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GC-MS AND FT-IR ANALYSIS OF METHANOL FRUIT EXTRACT OF *FICUS RACEMOSA* AND *FICUS AURICULATA*

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Keywords:

Ficus racemosa, Ficus auriculata, fruit, Methanol extract, GC-MS analysis, FTIR

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ABSTRACT: The fruits of the plant *Ficus racemosa* and *Ficus auriculata* are consumed as a wild edible fruit and have also been used extensively in traditional medicine to treat various illnesses ranging from diarrhea, dysentery, jaundice to diabetes, piles, asthma, and urinary diseases. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanol extract of the fruits was carried out to identify the possible bioactive compounds. The major constituents identified in *F. racemosa* were 9, 12, 15-octadecatrienoic acid (z,z,z)- (14.323%); (z)6,(z)9-pentadecadien-1-ol (10.190%); Resorcinol (5.613%); n-hexadecanoic acid (2.965%) and Chloroacetic acid, dodec-9-ynyl ester (0.659%). The compounds like 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (58.216%); L-(+)-Ascorbic acid 2,6-dihexadecanoate (5.459%); Geranylgeraniol (0.432%); 9,12-Octadecadienoyl chloride, (Z,Z)- (0.151%) and 2H-Benzo [f]oxireno [2, 3-E] benzofuran-8 (9H)-one, 9-[[[2-(dimethyl-amino) ethyl]amino]methyl]octahydro-2,5a-dimethyl- (0.132%) were identified in *F. auriculata*. The Fourier-Transform Infrared Spectroscopy (FT-IR) analysis indicated the presence of N-H, O-H, C=C, C=O, C-H, C-O, S=O, C-N, and N-O functional groups. The results confirm the presence of bioactive components, which are known to exhibit medicinal value as well as pharmacological activities.

INTRODUCTION: Ficus is a tropical evergreen tree genus belonging to the family Moraceae with more than 750 species [1]. A total of 115 species of Ficus have been recorded from India 1 among which 42 species is found in Assam [2]. Fruit, leaves, root, bark, and latex of various Ficus species are commonly used in the treatment of various illnesses. Ficus species are reported to be rich in several phytocompounds, including sterols, terpenes, coumarins, and flavonoids [3, 4, 5,] which are reported to be responsible for several medicinal and bioactive properties such as antimicrobial [5], antioxidant [6, 7,] anticancer [8], Anti-diabetic [9] and anti-inflammatory [10].

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Ficus auriculata Lour. is a tree with 4 to 10 m in height and dioecious in nature. It contains an abundant amount of white latex in every part of the plant. The bark is grayish-brown in color with a rough texture. Branchlets are reddish-brown. Fruits are pear-shaped and generally occur on leafless branchlets at base of trunk and main branches [11].

F. auriculata has been traditionally used in the treatment of diarrhea, dysentery, cuts, wounds, mumps, cholera, and jaundice [12] which implies the plant's medicinal efficacy. The fruits of *F. auriculata* were also eaten by various tribes for their nutritional properties [13]. *Ficus racemosa* Linn. is an evergreen, large-sized, lactiferous tree. Leaves, fruits, bark, latex, and sap of the root are medicinally used in the treatment of various ailments in the traditional medicine system, including bile infections, diarrhea, dysentery, ulcers, diabetes, piles, asthma, and urinary diseases [14, 15]. Apart from their usage in the traditional medicine system, scientific studies also suggest that

F. racemosa possesses various biological effects such as anticancer [8], anti-inflammatory [10], antidiabetic [16], hepatoprotective [17,] antipyretic [18], antitussive [19,] and antidiuretic [20]. The present investigation was carried out to identify the possible bioactive compounds present in the *Ficus auriculata* and *Ficus racemosa* fruit methanol extract with the help of GC-MS and FT-IR techniques, which may provide an insight into its use in traditional medicine system.

MATERIALS AND METHODS: Collection of plant materials: Fresh and mature fruits of *Ficus racemosa* and *Ficus auriculata* were collected during the months of January 2019 from Kamrup (N 26 °15' and E 99 °66') and Goalpara (N 26 °13' and E 90 °61') districts of Assam, respectively. The fruits were collected in polyethylene bags to prevent the loss of moisture during transportation to the laboratory. The plant specimens were authenticated by the Herbarium of Department of Botany, Gauhati University (Specimen Accession No. GUBH-18676 and GUBH-18677 respectively).

Preparation of Extract: The fruit samples of F. auriculata and *F. racemosa* were washed thoroughly, sliced, and dried in an oven at 40 ± 5 °C until they are completely dry. The dried sample was ground into a fine powder using a mechanical grinder, and the dried powder was macerated with methanol in an orbital shaker at 150 rpm and 37 °C for 24 h. The extracts were then filtered using a Whatman® no.1 filter paper to obtain a liquid extract which was then concentrated by using a rotary evaporator at a temperature of 40 ± 5 ºC. The dry crude extract thus obtained was stored in a glass vial at 4 °C for GC-MS analysis.

GCMS Condition: The methanol extract of the studied fruit samples was analyzed for the presence of possible bioactive compounds through GC-MS and was performed at Biotech-Park, IIT Campus, Guwahati in Clarus 680 GC & Clarus 600 C MS PerkinElmer, USA. The GC-MC system was equipped with a capillary column of 60 m in length and 0.25 mm in diameter and 0.25 µm in film thickness and composed of 5% diphenyl 95% dimethyl polysiloxane as a stationary phase. Helium gas (99.99%) was used as carrier gas with a flow rate of 1 ml/min. Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV, and mass ranges from 50-600 AMU with a solvent delay for 8 min. 1 µL of the sample was injected in the GC-MS system through autosampler in split mode (split ratio 10:1). Injector temperature was maintained at 280 °C, and ion source temperature was at 180 °C. The oven temperature was programmed at 60 °C (for 3 min) with an increase at the rate 5 °C/min to 200 °C (hold for 3 min) then again increased at a rate of 6 °C/min to 300 °C (hold for 10 min).

The total run time of the system was ~60.67 min, and solvent delay was kept for 8 min. Interpretation of the peaks appeared in the GC Chromatogram was done by library search of the mass spectrum of corresponding peaks using the database software of National Institute Standard and Technology-2008 (NIST-2008). The mass spectrums of the unknown components were compared with the spectrum known components of NIST library, and the compounds were identified with name, molecular weight, empirical formula, *etc.*

FTIR Analysis: A small quantity of dried fruit powder of each plant material was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet in order to prepare a translucent sample disc. The powdered sample of each plant specimen was then loaded in FTIR Spectroscopy (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The functional groups were analyzed in the region 4000 - 400 cm^{-1}.

RESULTS: Methanol fruit extract of *F. auriculata* and F. racemosa were evaluated for the presence of bioactive compounds through GC-MS analysis **Fig 1, 2**. The bio-active properties of the compounds along with their retention time (RT), molecular formula, molecular weight (MW), and peak area (%) are presented in (Table 1;2;3;4). Compounds identified in *F. racemosa* fruit methanol extract were 9, 12, 15-octadecatrienoic acid (z,z,z)- (14.323%); (z)6,(z) 9-pentadecadien -1-ol (10.190%); Resorcinol (5.613%); n- hexadecanoic acid (2.965%) and Chloroacetic acid, dodec-9-ynyl ester (0.659%). While in *F. auriculata* fruit methanol extract, identified bio-active components are 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- (58.216%); L-(+)-Ascorbic acid 2,6-dihexa-decanoate (5.459%); Gerany lgeraniol (0.432%);

9,12- Octadecadienoyl chloride, (Z,Z)- (0.151%) and 2H-Benzo [f]oxireno[2, 3-e] benzofuran-8 (9h)-one, 9-[[[2- (dimethylamino) ethyl] amino] methyl] octahydro-2,5a -dimethyl-(0.132%). The FT-IR spectrum was used to identify the functional groups of the bioactive components present in methanol fruit extracts based on the peak values in the region of IR radiation.

When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peak ratio. The results of FT-IR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O, C=O, N-O, S=O, and C-N functional groups in the studied fruit methanol extracts **Fig 3** and **4** and **Table 5** and **6**.

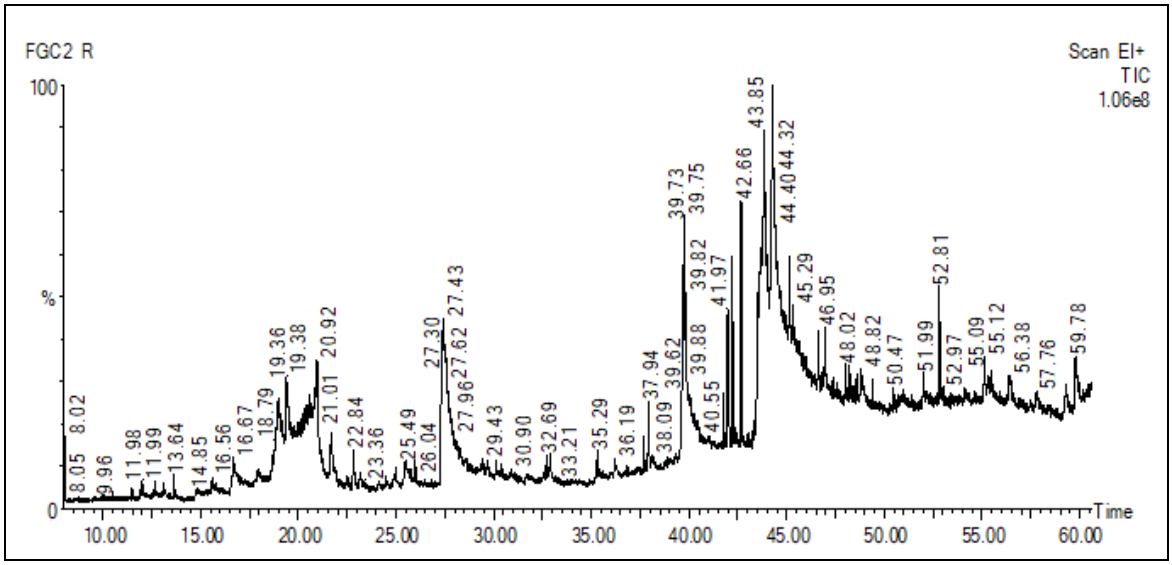

FIG. 1: GC-MS CHROMATOGRAM OF *FICUS RACEMOSA*

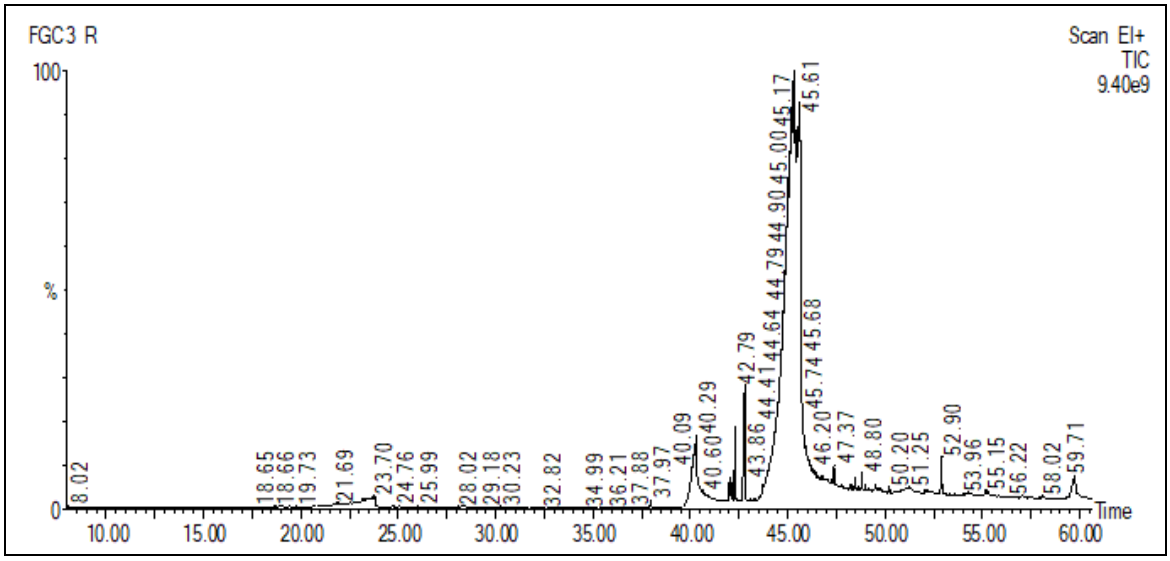

FIG. 2: GC-MS CHROMATOGRAM OF *FICUS AURICULATA*

TABLE 1: LIST OF COMPOUNDS IN *FICUS RACEMOSA* FRUIT METHANOL EXTRACT IDENTIFIED BY USING GC-MS

S. no.	Name of Compounds	RT (min)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
1	Resorcinol	27.426	$C_6H_6O_2$	110	5.613
2	N-Hexadecanoic acid	39.764	$C_{16}H_{32}O_2$	256	2.965
3	(Z)6,(Z)9-Pentadecadien-1-ol	43.853	$C_{15}H_{28}O$	224	10.190
4	9,12,15-Octadecatrienoic acid (z,z,z)-	44.273	$C_{18}H_{30}O_2$	278	14.323
5	Chloroacetic acid, dodec-9-ynyl ester	52.806	$C_{14}H_{23}O_2Cl$	258	0.659

TABLE 2: BIO-ACTIVITY OF COMPOUNDS IDENTIFIED IN *FICUS RACEMOSA* FRUIT METHANOL EXTRACT BY GC-MS

S. no.	Name of Compounds	Bio-activity
1	Resorcinol	Topical antipruritic and Antiseptic [21]
2	N-Hexadecanoic acid	Anti-inflammatory, Antioxidant, Hypocholesterolemic, Anti-androgenic and Hemolytic [22]
3	(Z)6,(Z)9-Pentadecadien-1-ol	Antibacterial [23]
4	9,12,15-Octadecatrienoic acid (z,z,z)-	Antioxidant, Antimicrobial, Anti-cancerous and Hypocholesterolemic [23]
5	Chloroacetic acid, dodec-9-ynyl ester	No activity reported

TABLE 3: LIST OF COMPOUNDS IN *FICUS AURICULATA* FRUIT METHANOL EXTRACT IDENTIFIED BY USING GC-MS

S. no.	Name of compounds	RT (min)	Molecular formula	Molecular weight (g/mol)	Peak Area (%)
1	L-(+)-Ascorbic acid 2,6-dihexadecanoate	40.316	$C_{38}H_{68}O_8$	652	5.459
2	9,12,15-Octadecatrienoic acid, (z,z,z)-	45.328	$C_{18}H_{30}O_2$	278	58.216
3	9,12-Octadecadienoyl chloride, (z,z)-	47.369	$C_{18}H_{31}OCl$	298	0.151
4	2H-Benzo[f]oxireno[2,3-e]benzofuran-8(9h)-one, 9-[[[2-(dimethylamino)ethyl]amino]methyl]octahydro-2,5a-dimethyl-	48.805	$C_{19}H_{32}O_3N_2$	336	0.132
5	Geranylgeraniol	52.906	$C_{20}H_{34}O$	290	0.432

TABLE 4: BIO-ACTIVITY OF COMPOUNDS IDENTIFIED IN *FICUS AURICULATA* FRUIT METHANOL EXTRACT BY GC-MS

S. no.	Name of Compounds	Bio-activity
1	L-(+)-Ascorbic acid 2,6-dihexadecanoate	Antioxidant, Cardio protective, Cancer preventive, Flavour and Anti-infertility [24]
2	9,12,15-Octadecatrienoic acid, (z,z,z)-	Antioxidant, Antimicrobial Anticancerous and Hypocholesterolemic [23]
3	9,12-Octadecadienoyl chloride, (z,z)-	Anti-inflammatory, Cancer preventive, Insectifuge, Nematicide, Anti-acne, Anti-eczemic and anti-coronary [25]
4	2H-Benzo[f]oxireno[2,3-e]benzofuran-8(9h)-one, 9-[[[2-(dimethylamino)ethyl]amino]methyl]octahydro-2,5a-dimethyl-	No activity reported
5	Geranylgeraniol	Antimicrobial and Anticancer [26]

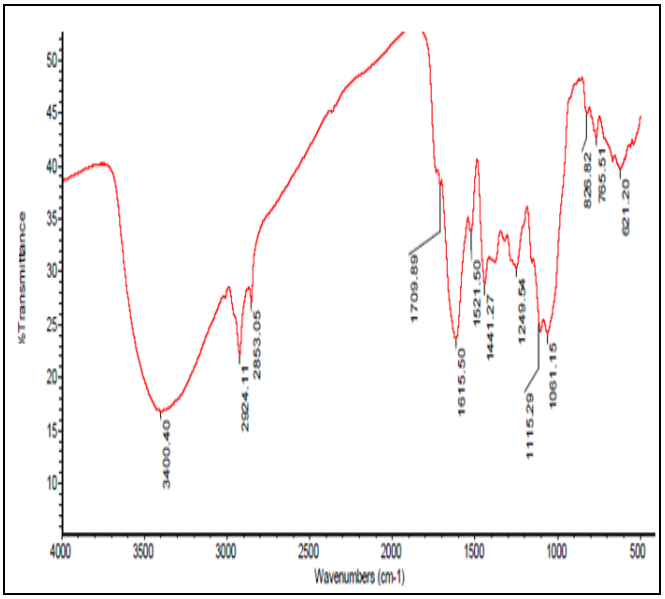

FIG. 3: FTIR SPECTRUM OF METHANOL EXTRACT OF *FICUS RACEMOSA*

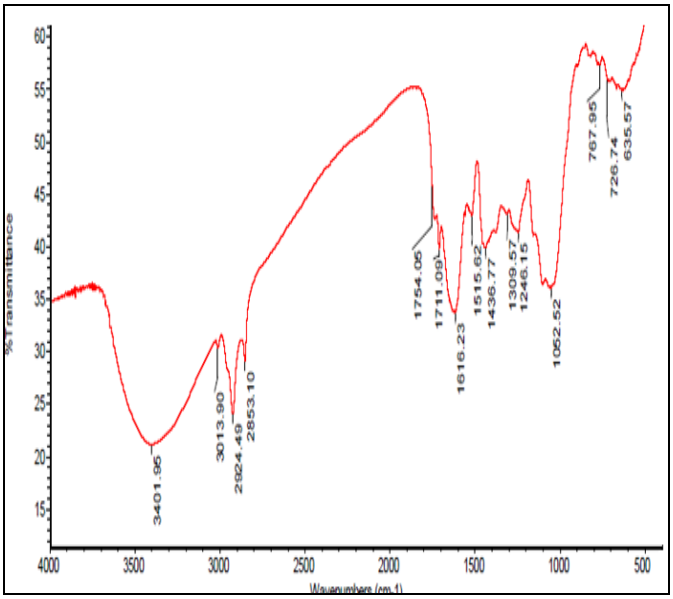

FIG. 4: FTIR SPECTRUM OF METHANOL EXTRACT OF *FICUS AURICULATA*

TABLE 5: FTIR PEAK VALUES OF METHANOL EXTRACT OF *FICUS RACEMOSA*

S no.	Peak values (cm^{-1})	Functional groups
1	2924.11	N-H
2	2853.05	N-H
3	1709.89	C=O
4	1615.50	C=C
5	1521.50	N-O
6	1441.27	O-H
7	1249.54	C-N
8	1115.29	C-O
9	1061.15	S=O

TABLE 6: FTIR PEAK VALUES OF METHANOL EXTRACT OF *FICUS AURICULATA*

S no.	Peak values (cm^{-1})	Functional groups
1	3013.90	C-H
2	2853.10	C-H
3	1754.05	C-H
4	1711.09	C=O
5	1616.23	C=C
6	1515.62	N-O
7	1436.77	O-H
8	1309.57	S=O
9	1246.15	C-O

DISCUSSION: The compounds identified through GC-MS possess various biological activities. 9, 12, 15-Octadecatrienoic acid (z, z, z)- is the major compound identified in both the studied fruit methanol extract with a peak area of 14.323% in *F. racemosa* and 58.216% in *F. auriculata*. 9, 12, 15-Octadecatrienoic acid (z, z, z)- which is also known as Linolenic acid has antioxidant, antimicrobial, anti-cancerous and hypocholesterolemic properties [23]. Of the identified compounds in *F. racemosa* fruit methanol extract, n-hexadecanoic acid (Palmitic acid) was also reported to be present in *Ziziphus trinervia* fruit methanolic extract [27] and possess anti-inflammatory, anti-oxidant, hypocho lestero-lemic, anti-androgenic, and hemolytic activites [22, 27]. L-(+)-Ascorbic acid 2, 6-dihexadecanoate which is a vitamin known for its antioxidant, cardio protective, cancer preventive and anti-infertility properties [24, 28] were identified in *F. auriculata* fruit methanol extract and was also reported to be present in *Bryonopsis laciniosa* fruit extract [28]. 9, 12-Octadecadienoyl chloride, (z,z)- or Linoleic acid has various biological properties like anti-inflammatory, anticancer, anti-eczemic, anti-coronary, insectifugal, and nematicidal [25]. Geranylgeraniol is a diterpenoid responsible for various bioactive properties such as antimicrobial and anticancerous [26].

The FTIR analysis of the methanol extracts of *F. racemosa* fruit reveals functional groups such as N-H stretching or amine (2853.05 cm^{-1}), C=O stretching or aliphatic ketone (1709.89 cm^{-1}), C=C stretching or unsaturated ketone (1615.50 cm^{-1}), N-O stretching or nitro compound (1521.50 cm^{-1}), O-H bending or carboxylic acid (1441.27 cm^{-1}), C-N stretching or amine (1249.54 cm^{-1}), C-O stretching or primary alcohol (1115.29 cm^{-1}) and S=O stretching or sulfoxide (1061.15 cm^{-1}) were detected. Functional groups of C-H stretching or alkene, alkane (3013.90 cm^{-1}, 2853.10 cm^{-1}), C=O stretching or aliphatic ketone (1711.09 cm^{-1}), C=C stretching or alkene (1616.23 cm^{-1}), N-O stretching or nitro compound (1515.62cm^{-1}), O-H bending or carboxylic acid (1436.77cm^{-1}), S=O stretching or sulfone (1309.57cm^{-1}), C-O stretching or ether (1246.15 cm^{-1}) were found to be present in *F. auriculata* fruit.

CONCLUSION: The phytocompounds identified through GC-MS analysis in the methanol extract of the fruits reported to have a wide range of bioactive properties such as antioxidant, antimicrobial, anti-inflammatory, and anti-cancerous, among others. The presence of such bioactive compounds in the studied fruits justifies their use in the traditional medicine system. Furthermore, the studied fruits can serve as a source of pure bioactive compounds, which may be isolated and used to prepare drugs in pharmaceutical industries.

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