalensis* are also explored as therapeutic use because of its immunomodulatory activity. In rural India aerial roots of *F. benghalensis* has been used to boost immune system and various other medicinal purposes [12, 13]. Aerial root extract (97% Chloroform: 3% Methanol) was also used for increasing hair growth and decreasing hair loss [14]. The chemical components present in aerial root of *F. benghalensis* are still unknown. In the progression of screening of active compounds including secondary metabolites we also described the antibacterial, antioxidant and cytotoxic activity of ethanol and methanol extracts of aerial roots of *F. benghalensis*. The metabolites in the methanol extract were also screened and identified through GC-MS analysis.

MATERIALS AND METHODS:

Plant material and extraction:

Healthy aerial roots (prop roots) of *F. benghalensis* were collected from campus of University of Delhi (New Delhi, India), washed with water and allowed to dry in shade at RT. Dried roots were ground and passed through 1mm sieve. 10 grams of sieved powder of *F. benghalensis* was added to 50 ml of methanol and ethanol respectively and stirred overnight on Magnetic stirrer at 25°C. Slurry was filtered through Whatman® No. 41 filter paper. Filtrate was dried using rotary evaporator at 40°C and stored at -20°C until use.

Antimicrobial assays:

Selection and culture of microorganisms: Bacterial isolates were inoculated in the 250 ml conical flasks containing 50 ml Luria-Bertani culture media (pH 7.4) and 1% or 2% NaCl concentration for fresh water (*Aeromonas hydrophila, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) and marine bacteria (*Vibrio anguillarium* and *Vibrio harveyi*) respectively. The inoculated bacterial flasks were allowed to grow overnight at 37°C under gentle shaking conditions.

Determination of antibacterial activity: Antibacterial activity of ethanol and methanol extracts were determined by Disc Diffusion Assay (DDA) against above mentioned bacterial strains. Sterilized LB agar plates (1.5%) were prepared with 1% or 2% NaCl (for freshwater and marine water bacteria respectively) and respective bacteria were seeded with a standard inoculum of $1x10^8$

cells. Sterile circular paper discs (thickness 1mm; diameter 6mm) were placed on agar plate and were impregnated with 100µl plant extract prepared at six different concentrations (200, 100, 50, 25, 12.5 and 6.25µg per disc) in 0.2% DMSO. DMSO (0.2%) was used as a negative control while Ampicillin (Broad spectrum antibiotic) was taken as positive control.

Antioxidant assays:

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay: Antioxidant property of *F. benghalensis* aerial root extract was determined using Brand-Williams et al. (1995)method modified by Miliauskas et al. (2004) [15, 16]. 10µl of freshly prepared methanol sample (0.5mg/ml) was added to 300µl of DPPH solution (6×10^{-5} M in methanol) in a 96 well microtiter plate. Mixture was incubated at 37°C for 20 min. and absorbance was recorded at 515nm. For control 10µl of methanol was added to 300µl DPPH solution. Free Radical scavenging property of plant extract was calculated as percentage inhibition using standard formula $[(A_B-A_S)/A_B] \times 100$; where A_B was absorbance of blank and A_S was absorbance of sample [17, 18]. The assay was performed similarly for the ethanol extract, with ethanol as carrier blank control. Serial double dilution of ascorbic acid was used as a positive standard (20 to 0.078 mg/ml). Samples were processed in quadruplicates in this assay.

FRAP (Ferric Reducing Ability of Plasma) assay: 10µl of respective plant extract solution (0.5mg/ml) was mixed with 30µl of distilled water and 300µl of freshly prepared FRAP solution [containing 10 parts of 300mM acetate buffer (pH 3.6), 1 part of 10mM TPTZ (2,4,6-tripyridyl triazine) in 40mMHCl and 1 part of 20mM ferric chloride]. Samples were incubated at 37°C for 30 min. A standard curve was prepared by serial double dilution of ferrous sulphate (20.0 to 0.009 mg/ml) as substrate. Absorbance was recorded at 593nm. For control acetate buffer was used in placing of sample.

Determination of total phenolic content (TPC):

Phenolic content in methanol and ethanol extracts of *F. benghalensis* were determined according to Djeridane et al. (2006) [19]. One ml of extract containing 2 mg extract was dissolved in 1.5 ml distilled water and 0.5 ml Folin-Ciocalteu's Phenol reagent. Sodium carbonate solution (20%) was added after 1 min and mixture was incubated for 2 hours in dark at 25°C with intermittent shaking. Absorbance was recorded at 760nm and a standard curve was obtained using serial double dilutions of gallic acid (20-0.5µg/ml) as standard [18].

Estimation of total flavonoids:

Flavonoids contents of methanol and ethanol extracts of aerial roots from *F. beghalensis* were estimated using Dowd method as modified by Arvouet-Grand et al. (1994) [20]. 1 ml of 2% aluminium tri-chloride solution (prepared in methanol) was mixed with 1 ml of plant extract. After 10 min of incubation at RT, absorbance was recorded at 415 nm. For control (carrier blanks), 1ml sample was replaced by methanol/ethanol solution [18]. Concentration of flavonoids in extracts was calculated using serial double dilution of quercetin(8.33-0.032 mg/ml) as standard.

Gas Chromatography Mass Spectrophotometry (GC-MS) analysis:

Sample preparation for GC-MS: *F. benghalensis* powder was soaked in methanol and stirred on magnetic stirrer at RT for 24 hours. Filtrate was obtained from the slurry and lyophilized. 1 mg of lyophilized extract of plant was dissolved in 1 ml HPLC grade methanol for quality results. The samples were filtered through 0.22 µm syringe filter. One microliter sample was loaded by automatic programmed syringe injector for GC-MS analysis.

Chromatographic conditions used in GC-MS: GC-MS analysis was carried out on a thermal desorption TD-20 system, GCMSQP-2010 Plus (Shimadzu, Japan) comprising of auto-sampler. The gas chromatograph was interfaced from a mass spectrometer instrument employed with RTx-5MS column (30m X 0.25mm X 0.25µm) operating in electron impact mode at 70eV. Helium gas (99.99%) was used as the carrier gas in instrument with a steady flow rate of 1.2 ml/min. The column's initial oven temperature was 80°C (isothermal for 4 min.) with gradual increase of 5°C/min to 310°C, flow rate 1.21 ml/min and column pressure of 81.7kPa. A Mass spectrum was

prepared at a scan interval of 0.50 sec with a mass scan from 40 m/z to 650 m/z.

Identification of compounds: For interpretation of data obtained from GC/MS, NIST/NIH/EPA Mass spectral Database with NIST05 (National Institute of Standards and Technology) MS programme v.2.0d and WILEY08 libraries were used. Unknown components were also identified with the help of spectrum in NIST and Wiley libraries according to their retention time. The names, chemical formulas, molecular mass and structure of components of identified compounds were determined. Chemical and biological activity of identified compounds were found using Dr. Duke's phytochemical and ethanobotanical databases, NCBI-Pubchem, ChemSpider from Royal Society of Chemistry and various literatures.

Statistical analysis:
Minimum required sample size was maintained throughout the study. Student's t-test and ANOVA were employed to analyze the data for significance by using Sigma Plot 12.0 software. The p values less than 0.05 were considered as significant.

RESULTS:

Antimicrobial assays:
Table 1 represents the zone of inhibition after overnight growth on agar plate. Crude ethanol and methanol extracts of aerial roots of *F. benghalensis* showed antimicrobial activity at 200, 100, 50, 25, 12.5µg/disc against *A. hydrophila*, *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *V. anguillarium* and *V. harveyi*.

Extracts are more sensitive against gram-negative *V. anguillarum*, but lower concentration of ethanol extract (< 25 µg/disc) did not inhibit the growth of gram-negative *E. coli* and gram-positive *E. faecalis*. The methanol extract failed completely to impede the growth of gram-negative bacteria *P.aeruginosa* and *V. harveyi*.

TABLE 1: ANTIBACTERIAL ACTIVITY OF METHANOL AND ETHANOL EXTRACTS OF *F. BENGHALENSIS* (AERIAL-ROOT) AGAINST GRAM POSITIVE (*E. FAECALIS* AND *S. AUREUS*) AND GRAM NEGATIVE (*A. HYDROPHILA, E. COLI, P. AERUGINOSA, V. ANGUILLARUM* AND *V. HARVEYI*) BACTERIA BY DISC DIFFUSION ASSAY.

Bacterial strain	200 µg/disc		100 µg/disc		50 µg/disc		25 µg/disc		12.5 µg/disc	
	Diameter of zone of Inhibition (in mm)									
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Methanol extract										
A. hydrophila	18.40^{a}	0.21	13.57^{b}	0.23	11.33^{c}	0.17	10.5^{d}	0.17	8.5^{e}	0.17
E. coli	14.67^{a}	0.44	11.07^{b}	0.3	10.17^{b}	0.37	8.33^{c}	0.17	7.6^{c}	0.3
E. faecalis	21.10^{a}	0.45	17.97^{b}	0.39	13.77^{c}	0.43	11.87^{d}	0.24	10.3^{e}	0.29
P. aeruginosa	15.33^{a}	0.24	11.83^{b}	0.2	9.83^{c}	0.2	8.77^{d}	0.43	-	-
S. aureus	15.33^{a}	0.64	12.90^{b}	0.21	11.83^{b}	0.2	10.43^{c}	0.23	8.4^{d}	0.59
V. anguillarum	21.90^{a}	0.21	19.20^{b}	0.5	16.9^{c}	0.38	12.3^{d}	0.25	11.03^{e}	0.39
V. harveyi	12.17^{a}	0.2	10.37^{b}	0.29	9.10^{c}	0.21	7.77^{d}	0.38	-	-
Ethanol extract										
A. hydrophila	17.77^{a}	0.38	12.70^{b}	0.1	10.63^{c}	0.28	9.83^{d}	0.2	8.37^{e}	0.3
E. coli	13.30^{a}	0.4	10.90^{b}	0.38	9.50^{c}	0.17	7.43^{d}	0.23	-	-
E. faecalis	18.10^{a}	0.32	14.77^{b}	0.26	11.00^{c}	0.42	8.10^{d}	0.26	-	-
P. aeruginosa	14.97^{a}	0.48	14.30^{a}	0.26	12.73^{b}	0.35	10.73^{c}	0.29	9.43^{d}	0.23
S. aureus	18.87^{a}	0.2	17.07^{b}	0.18	15.83^{c}	0.2	13.47^{d}	0.13	11.83^{e}	0.2
V. anguillarum	20.30^{a}	0.15	17.90^{b}	0.17	16.63^{c}	0.3	14.27^{d}	0.41	12.00^{e}	0.29
V. harveyi	12.83^{a}	0.2	11.73^{b}	0.12	10.47^{c}	0.09	9.60^{d}	0.2	8.47^{e}	0.26

Values are represented as Mean ± SEM. ANOVA employing Newman-Keuls' multiple range test was performed to calculate p value. P values less than 0.05 were only considered significant. Dissimilar superscripts $^{(a\text{-}e)}$ on the mean values represent that they are statistically different.

Antioxidant Assays: The antioxidant activity of plant extracts were calculated through DPPH and FRAP assays by the measure of reducing activity through electron donation, hydrogen donating or

radical scavenging ability. The discoloration of violet coloured DPPH free radical to non-radical solution when mixed with plant extracts indicated reducing potential, expressed in percentage inhibition of free radicals. Methanol extract of *F. benghalensis* aerial-root showed higher free radical scavenging ability in comparison to ethanol extract as well as to naturally occurring mild reducing and standard antioxidant, ascorbic acid (Vitamin C). Reducing ability of extracts by FRAP assay were correlated with the ferric reducing antioxidant potential which were expressed in millimoles of Fe^{2+}/g of the plant extract ($FeSO_4$ was used as standard). The methanol extract had the higher reducing potential than ethanol extract but less than the purified positive control antioxidants, gallic acid (**Table 2**).

TABLE 2: ANTIOXIDANT POTENTIAL OF METHANOL AND ETHANOL EXTRACTS OF *F. BENGHALENSIS* AERIAL-ROOT THROUGH DPPH AND FRAP ASSAYS.

	DPPH Assay (% scavenging activity)	FRAP Assay (mM Fe^{2+}/mg extract)
	Mean ± SEM	Mean± SEM
Ethanol Extract	38.66 ± 2.44[a]	261.24 ± 10.77[a]
Methanol Extract	80.14 ± 0.56[b]	982.93 ± 61.17[b]
Ascorbic acid	77.44 ± 2.01	- -
Gallic Acid	- -	2907.48 ± 299.71

Values are represented as Mean ± SEM. Antioxidant potential of methanol and ethanol extracts were compared by calculating p values using t-test and p values less than 0.05 were considered significant. Dissimilar superscripts [(a-b)] on the mean values represent that they are statistically different. Ascorbic acid and gallic acids were used as positive controls for DPPH and FRAP assays respectively.

Total phenolic content:

Total phenolic content (TPC) was measured by Folin-Ciocalteu reagent, showed the presence of large amounts of phenolic compounds. However phenolic content was significantly lower in ethanol extract compared to methanol extract (**Table 3**).

TABLE 3: TOTAL PHENOLIC AND FLAVONOIDCOMPOUNDS IN µg EQUIVALENT TO GALLIC ACID AND QUERCETIN RESPECTIVELY PRESENT PER MG IN METHANOL AND ETHANOL EXTRACTS OF *F. BENGHALENSIS* AERIAL-ROOT.

	Total phenolics (µg GAEs / mg extract)		Total flavanoids (µg QEs / mg extract)	
	Mean	SEM	Mean	SEM
Ethanol extract	22.41[a]	0.47	6.14[a]	0.41
Methanol extract	55.35[b]	3.95	6.11[a]	0.34

Values are represented as Mean ± SEM. p values was calculated using t-test and p values less than 0.05 were considered significant. Dissimilar superscripts [(a-b)] on the mean values represent that they are statistically different. GAEs and QEs denote gallic acid equivalent and quercetin equivalent respectively.

Total flavonoid content:

The ethanol and methanol extracts of *F. benghalensis* aerial root showed proportionate amounts of flavonoids. Flavonoid content was calculated with the help of quercetin as a standard so the flavonoid content was expressed in milligram of quercetin equivalent flavonoids present per gram of the extract (**Table 3**).

GC-MS analysis of methanol extract of *F. benghalensis* (aerial root):

GC-MS analysis of methanol extract of aerial root of *F. benghalensis*, resolved in 33 major peaks (**Fig. 1**, Chromatogram) containing different secondary metabolites as well as some other important chemicals with known valuable properties (**Table 4**). **Fig.2** represents the proportion of various phytochemicals in the extract and structures of some of the major components in the methanol extract of *F. benghalensis*(aerial root) are depicted in **Fig. 3**.

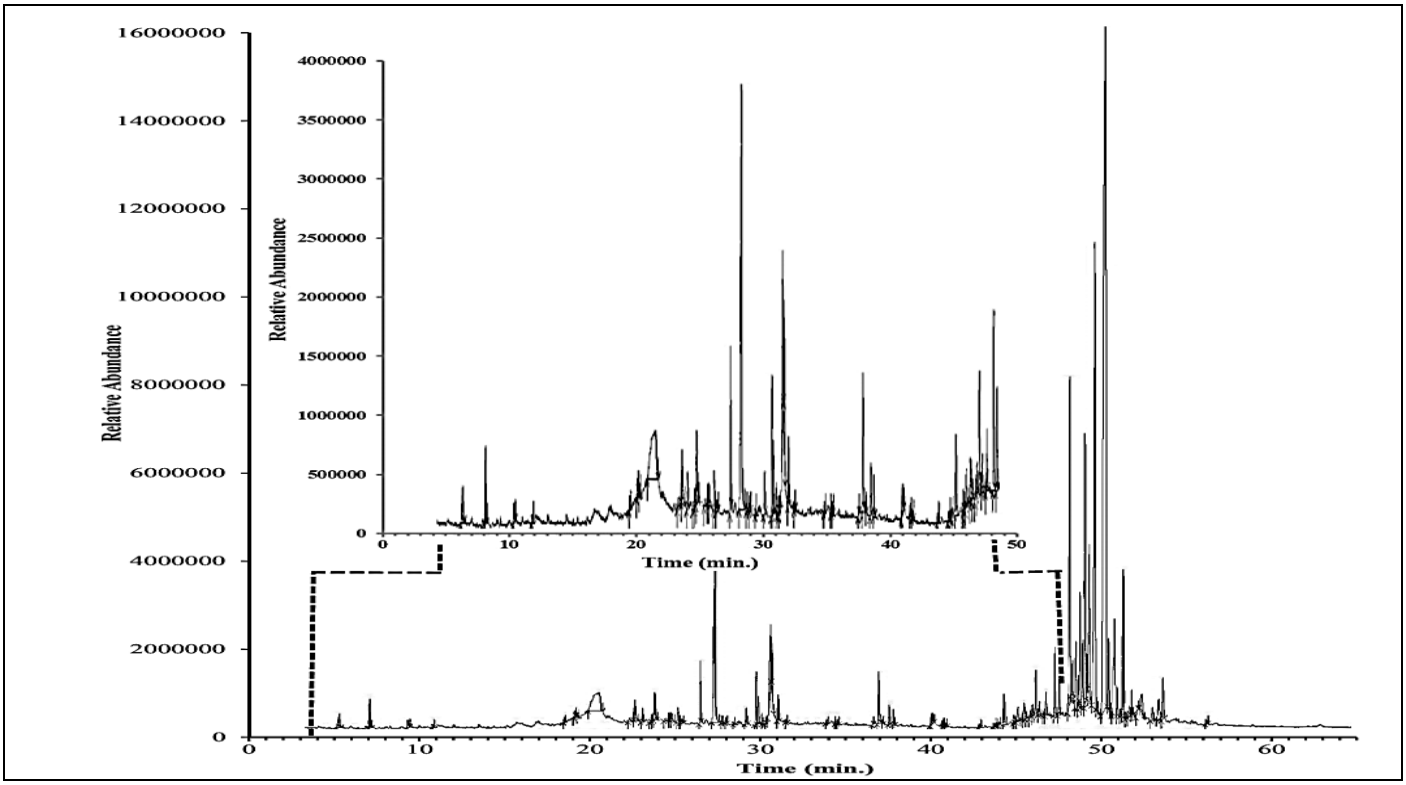

FIG. 1: GC CHROMATOGRAM OF METHANOL EXTRACT OF *F. BENGHALENSIS* (AERIAL ROOT).

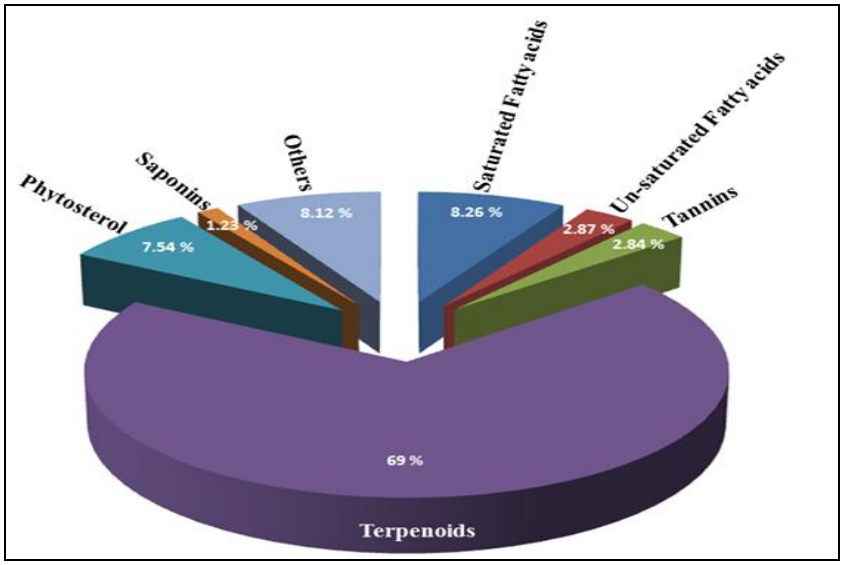

FIG.2: PROPORTION OF VARIOUS PHYTOCHEMICALS IN THE METHANOL EXTRACT OF *F. BENGHALENSIS* BASED ON GC MS ANALYSIS.

DISCUSSION: In this investigation we highlighted biological medicinal important activities of methanol and ethanol extracts of aerial roots from a remarkable sacred tree, *F. benghalensis*. These extracts showed antibacterial activity against gram positive as well as gram negative pathogenic bacteria, which primarily affect aquatic animals and may cause severe infections to human beings. Earlier investigators have reported antibacterial activity of different plant parts of *F. benghalensis* such as bark, fruits and leaves against different bacteria [109-111]. Govindarajan (2010) suggested larvicidal activity of acetone, benzene and methanol leaves from *F. benghalensis* against *Culexquinque fasciatus*, *Aedesaegypti* and *Anopheles Stephens* [112]. Simonsen et al. in 2001 reported antimalarial activity of bark of the tree against *Plasmodium falciparum* [111].

Presence of antioxidants in food is considered to be an important factor, which not only prevents various components such as polyunsaturated fatty acids from oxidation but also protects it from rapid deteriorative changes during storage and processing. Oxidation of food components can be responsible for causing early ageing or carcinogenesis [113], while synthetic antioxidants like butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) have adverse effect on health status of human beings [114]. Hence to investigate the potential antioxidants in food sources is of great relevance to food processing industries and human beings.

The DPPH radical scavenging activity can be ascertained on the basis of formation of non-radicals of DPPH i.e. DPPH-H, in the presence of hydrogen donating antioxidants [115]. The discolouration of violet colour of DPPH was predominantly more in methanol extract compared to ethanol extract of *F. benghalensis*. The naturally available antioxidant, ascorbic acid was used as standard which showed non-significant difference with the methanol extract. Methanol extract showed higher antioxidant activity when calculated through FRAP assay with respect to standard gallic acid.

The perusal of literature reveals that the antioxidant potential of plant extracts depends on secondary metabolites including phenols, flavonoids, tannins, terpenes and other important components. Polyphenols are known to play an important role in the inhibition of reactive oxygen species (ROS), inhibit lipid oxidation [116, 117]and prevent organisms from mutations which may cause cancer and other lethal diseases [118, 119]. Phenolic compounds are invariably the most extensively studied secondary metabolites of plants and are prevalent in all parts of the plants. Flavanoids, a kind of natural phenolic compounds exhibit various important biological activities including anti-bacterial, anti-viral, anti-oxidation, anti-inflammatory, anti-cancer and cardio-protective etc. [120]. **Table 3** indicates the presence of total phenolic compounds as well as total flavonoids present in the extract. The amount of total flavonoids are equivalent in both extracts while total phenolic compounds present in the methanol extract are about two and a half times higher than in ethanol extract of *F. benghalensis*. Higher amounts of total phenolic components present in methanol extract may be responsible for its higher antioxidant activity. Various researchers have emphatically illustrated beneficial biological activities when extracts from different plants and their parts are prepare in methanol as a solvent compared to other solvents like hexane, benzene, ethyl acetate, ethanol or aqueous [4, 121].

Terpenes or terpenoids are by far the largest class of secondary metabolites present in all plants which can be used to synthesize different terpenes at different sites of plant for a variety of purposes. A few terpenes are known to be poisonous to human beings however they provide protection to the plants against insects and other herbivores. Through GC-MS analysis of aerial roots from *F. benghalensis* we report in this manuscript various terpenes mainly tri-terpenes. Tri-terpenes are known to provide protection against colon cancer, breast cancer, oral mucosa cancer and human T-cell leukemia [78, 80, 81]. Besides this it also exhibit analgesic, antioxidant, anti-inflammatory, antibacterial and hepatoprotective properties [78, 83, 92, 93, 97]. The benghalensin is the only important component reported from the *F. benghalensis* stem latex, besides there is no report describing different components present in their aerial root. Vipin *et al* supported the immuno-stimulatory biological activities of aerial roots of *F. benghalensis* in two different representatives of fish, i.e., bony fish, *Channa punctata* and catfish, *Clariasgariepinus* [10, 122].

GC-MS data showed numerous compounds that exhibit various biological activities which may be useful in the prevention and cure of different diseases as well as for health improvement of humans and other animals. The aerial root of methanol extract of *F. benghalensis* showed the presence of various useful saturated/poly-unsaturated fatty acids, terpenes, phytosterols, polyphenols, other secondary metabolites and chemotypes with potential biological activities (**Table 4**). GC-MS analysis on methanol extract of *F. benghalensis* represents the presence of components like quinic acid, Palmitic acid, methyl ester, ergosterol acetate and α-amyrenyl acetate which exhibit antioxidant activities. It shows

majority of compounds exhibit potential anti-inflammatory functions/anti-cancer activity. Lupenyl acetate and α-amyrenyl acetate were found to be present in the highest amounts (representing 35.4% and 16.34% respectively) in methanol extract of *F. benghalensis*. The anti-malarial, anti-bacterial, anti-fungal, anti-oxidant, anti-ulcer, anti-hyperglycemic and anti-cancer activity of these two compounds reflects that *F. benghalensis* has valuable medicinal importance. **Fig.3** highlight the structures of few of the biologically active compounds which are present in high amounts in the methanol extract of *F. benghalensis*.

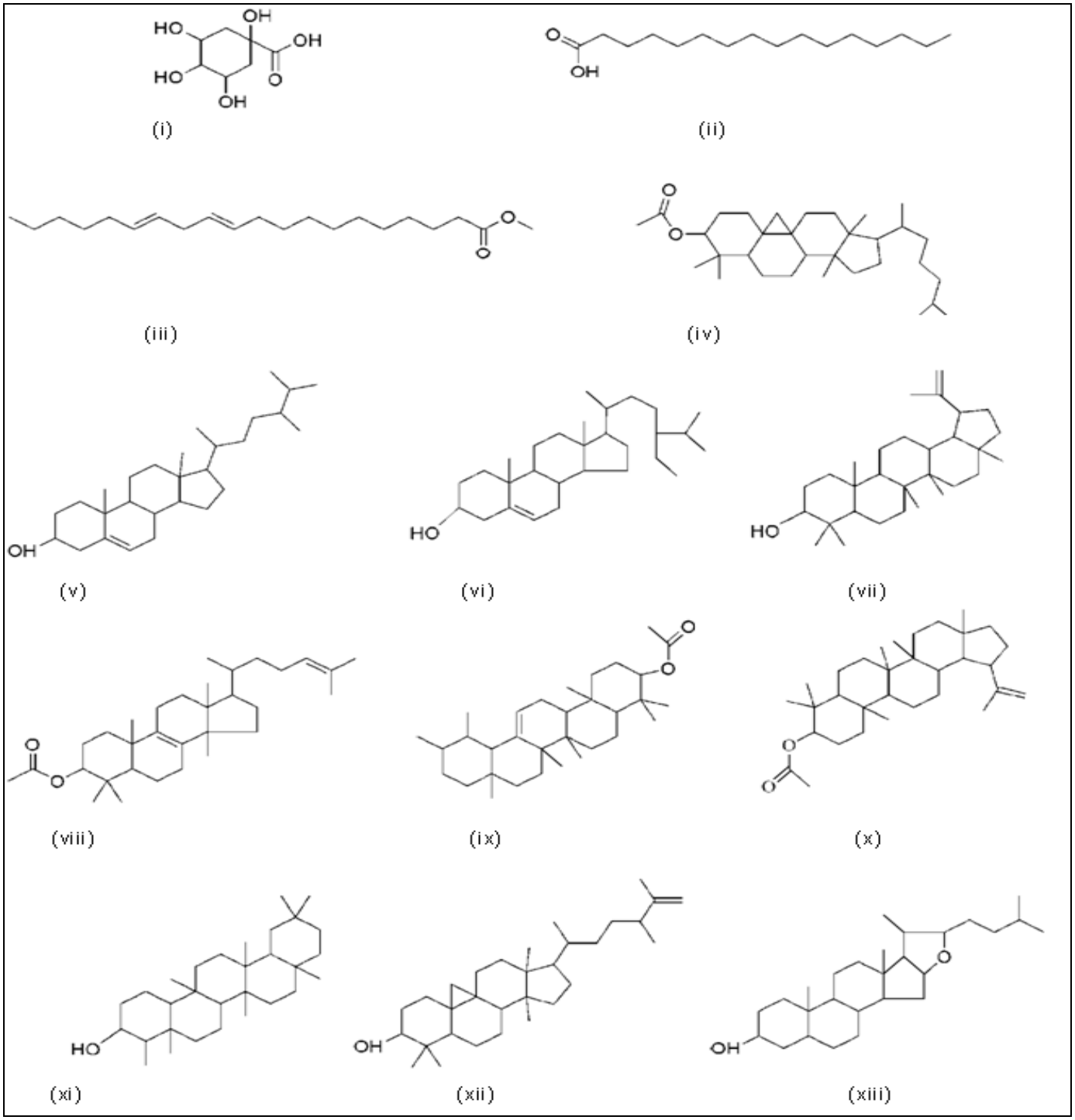

FIG.3: CHEMICAL STRUCTURE OF MAJOR COMPOUNDS IN THE METHANOL EXTRACT OF *F. BENGHALENSIS* (AERIAL-ROOT) (i) Quinic acid; (ii) Palmitic acid; (iii) Eicosadinoic acid methy ester; (iv) Cycloartanyl acetate; (v) Dihydrobrassicasterol; (vi) γ- sitosterol; (vii) Lupeol; (viii) Lanosterol acetate; (ix) Amyrin acetate; (x) Lupeolacetate; (xi) Epifriedelanol; (xii) Cycloaudenol; (xiii) Protodioscin

TABLE 4: COMPOUNDS IDENTIFIED BY GC-MS ANALYSIS OF METHANOL EXTRACT OF *F. BENGHALENSIS* (AERIAL-ROOT)

Peak	Retention Time	Area%	Chemical Formula	Common Name	Benefits
9	21.415	2.84	$C_7H_{12}O_6$	Quinic acid	Anti-oxidant [21], Anti-Hepatitis B activity [22], Anti-HIV therapeutic [23], Growth-promoting [24].
12	23.876	0.23	$C_{14}H_{28}O_2$	Myristic acid	Anti-bacterial [25], Anti-cancer [26], Anti-oxidant [27], Decrease renal damage [28], Hypercholesterolemic [29],lipid anchor of biomembrane [30], Nematicide [31].
15	24.589	0.68	$C_{21}H_{30}O_2$	Beta-Progesterone	Anti-inflammatory [32], normalize blood clotting [33], Prevent from endometrial cancer [34, 35].
21	27.188	0.85	$C_{19}H_{38}O_2$	Palmitic acid, methyl ester	Antioxidant [36], Anti-inflammatory [37], Anti-fibrotic [37], Vasodialator [38].
22	28.043	5.17	$C_{16}H_{32}O_2$	Palmitic acid	Anti-inflammatory [39], Anti-androgenic*, Anti-fibrinolytic*, Hypercholesterolemic*, Nematicide*.
26	29.815	0.26	$C_{17}H_{34}O_2$	Heptadecanoic acid	Anti-oxidant*.
27	30.395	0.71	$C_{19}H_{34}O_2$	Linoleic acid, Methyl ester	Prevent hair loss and skin scaling [40], Wound healing [41].
28	30.507	0.42	$C_{18}H_{31}ClO$	Linoleoyl chloride	-
29	30.732	0.19	$C_{20}H_{40}O$	Phytol	Adjuvant [42, 43], Anti-diabetic[44], Anti-diuretic [45], Anti-inflammatory [46],Anti-microbial [47, 48], Anti-oxidant [47], Anti-schistosomal [48], Anti-convulsant [49], Cancer-preventive [50], immunostimulatory [51], Nematicide [48].
31	31.215	1.74	$C_{21}H_{38}O_2$	Eicosadienoic acid, Methyl ester	-
33	31.661	0.4	$C_{18}H_{36}O_2$	Stearic acid	Anti-inflammatory [52], Decrease plasma cholesterol [53], Hypocholesterolemic *.
39	37.384	0.86	$C_{21}H_{42}O_4$	Alpha.-Monostearin	-
41	37.997	0.28	$C_{24}H_{38}O_4$	Phthalic acid,Dioctyl ester	-
43	40.467	0.35	$C_{23}H_{46}$	Muscalure	Insect Sex Pheromone [54].
50	44.566	0.49	$C_{30}H_{62}O$	Triacontanol	Anti-dermatitic*, Anti-inflammatory [55, 56], Anti-oxidant [55], Anti-ulcer [56].
53	45.364	0.26	$C_{15}H_{26}O$	Globulol	Anti-microbial [57]
55	45.695	0.53	$C_{30}H_{50}O$	Lanosterol	Cancer-preentive*
59	46.378	2.89	$C_{32}H_{54}O_2$	Cycloartanyl acetate	Antibacterial [58], Anti-fungal [58], Anti-inflammatory [59].
63	47.461	1.00	$C_{28}H_{48}O$	Dihydrobrassicasterol	Anti-inflammatory [60], Apoptotic [60].
64	47.738	0.61	$C_{29}H_{48}O$	Stigmasterol	Anti-hepatotoxic*, Anti-inflammatory [61], Anti-osteoartheritis [62], Antioxidant*, Anti-virus [63], Hypocholesterolemic*, Inhibit HIV Reverse transcriptase [64], Inhibit Tumor Promotion[65], Lowers plasma LDL [66].
65	48.305	5.45	$C_{29}H_{50}O$	Sitosterol	Anti-bacterial [67, 68], Anti-cancer [69, 70], Antidiabetic [71, 72],Antifungal [67], Anti-inflammatory [68, 73], Anti-oxidant [71, 72], Anti-viral [68], Decrease Blood Cholesterol [74], Treat Hypercholesterolemia[75], Treat prostatic hyperplasia [76].
71	49.175	4.44	$C_{30}H_{50}O$	Lupeol	Anti-angeogenic[77-79], Anti-arthritic [80], Anti-cancer [81], Anti-inflammatory [82-84], Anti-microbial [85], Anti-malarial*, Anti-viral*, Cardioprotective [86], Chemopreventive [87], Hepatoprotective [88], Hypercholestrolemia related renal protection [89], Nephroprotective [90].
72	49.343	0.48	$C_{30}H_{46}O_2$	Ergosterol acetate	Anti-apoptotic [91].
73	49.464	3.58	$C_{32}H_{52}O_2$	Lanosterol acetate	-
74	49.75	16.34	$C_{32}H_{52}O_2$	Amyrin acetate	Antiarthritic [92], Anti-bacterial [93], Antifungal [93], Antihyperglycemic [94, 95], Anti-inflammatory [96-98],Anti-lipoxygenase [92], Antioxidant [98].
76	50.385	35.4	$C_{32}H_{52}O_2$	Lupenyl acetate	Antihyperglycemic*, Anti-inflammatory [99], Antimicrobial [100], Anti-mutagenic [101], Anti-ulcer*.
77	50.529	1.5	$C_{30}H_{52}O$	Friedelanol	Analgesic[102], Anti-inflammatory [103], Anti-tumor [103].
79	50.879	3.79	$C_{31}H_{52}O$	Cyclolaudenol	-
84	51.700	0.22	$C_{29}H_{46}O$	4,22-Stigmastadiene-3-one	Anti-microbial [104]
86	51.931	0.10	$C_{37}H_{76}O$	1-Heptatriacotanol	-
91	53.663	1.23	$C_{27}H_{46}O_2$	Furostanol	Antifungal[105], Anti-gliomic*, Anti-inflammatory[106], Anti-microbial [107], Anti-proliferative*, Anti-tumor[108].

* represent the biological activity based on Dr. Duke's phytochemical and ethnobotanical databases

Thus through this study we report that the aerial roots of *F. benghalensis treasure* ahuge number of secondary metabolites which could be further exploited for its potential usage in medicine.

CONCLUSION: *F. benghalensis* treasure various phytochemicals which are excellent source of traditional medicine, especially Ayurveda for potential treatment of kidney damage, removal of blood clots, schizophrenia, heart disease, nervous disorders low cholesterol and osteo-arthritis. A few studies support the role of crude extract for various disease treatments; however this is a first report on the phytochemical analysis of aerial roots of *F. benghalensis*. The phytochemicals reported in this study could be of immense help to expand our therapeutics, correlate it with traditional medicine and support their use in modern medicines for treatment of diseases.

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