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GC-MS Analysis of Phytocompounds of Leaf and Stem of *Marsilea quadrifolia* (L.)

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Authors' contributions

The first author KG performed the research work and wrote the initial draft of manuscript. The corresponding author RU designed the research problem and corrected the final format of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate the phytochemical constituents of methanolic extracts of leaf and stem of *Marsilea quadrifolia* (Linn.).
Methods: The methanolic leaf and stem extracts of *M. quadrifolia* were prepared by standard procedure and concentrated at 40ºC using hot air oven. The concentrated methanolic extracts were subjected to phytochemical analysis using GC-MS.
Results: The GC-MS analyes showed that the presence of 39 phytocompounds in the methanolic extract of leaf of *M. quadrifolia* including 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-(21.41%)**;** n-Hexadecanoic acid (17.47%); 9,12,15-Octadecatrienoic acid,methylester (Z,Z,Z)-(12.96%); 2-Furancarboxaldehyde,5-(hydroxyl methyl)-(9.39%) and 9,12,15-Octadecatrien-1-ol (Z,Z,Z)-(3.54%). The methanolic extract of stem of *M. quadrifolia* revealed that the presence of 29 bioactive compounds including 2-Furancarboxaldehyde, 5-(hydroxymethyl)-(60.42%); 4H-Pyran-4-one, 2, 3-dihydro-3, 5-di hydroxyl-6-methyl-(13.88%); n-Hexadecanoic acid-(6.00%); 6-Octadecenoic acid (Z)-(2.69%) and Furfural-(2.23%).
Conclusions: The result of the GC-MS analysis showed that the methanolic extract of *M. quadrifolia* contains many pharmacologically important bioactive compounds. However *M. quadrifolia* is an important medicinal plant and used in the traditional system of medicine to cure many diseases including diabetes mellitus. So there are need of further studies to isolate and identify the specific phytocompound involved in controlling diseases and ultimately which may lead to drug development.

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Keywords: Marsilea quadrifolia; bioactive compounds; chromatogram; GC-MS analysis.

1. INTRODUCTION

Today natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action [1]. Plants are used as medicine in many countries and also act as a source for many potent drugs [2]. A large number of medicinal plants and their purified constituents have shown therapeutic activities [3]. Natural remedies from medicinal plants proved as safe and effective. Many plant species have been used in folklore medicine to treat various ailments [4]. Phytocompounds from medicinal plants are important in pharmaceutical industry for drug development and preparation of therapeutic agents [5]. The development of pharmaceuticals begins with identification of active principles, detailed biological assays and dosage formulations followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug [6]. *Marsilea quadrifolia* is an important medicinal plant belongs to the family of *Marsileaceae*. *M. quadrifolia* is beneficial for nutrient mitigation from the fresh water of lake and significant progress has been made for wetland restoration [7,8]. It is used in the ayurvedic system of medicine for curing several ailments. Antibacterial, cytotoxic and antioxidant activities of petroleum ether, chloroform and ethyl acetate extracts of aerial parts of *M. quadrifolia* have been reported [9]. Antimicrobial activity of leaf extract of *M. quadrifolia* against various bacterial pathogens was reported [10]. In the previous study, we reported that the antibacterial and antifungal activities of methanol, ethanol, diethyl ether and aqueous extracts of leaf and stem of *M. quadrifolia* against some selected human bacterial and fungal pathogens [11]. Antidiabetic activity of different solvent extracts of *M. quadrifolia* have been reported [12,13].

In vitro cytotoxic activity of methanol, aqueous and ethyl acetate extracts of leaves of *M. quadrifolia* on MCF-7 cells from human breast cancer was evaluated [14]. Juice made from the leaves of *M. quadrifolia* possess anti-inflammatory, anti-diuretic and febrifuge activities and used to treat snakebite and abscesses [15,16]. *M. quadrifolia* is also used to treat cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea and skin diseases [17]. It is highly nutritious, good for reducing body heat, thirst and act as anti-inflammatory drug [18]. As per the traditional claims, *M. quadrifolia* has been used for astringent, hypnotic, expectorant, aphrodisiac, anodyne, ophthalmic, constipating, strangury, dyspepsia and also used in the treatment of leprosy, haemorrhoids, fever and insomnia [19,20]. The crude extract of *M. quadrifolia* caused prompt hypotensive response and also found to be effective against electro convulsions [21]. But there is no study on phytochemical analysis of *M. quadrifolia* using GC-MS. So, the present study was aimed to identify the phytocompounds of *M. quadrifolia* by GC-MS analysis. Up to date, this may be the first report on phytochemical analysis of *M. quadrifolia* using GC-MS.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh plants of *M. quadrifolia* were collected from natural habitats of Pathur Village, Thiruvarur District, Tamilnadu, India. The collected plants were washed thoroughly in running tap water to remove soil particles and adhered debris from it and finally washed with sterile distilled water. The stem and leaves of *M. quadrifolia* were separated and shade dried for 15 days at room temperature. Then the plant materials were ground well into fine powder. The powdered materials were stored in air tight containers until the time of use.

2.2 Preparation of Plant Extracts

The leaf and stem of *M. quadrifolia* extracts were prepared according to the previous research reports [3,11]. For this 20g of leaf and stem powder of *M. quadrifolia* was soaked in 200ml of methanol and kept in orbit shaker for 48h. After 48h, it was filtered through Whatman no 1 filter paper and then the volume of supernatant was concentrated at 40ºC using hot air oven. The concentrated methanolic extracts of leaf and stem of *M. quadrifolia* were used for GC-MS analysis.

2.3 GC- MS Analysis

The GC–MS analysis was carried out using a Perkin Elmer Clarus 500 [3]. The data were obtained on a Capillary Column Elite-5MS (5% phenyl 95% dimethyl poly siloxane). Helium (99.999%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 1µl of methanol solution of the sample was injected into the column with the injector temperature at 270ºC. GC oven temperature started at 110ºC and holding for 2min and it was raised to 200ºC at the rate of 10ºC/min without holding. Holding was allowed at 280ºC for 9min with program rate of 5ºC /min (50ºC @8ºC/min to 150ºC (5min)@ 8ºC/min to 250ºC (10min)). GC interface and Ion source temperature was maintained at 200ºC. The mass spectrum of compounds in the samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 40-450 amu (atomic mass units). A scan interval of 0.5 second and fragments from 40 to 450 Da were maintained. The total running time was 36minutes.

2.4 Identification of Phytocompounds

Interpretation of mass spectrum of plant extracts were conducted using the database of National Institute of Standard and Technology (NIST) library having more than 62,000 spectral patterns. The spectrum of the compounds was compared with the spectrum of NIST library database. The identity of the spectra above 95% was needed for the identification of compounds.

The name, molecular weight and structure of the components of the plant extracts were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area with the total area. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library, Turbomass ver. 5.2.0.

3. RESULTS

GC-MS is one of the best techniques to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids, esters etc. The result pertaining to GC-MS analysis leads to the identification of the number of compounds from the GC fractions of the methanolic extract of leaf and stem of *M. quadrifolia* and these compounds were identified through mass spectrometry attached with GC. The various components present in the leaf and stem of *M. quadrifolia* were detected by the GC-MS and which are shown in Tables 1 and 2. The compounds such as 2-Furanmethanol**;** 2-Furancarboxaldehyde, 5-methyl-; Phenol; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 2-Furancarboxaldehyde, 5-(hydroxymethyl)-; DL-Proline, 5-oxo-, methylester; D-Allose; 1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran -3a-ol and n-Hexadecanoic acid were

commonly present in both leaf and stem extracts of *M. quadrifolia.* The compounds like Butanoic acid, 2-methyl-; Butanoic acid, 4-hydroxy-; Ethyl(dimethyl)ethoxysilane; 1-Piperazinecarboxaldehyde; Benzeneacetaldehyde; 3,5-Octadien-2-one; 2-Pyrrolidinone; 1,2-Cyclopentanediol, 3-methyl-; (1S,2S)-(+)-2-Amino-3-methoxy-1-phenyl-1-propanol; 1,2,3-Propanetriol, monoacetate; 2-Methoxy-4-vinylphenol; Piperidin-2-one-5-carboxylic acid; Naphthalene,1,2-dihydro-1,5,8-trimethyl-; 1,3;2,5-Dimethylene-4-methyl-d-rhamnitol; à,à-Dimethyl-á-methylsuccinimide; Methyl-2,4-di-O-methylàd-glucopyranoside; Megastigmatrienone; 1,6;3,4-Dianhydro-2-O-acetyl-á-d-allopyranose; 3-Buten-2-one,4-(5-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-; 1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol; Glycyl-L-proline; Bicyclo [4.3.0] nonane, 2,2,6,7-tetramethyl-7-hydroxy-; Hexadecanoic acid, methyl ester; 9,12,15-Octadecatrien-1-ol (Z,Z,Z)-; 6,11-Eicosadienoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)-; Phytol; Z,E-2-Methyl-3,13-octadecadien-1-ol; 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)- and Eicosanoic acid were present in leaf extract of *M. quadrifolia*.

The stem extract of *M. quadrifolia* revealed that the presence of Furfural; N,N-Dinitropiperazine; 1,2-Cyclopentanedione; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; Pantolactone; 2,5-Furandicarboxaldehyde; 2,5-Furandicarboxaldehyde; Acetyl valeryl; 2(3H)-Furanone, dihydro-4-hydroxy-; 4-Ethyl-2-hydroxycyclopent-2-en-1-one; 2,5-Pyrrolidione, N-[2-(thienyl)acetyloxy]-; Methyl 4-O-acetyl-2,3-di-O-methyl-à-D-xylopyranoside; Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-; Ethyl N-(o-anisyl)formimidate; Phenol 4-methoxy-2-nitro-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Tetradecanoic acid, 12-methyl-, methyl ester, (S)-; Isophytol; 6-Octadecenoic acid, (Z)- and Octadecanoic acid. The GC-MS chromatogram of methanolic extracts of leaf and stem of *M. quadrifolia* revealed the presence of various compounds with corresponding peaks at different retention time (Figs. 1 and 2). The molecular formula, molecular weight, peak area %, retention time, nature of compound and biological activity of identified compounds of leaf and stem of *M. quadrifolia,* were represented in Tables 1 and 2. The biological activities of the phytocompounds of *M. quadrifolia* mentioned in the Tables 1 and 2 based on the phytochemical and ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

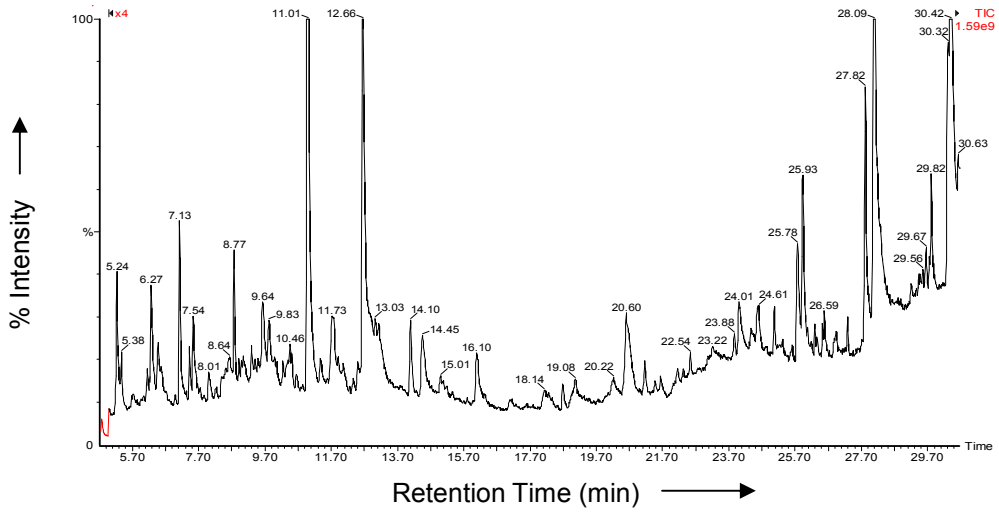

Fig. 1. GC-MS Chromatogram of methanolic extract of leaf *of M. quadrifolia*

Table 1. List of Identified phytocompounds of methanolic extracts of leaf of *M. quadrifolia* by GC-MS analysis

Name of the compound	Molecular formula	MW	Peak area %	RT	Nature of compound	Activity *
2-Furanmethanol	$C_5H_6O_2$	98	1.84	5.24	Furan alcohol	Antioxidant
Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	102	0.89	5.38	Fatty acid	Nf
Butanoic acid, 4-hydroxy-	$C_4H_8O_3$	104	1.24	6.27	--	Nf
2-Furancarboxaldehyde, 5-methyl-	C6H6O2	110	2.08	7.13	Furan aldehyde	Antimicrobial, Preservative
Ethyl(dimethyl)ethoxysilane	$C_6H_{16}O$	132	1.16	7.54	Silane compound	Nf
Phenol	C_6H_6O	94	0.47	8.01	Phenolic compound	Antioxidant, Antibacterial
1-Piperazinecarboxaldehyde	$C_5H_{10}N_2O$	114	0.19	8.64	Alkaloids	Nf
Benzeneacetaldehyde	C_8H_8O	120	1.20	8.77	Aromatic aldehyde	Nf
3,5-Octadien-2-one	$C_8H_{12}O$	124	1.17	9.64	Ketone	Nf
2-Pyrrolidinone	C_4H_7NO	85	0.62	9.83	Alkaloids	Nf
1,2-Cyclopentanediol, 3-methyl-	$C_6H_{12}O_2$	116	0.20	10.46	Alcohol	Nf
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	21.41	11.01	Flavonoid	Antimicrobial, Anti-inflammatory, Antiproliferative Antioxidant, Automatic nerve activity
(1S,2S)-(+)-2-Amino-3-methoxy-1-phenyl-1-propanol	$C_{10}H_{15}NO_2$	181	2.88	11.73	Alkaloid	Nf
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	9.39	12.66	Furan aldehyde	Antimicrobial, Preservative Clastogenic activity, Uterotonic activity
1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	134	0.10	13.03	Glycerol	Nf
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	1.24	14.10	Phenolic compound	Antioxidant
Piperidin-2-one-5-carboxylic acid	$C_6H_9NO_3$	143	2.38	14.45	Alkaloid	Nf
Naphthalene, 1,2-dihydro-1,5,8-trimethyl-	$C_{13}H_{16}$	172	0.14	15.01	Aromatic hydrocarbon	Nf
DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	2.04	16.10	Amino acid	Nf
1,3;2,5-Dimethylene-4-methyl-d-rhamnitol	$C_9H_{16}O_5$	204	0.21	18.14	Sugar	Nf
à,à-Dimethyl-á-methylsuccinimide	$C_7H_{11}NO2$	141	0.26	19.08	Cyclic amide	Nf
Methyl-2,4-di-O-methylàd-glucopyranoside	$C9H_{18}O_6$	222	0.96	20.22	Sugar	Nf
D-Allose	$C_6H_{12}O_6$	180	3.44	20.60	Sugar	Anticancer, Antioxidant
Megastigmatrienone	$C_{13}H_{18}O$	190	0.25	22.54	Terpene ketone	Nf
1,6;3,4-Dianhydro-2-O-acetyl-á-d-allopyranose	$C_8H_{10}O_5$	186	0.10	23.22	Sugar	Nf
3-Buten-2-one, 4-(5-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{20}O_2$	208	0.25	23.88	Ketone	Nf
1,2-tetradecanediol	$C_{14}H_{30}O_2$	230	0.29	24.01	Fatty alcohol	Nf
1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	$C_{14}H_{26}O_2$	226	1.36	24.61	Furan alcohol	Nf
Glycyl-L-proline	$C_7H_{12}N_2O_3$	172	2.17	25.78	Amino acid	Antimicrobial
Bicyclo[4.3.0]nonane, 2,2,6,7-tetramethyl-7-hydroxy-	$C_{13}H_{24}O$	196	2.10	25.93	--	Nf
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.02	26.59	Fatty acid methyl ester	Nf
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264	3.54	27.82	Fatty acid	Antioxidant, Antibacterial
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	17.47	28.09	Fatty acid	Antitumor, Antioxidant, Anti-inflammatory
6,11-Eicosadienoic acid, methyl	$C_{21}H_{38}O_2$	322	0.06	29.56	Methyl fatty acid	Nf
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.26	29.67	Fatty acid ethyl ester	Nf
Phytol	$C_{20}H_{40}O$	296	1.38	29.82	Diterpene	Nf
Z,E-2-Methyl-3,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	0.69	30.32	Fatty alcohol	Nf
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	12.96	30.42	Fatty acid methyl ester	Antimalarial, Antioxidant
Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.35	30.63	Fatty acid	Nf

RT – Retention Time, MW – Molecular Weight, Nf – Not found; * Dr. Duke's ethanobotanical databases

Table 2. List of Identified phytocompounds of methanolic extracts of stem of *M. quadrifolia* by GC-MS analysis

Name of the compound	Molecular formula	MW	Peak Area %	RT	Nature of compound	Activity *
Furfural	$C_5H_4O_2$	96	2.23	4.76	Heterocyclic aldehyde	Nf
2-Furanmethanol	$C_5H_6O_2$	98	1.69	5.22	Furan alcohol	Antioxidant
N,N-Dinitropiperazine	$C_4H_8N_4O_4$	176	0.40	6.27	Nitramines	Antimicrobial
1,2-Cyclopentane dione	$C_5H_6O_2$	98	0.89	6.55	Aliphatic	Nf
2-Furancarbox aldehyde, 5-methyl-	$C_6H_6O_2$	110	1.54	7.12	Furan aldehyde	Antimicrobial, Preservative
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	0.60	7.41	Furan ketone	Antioxidant
Phenol	C_6H_6O	94	0.64	8.00	Phenolic compound	Antioxidant, Antibacterial
Pantolactone	$C_6H_{10}O_3$	130	0.20	8.98	--	Nf
2,5-Furandicarbox aldehyde	$C_6H_4O_3$	124	0.08	9.54	Furan aldehyde	Antifungal
2,5-Furandicarbox aldehyde	$C_6H_4O_3$	124	1.47	9.63	Furan aldehyde	Antifungal
Acetyl valeryl	$C_7H_{12}O_2$	128	0.31	10.46	--	Antibacterial
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	13.88	11.04	Flavonoid	Antimicrobial, Anti-inflammatory, Antiproliferative Antioxidant, Automatic nerve activity
2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	102	0.37	11.90	Furan ketone	Nf
2-Furancarbox aldehyde, 5-(hydroxymethyl)-	$C_4H_6O_3$	126	60.42	12.85	Furan aldehyde	Antimicrobial, Preservative Clastogenic activity, Uterotonic activity
4-Ethyl-2-hydroxy cyclopent-2-en-1-one	$C_7H_{10}O_2$	126	0.26	13.09	Aliphatic ketone	Nf
2,5-Pyrrolidione, N-[2-(thienyl)acetyloxy]-	$C_{10}H_9NO_4S$	239	1.54	14.51	--	Nf
DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	0.85	16.11	Amino acid	Nf
Methyl 4-O-acetyl-2,3-di-O-methyl-à-D-xylopyranoside	$C_{10}H_{18}O_6$	234	0.05	18.28	Sugar derivative	Antiproliferative activity
Butanamide, 2 hydroxy-N,2,3,3-tetramethyl-	$C_8H_{17}NO_2$	159	0.47	19.07	Amino compound	Nf
D-Allose	$C_6H_{12}O_6$	180	1.21	20.62	Sugar	Anticancer, Antioxidant
Ethyl N-(oanisyl) formimidate	$C_{10}H_{13}NO_2$	179	0.14	22.16	-	Nf
Phenol, 4-methoxy-2-nitro-	$C_7H_7NO_4$	169	0.75	24.02	-	Antioxidant
1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	$C_{14}H_{26}O_2$	226	0.27	24.57	Furan alcohol	Nf
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.44	25.92	Diterpene	Nf
Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	$C_{16}H_{32}O_2$	256	0.29	27.29	Fatty acid methyl ester	Nf
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.00	28.10	Fatty acid	Antitumor, Antioxidant, Anti-inflammatory
Isophytol	$C_{20}H_{40}O$	296	0.10	29.80	Diterpene	Antimicrobial,anti diabetic, antiarthiritis
6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	2.69	30.35	Fatty acid	Nf
Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.09	30.58	Fatty acid	Antibacterial, Antifungal

RT – Retention Time, MW – Molecular Weight, Nf – Not found; * Dr. Duke's ethanobotanical databases

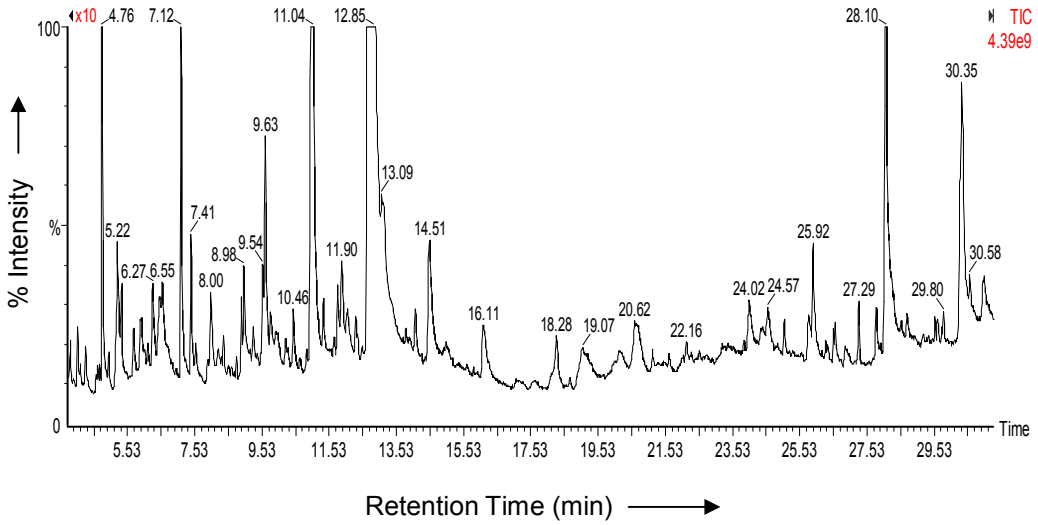

Fig. 2. GC-MS Chromatogram of methanolic extract of stem of *M. quadrifolia*

4. DISCUSSION

Plants have been used in the traditional systems of medicine for thousands of years. Traditional knowledge of medicinal plants has always guided the search for new drugs. Authentication of medicinal plants in genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations [4]. In spite of the advantage of modern high throughput drug discovery and screening techniques, traditional medicinal knowledge have also given clues to the discovery of valuable drugs [22,23]. There is growing awareness in correlating the phytochemical compounds with their biological activities [24,25,26,27].

In the present study, we characterized the phytochemical profile of leaf and stem of *M. quadrifolia* using GC- MS. As per Dr. Duke's ethnobotanical database, the compounds such as furan compounds, phenolic compounds, flavonoids, sugar derivatives, fatty acid esters and diterpenes posses antioxidant, antibacterial, antimicrobial, anti-inflammatory, antiproliferative, automatic nerve activity, anticancer, antitumor, antidiabetic, antiarthiritic and antimalarial activities, which are present in the leaf and stem of *M. quadrifolia*. In our previous study, we confirmed that the antibacterial and antifungal activities of leaf and stem of *M. quadrifolia* [11]. In that study antibacterial activity against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens and Streptococcus pyogenes* and antifungal activity against *Aspergillus niger*, *Aspergillus flavus, Aspergillus terreus*, *Trichoderma viride* and *Fusarium solani* were confirmed. The presence of phytocompounds like phenolic compounds and flavonoids in leaf and stem of *M. quadrifolia* may be responsible for the antibacterial and antifugal activities. Several phytochemical screening studies have been carried out in different parts of the world using GC-MS [28,29,30,31,32,33].

The gas chromatogram showed that the relative concentration of various compounds getting eluted at different retention times. The heights of the peak indicate that the relative concentrations of the components present in the plant extract. The mass spectrometer

analysis used to identify the nature and structure of the compounds eluted at different retention times. The large fragments into small compounds giving rise to appearance of peaks at different m/z ratios. The mass spectra of plant extract are the fingerprint of phytocompounds of methanolic extracts of leaf and stem of *M. quadrifolia* which were identified from the NIST data library. Hence, the results of the GC-MS profile of *M. quadrifolia* showed the presence of many phytocompounds including phenolic compounds, alkaloides and flavonoids. The prediction of the biological activities of the phytocompounds in *M. quadrifolia* by applying the Duke's ethnobotanical databases was confirmed, supported and supplemented.

5. CONCLUSION

In the present study, the identified phytocompounds of *M. quadrifolia* with molecular formula and structure, which may be used for drug development. This study may also be enhances the traditional usage of *M. quadrifolia* due to its bioactive compounds identified by GC-MS analysis. Further investigation is required for the pharmacological activity of specific compound of *M. quadrifolia,* which may lead to the development of new drug for the treatment of specific disease. Thus the GC-MS analysis is the first step towards understanding the nature of active principles in *M. quadrifolia.* This study will also be helpful to further pharmacological study for researchers and drug development in pharmaceutical industry.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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