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INVESTIGATION OF PHYTOCONSTITUENTS IN STEM BARK OF *FICUS BENGHALENSIS* LINN. USING GC-MS TECHNIQUE

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Keywords:

Phytoconstituents, Stem bark , *Ficus benghalensis* Linn., GC-MS

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ABSTRACT: Objective of the present study is to investigate the chemical composition of methanol extract of stem bark of *Ficus benghalensis* Linn. (Indian Banyan Tree). Shade and air dried stem bark were powdered and extract was prepared according to selective sequential extraction using different solvents of increasing polarity. Methanol extract of stem bark of this plant was further subjected to gas chromatography and mass spectrometry as to the best of authors' s knowledge no published literature exist about the characterization of chemical constituents of methanol extract by GC-MS technique. Total 63 compounds were reported by GCMS studies out of which two were never reported earliar. Qualitative and quantitative presence of different biologically important phytoconstituents were reported and two new phytoconstituents were investigated. Results of this study suggested the phytopharmacological importance of reported constituents of stem bark extract of *Ficus benghalensis* Linn. and justify the use of *Ficus benghalensis* Linn. in ancient literature of Ayurveda.

INTRODUCTION: *Ficus benghalensis* Linn. 'The national tree of India', is native to India and commonly known as Banyan tree [1]. Its stem bark is greyish, hard, with uneven surface, on rubbing white papery flakes come out from the outer surface and inner surface is light brown without any characteristics odour [2 - 3]. Traditionally, stem bark is used as antioxidant [4], anti-inflammatory [5] analegesic [6], antipyretic [6], antiasthmatic [7] and in wound healing [8]. According to Ayurveda the bark of *Ficus benghalensis* Linn. is astringent and is useful in leucorrhoea, lumbago, sores and bruises [9].

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The bark is considered useful in burning sensation, ulcer pain, and toothache [10 - 11] methanol extract of *Ficus benghalensis* Linn. bark possess phyto-constituents with antidiabetic [12] antiproliferative [13] anti-inflammatory and analgesic properties in animal models [14]. Methanol extract of stem bark of *Ficus benghalensis* Linn. is reported to produce marked inhibitory effect on edema due to arthritis based on various animal models [15]. The majority of activities are reported in methanol extract, thus in the present study, methanol extract of this plant was subjected to gas chromatography and mass spectrometry as to the best of author's knowledge no published literature exists about the characterization of chemical constituents of methanol extract by GC-MS technique.

MATERIAL AND METHODS:

Plant Sample: The plant material (stem bark) was collected from the campus of Guru Jambheshwar

University of Science and Technology, Hisar in the month of February, 2016 and was Identified by Dr. Anjula Pandey, Principal Scientist, ICAR-NBPGR, New Delhi, vide reference no. NHCP/ NBPGR /2016-1, as *Ficus benghalensis* Linn.

Extraction: 100 g of shade and air dried stem bark were powdered and different extracts were prepared according to selective sequential extraction using solvents of increasing polarity in order of petroleum ether, chloroform, methanol and distilled water in soxhlet extractor. Percentage yield of methanol extract was calculated and was stored in dessicator for further use.

GC-MS Analysis: GC-MS analysis of extract for identification of phytoconstituents was done using Shimadzu GCMS-QP 2010 Plus Model from Shimadzu corporation, Kyoto, Japan with Restek Rxi ^{R}-5Sil MS crossbond ^{R} similar to 5 % diphenyl /95 % dimethyl polysiloxane with 30 meter in length and 0.25 mm diameter and 0.25 micrometer in thickness.

Identification of Phytoconstituents: Bioactive phytoconstituents extracted from methanol extracts of *Ficus benghalensis* Linn. were identified based on GC retention time on column and by matching of this with computer software data of standards from National Institute of Standards and Technology (NIST) and Wiley Library. Molecular weights were calculated by mass spectroscopy.

RESULTS:

Percentage Yield of Extracts: Percentage yield of methanol extract of *Ficus benghalensis* Linn. stem bark was 4.07 % w/w.

Physical Properties of Extracts: Methanol extract of *Ficus benghalensis* Linn. was brown in colour and after fluorescent study of extracts, colour changes to dark brown when viewed under short UV(254 nm) and black when viewed under long UV(365 nm) .

Phytoconstituents Reported in Extract after GC -MS: Bioactive compounds present in methanol extract of stem bark of *Ficus benghalensis* Linn. are shown in table no.1and as chromatogram in **Fig. 1**. Identification and characterization were based on their elution order in 5 Sil MS column. Elution time, molecular formula and the percentage of these phytoconstituents are also shown in the **Table 1**.

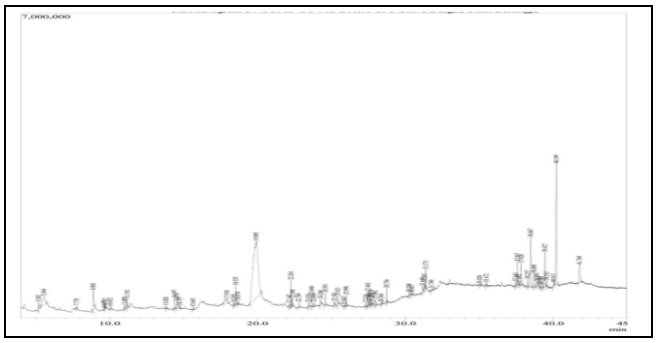

FIG. 1: GC-MS CHROMATOGRAM OF METHANOL EXTRACT OF *FICUS BENGHALENSIS* LINN.

Quinic acid (37%), α-Amyrin (4.49%), Androstan-17-one (5.53%) Lupeol acetate (2.67%) and Diglycerol (2.74%) were reported in majority according to chromatogram area percentage (**Fig. 2-6**). Total 63 compounds were present and two new compounds were also found in the chromatogram as their peak does not match (**Fig. 7** and **8**) with any compound in NIST and Wiley library. Some important phytosterols like fucosterol, lanosta-8, 24-dien-3-one, stigmasterol were present in traces. A large no. of fatty acids and their esters (8-Octadecanone) has also been identified. Tri-terpenoids like lupeol acetate, Terpenoids(α-Amyrin) were also been identified.

TABLE 1: BIOACTIVE COMPOUNDS OF *FICUS BENGHALENSIS* LINN. STEM BARK

Retention Time	Percentage	Molecular Formula	Compound Name
5.202	0.62	$C_5H_{12}O_2Si$	Silanol
5.564	2.74	$C_3H_8O_3$	Diglycerol
7.770	0.17	$C_{10}H_{18}O$	Linalool
8.903	2.25	$C_6H_8O_4$	Pyranone
9.637	0.11	$C_{11}H_{19}Cl_2O$	Menthyl chloroformate
9.707	0.18	$C_{10}H_{18}O$	Thujanol
10.052	0.13	$C_{20}H_{40}O$	Octadecane
11.005	0.22	$C_{14}H_{26}$	Cyclohexane
11.192	0.56	$C_{12}H_{20}O_2$	Bergamol
13.820	0.16	$C_{17}H_{34}$	Undecane
14.397	0.63	$C_{16}H_{32}$	Hexadecene

14.571	0.26	$C_{15}H_{32}$	Pentadecane
14.757	0.16	$C_{15}H_{30}$	Germacrane-A
15.645	0.16	$C_{17}H_{34}$	Cyclohexane
17.938	1.51	$C_8H_{19}N$	Butylamine
18.395	0.19	$C_{11}H_{22}$	1-Decene
18.535	1.18	$C_{16}H_{32}$	1-Hexadecene
18.678	0.26	$C_{20}H_{42}$	Eicosane
19.905	37.38	$C_7H_{12}O_6$	Quinic acid
22.147	0.36	$C_5H_{11}Br$	Isopentenyl bromide
22.263	1.49	$C_{18}H_{36}$	1-Octadecene
22.368	0.71	$C_{12}H_{21}N$	2,3Bis(1methylallyl)pyrollidine
22.789	0.35	$C_{17}H_{34}O_2$	Isopropyl myristate
23.436	0.31	$C_{19}H_{28}O_4$	Phthalic acid
23.660	0.79	$C_{18}H_{36}O$	8-Octadecanone
23.809	0.37	$C_{16}H_{34}O$	Loxanol
24.260	0.45	$C_{16}H_{22}O_4$	Diisobutyl benzene
24.581	0.92	$C_{17}H_{34}O_2$	Pentadecanoic acid
25.193	0.32	$C_{16}H_{22}O_4$	Phthalic acid
25.421	0.94	$C_{14}H_{28}O_2$	Myristic acid
25.847	0.16	$C_{12}H_{24}O_2$	Butanoic acid
25.998	0.93	$C_{16}H_{29}F_3O_2$	Trifluroacetic acid
27.336	0.26	$C_{13}H_{24}O_2$	Decanoic acid
27.483	0.92	$C_{15}H_{32}O$	Neodol
27.565	0.19	$C_{18}H_{34}O$	Linoleyl alcohol
27.658	0.41	$C_{19}H_{36}O_2$	Methyl Dihydromalvalate
27.759	0.16	$C_{15}H_{30}O_2$	Oxirane
27.991	0.36	$C_{19}H_{38}O_2$	Octadecanoic acid
28.368	0.12	$C_{15}H_{26}O$	6-Epi-shyobunol
28.736	0.64	$C_{22}H_{46}O$	Docosanol
30.250	0.30	$C_5H_{11}NO_2$	Valeramide
30.437	0.27	$C_{18}H_{38}O$	Crodacol
31.148	0.71	$C_{21}H_{44}O_3S$	Sulfurous acid
31.285	0.53	$C_{19}H_{38}O_4$	Palmitic acid
31.373	0.65	$C_{24}H_{38}O_4$	Genomoll
31.760	0.28	$C_{22}H_{46}$	Docosane
35.020	0.15	$C_{31}H_{50}O_2$	Stigmasterol
35.472	0.26	$C_{27}H_{46}O$	Cholesterol
37.443	0.39	$C_{30}H_{48}O$	Lanosta-8,24-dien-3-one
37.567	2.25	$C_{29}H_{50}O$	Fucosterol
37.692	0.21	$C_{29}H_{46}O$	Cycloprop[7,8]ergost-22-en-3-one
37.826	2.07	$C_{31}H_{50}O_4$	Methyl Commate C
38.257	0.83	$C_{30}H_{48}O$	Lanosta-8,24-dien-3-one
38.467	5.53	$C_{21}H_{34}O$	Androstan-17-one
38.688	1.60	$C_{30}H_{50}O$	Cycloartenol
38.891	0.69	$C_{30}H_{50}O_2$	Lup-20(29)-ene-3,28-diol
39.045	0.23	$C_{15}H_{22}O$	Longipinocarvone
39.245	0.36	$C_{19}H_{30}O$	Androstan-17-one
39.427	4.49	$C_{30}H_{50}O$	Alpha-amyrin
39.553	0.66	$C_{31}H_{52}O$	Cyclolaudenol
40.013	0.43	New	New
40.199	14.40	New	New
41.769	2.67	$C_{32}H_{52}O_2$	Lupeol acetate

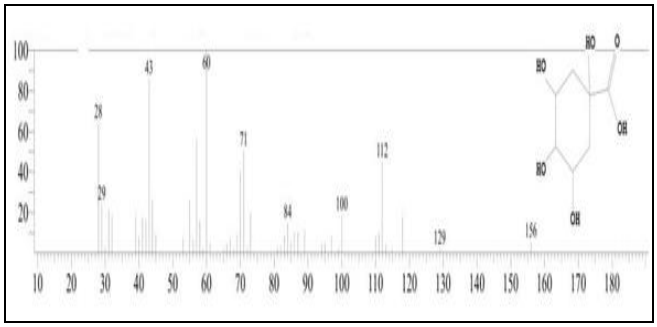

FIG. 2: GC-MS CHROMATOGRAM OF QUINIC ACID

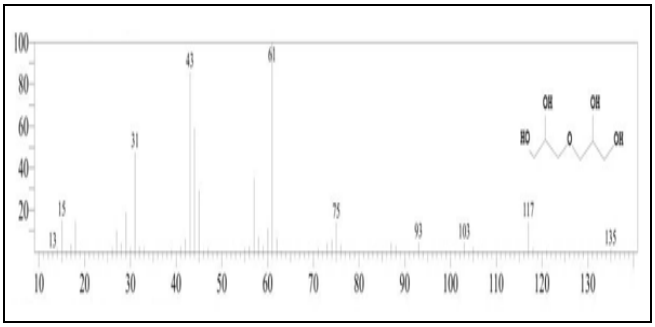

FIG. 3: GC-MS CHROMATOGRAM OF DIGLYCEROL

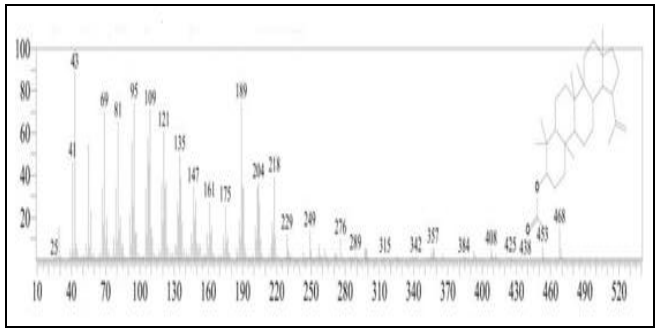

FIG. 4: GC-MS CHROMATOGRAM OF LUPEOL ACETATE

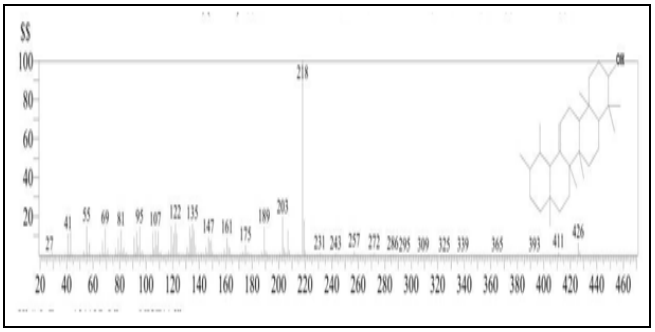

FIG. 5: GC-MS CHROMATOGRAM OF ALPHA-AMYRIN

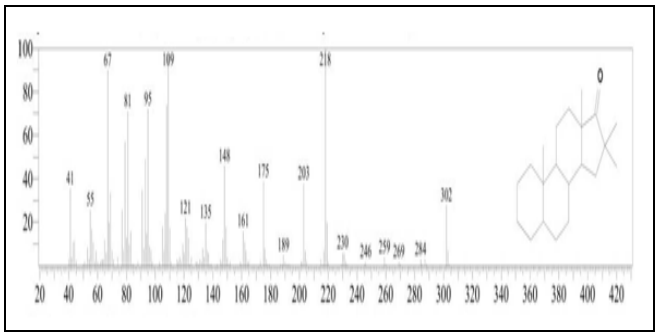

FIG. 6: GC-MS CHROMATOGRAM OF ANDROST-17-ONE

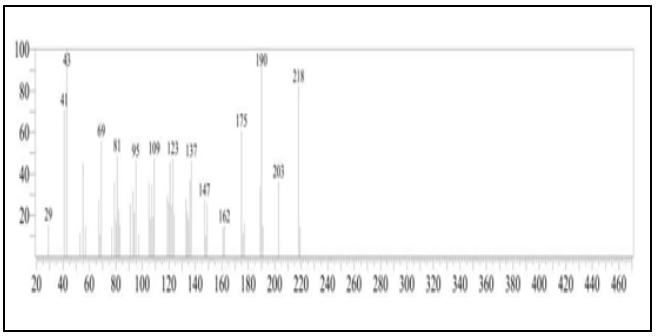

FIG. 7: GC-MS CHROMATOGRAM OF NEW COMPOUND-1, R.TIME-40.199, PERCENTAGE-14.40

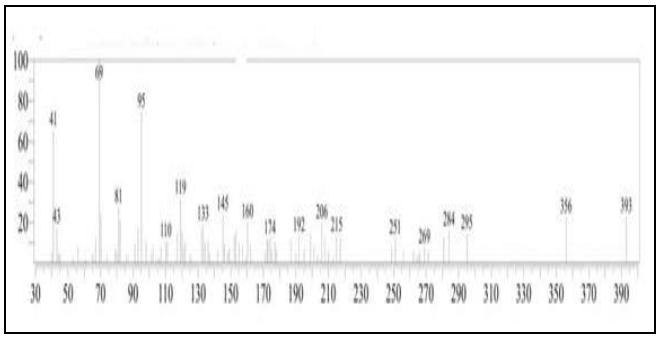

FIG. 8: GC-MS CHROMATOGRAM OF NEW COMPOUND-2, R.TIME-40.013, PERCENTAGE-0.43

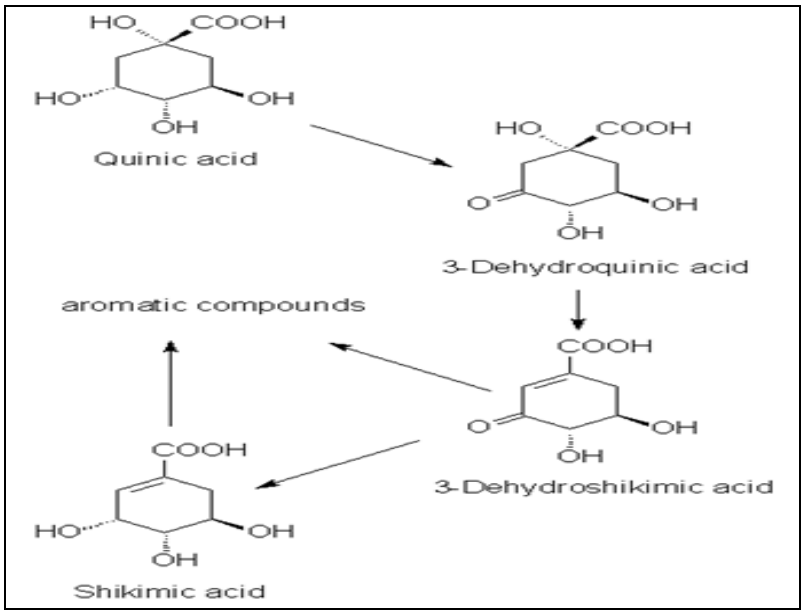

FIG. 9: QUINIC ACID IS SUGAR ACID AND STARTING MATERIAL FOR SYNTHESIS OF AROMATIC COMPOUNDS IN PLANTS

DISCUSSION: Chemically, quinic acid is sugar acid and starting material for synthesis of aromatic compounds in plants (**Fig. 9**). In a comparative study, quinic acid has been reported to be having greater anticancer potential than curcumin based on *in silico* study of compound [16]. It is reported to exhibit antitumor activity without inducing normal cell death [17]. Another study report about significant increases in number of spleen lymphocyte cells by quinic acid [18]. Hepatoprotective activity of quinic acid is reported by suitable animal models [19]. Quinic acid is an antioxidant compound [20]. Chemically, quinic acid is cyclic polyol in nature suggesting that the six-carbon ring structure bound to a carboxylic group plays an important role in the action of polyphenols. Androstan-17-one produces antiobesity and tumor chemopreventive activity [21]. α- Amyrin show anxiolytic, analgesic, anti-inflammatory and antidepressant effect [22]. It is pentacyclic triterpenoid in nature [23].

Another pentacyclic triterpenoid or phytosterol (Lupeol acetate) have a wide spectrum of biological activities [24]. In a study, lupeol acetate presents an anti-inflammatory activity in animal model by TNF-alpha and IL-2 specific mRNA regulation [25 - 26]. Diglycerol fatty acid esters were successfully investigated for their antibacterial activity [27]. Similarly other major phytoconstituents are also antioxidants in nature. Antioxidants are the substance responsible for treatment of various diseases like diabetes, cancer, inflammation *etc* [28].

In a previous study, lanostadienyl glucosyl cetoleate and bengalensisteroic acid ester reported as the new phytoconstituents from the methanolic extract of the stem bark of *Ficus benghalensis* Linn. [29.] Another study report about the mechanism of action of flavonoids isolated from *Ficus benghalensis* Linn. having antiatherogenic potential [30]. GCMS act as valuable technique which provides information about bioactive phytoconstituents in any plant [31]. According to report of a study conducted in 2014, various antioxidant compounds were reported from *Ficus bengalensis Linn.* tree using HPLC method [32] but our study focus on investigation of phyto-constituents using GC-MS technique. Results of the present study emphasize that the methanol extract of *Ficus benghalensis* Linn. is a good source of bioactive phytoconstituents and can be used further in the field of therapeutics.

CONCLUSION: The existing knowledge regarding its phytoconstituents may be increased by the present phytochemical investigation as the two new phytoconstituents were reported from the GC-MS chromatogram of methanol extract of the stem bark of *Ficus benghalensis* Linn. This plant has been used in the traditional Indian System of Medicine since long time, presence of maximum phyto-constituents reported by present study also justifies the use of stem bark of *Ficus benghalensis* Linn. in various diseases.

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CONFLICT OF INTERST: Nil

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