

Original Research Article

GC-MS Analysis of Phytocompounds of Leaf and Stem of *Marsilea quadrifolia*

ABSTRACT

Objective: To investigate the phytochemical constituents of methanolic extracts of leaf and stem of *Marsilea quadrifolia* (Linn.).

Methods: 20 g powder of leaf and stem of *M. quadrifolia* was soaked with 200 ml of methanol and kept in shaker for 48 hours at room temperature. After 48h, the mixer was filtered using a Whatman no.1 filter paper and the supernatant was concentrated at 40 °C using hot air oven. The concentrated methanolic extracts were subjected to phytochemical analysis using GC-MS.

Results: The GC-MS analyses 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 different bioactive compounds including 2-Furancarboxaldehyde,5-(hydroxymethyl)-(60.42%); 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxyl-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 different 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.

Keywords: Bioactive compounds; chromatogram; GC-MS analysis; methanolic extracts; *Marsilea quadrifolia*.

1. INTRODUCTION

Plants are used as medicine in many countries and also act as a source for many potent drugs [1]. A large number of medicinal plants and their purified constituents have shown therapeutic activities [2]. Natural remedies from medicinal plants proved as safe and effective. Many plant species have been used in folklore medicine to treat various ailments [3]. Phytochemicals from medicinal plants are important in pharmaceutical industry for drug development and preparation of therapeutic agents [4]. 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 [5]. *Marsilea quadrifolia* is an important medicinal plant belongs to the family of Marsileaceae. It is used in the ayurvedic system of medicine for curing several ailments. *M. quadrifolia* showed antibacterial, cytotoxic and antioxidant activities [6]. A juice made from the leaves of *M. quadrifolia* possess anti-inflammatory, anti-diuretic and febrifuge activities and also used to treat snakebite and abscesses [7, 8]. *M. quadrifolia* is also used to treat cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea and skin diseases [9]. But there is no study on phytochemical analysis of *M. quadrifolia* using GC-MS. So, the present study was aimed to identify the phytochemicals 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. MATERIAL 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

20 g of leaf and stem powder of *M. quadrifolia* was soaked in 200 ml of methanol and kept in orbit shaker for 48 h. After 48h, it was filtered through Whatman no 1 filter paper and then the volume of the supernatant was concentrated at 40 °C 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. 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 270 °C. GC oven temperature started at 110 °C and holding for 2 min and it was raised to 200 °C at the rate of 10 °C /min without holding. Holding was allowed at

280 °C for 9 min with program rate of 5 °C /min (50 °C @8 °C /min to 150 °C (5 min)@ 8 °C /min to 250 °C (10 min)). GC interface and Ion source temperature was maintained at 200 °C. 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 36 minutes.

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 extracted 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 (2005).

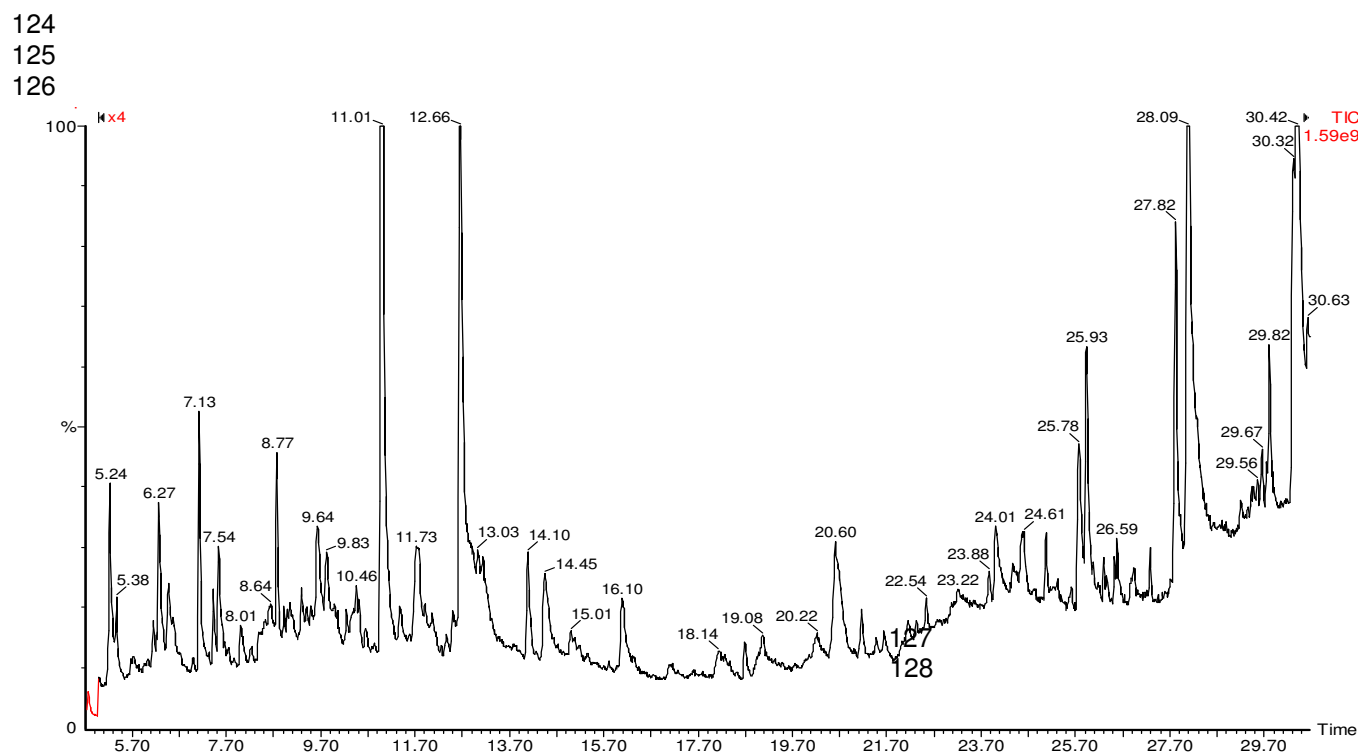
3. RESULTS

GC-MS is one of the best technique 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 stem and leaf 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

89 Table 1 and 2. The compounds such as 2-Furanmethanol; 2-Furancarboxaldehyde, 5-
90 methyl-; Phenol; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-;
91 2-Furancarboxaldehyde, 5-(hydroxymethyl)-; DL-Proline, 5-oxo-, methylester; D-Allose;
92 1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran -3a-ol and n-Hexadecanoic acid were
93 commonly present in both leaf and stem extracts of *M. quadrifolia*. The compounds like
94 Butanoic acid, 2-methyl-; Butanoic acid, 4-hydroxy-; Ethyl(dimethyl)ethoxysilane; 1-
95 Piperazinecarboxaldehyde; Benzeneacetaldehyde; 3,5-Octadien-2-one; 2-Pyrrolidinone;
96 1,2-Cyclopentanediol, 3-methyl-; (1S,2S)-(+)-2-Amino-3-methoxy-1-phenyl-1-propanol;
97 1,2,3-Propanetriol, monoacetate; 2-Methoxy-4-vinylphenol; Piperidin-2-one-5-carboxylic
98 acid; Naphthalene,1,2-dihydro-1,5,8-trimethyl-; 1,3;2,5-Dimethylene-4-methyl-d-rhamnitol;
99 à,à-Dimethyl-à-methylsuccinimide; Methyl-2,4-di-O-methylà-glucopyranoside;
100 Megastigmatrienone; 1,6;3,4-Dianhydro-2-O-acetyl-à-d-allopyranose; 3-Buten-2-one,4-(5-
101 hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-; 1-Isobutyl-7,7-dimethyl-octahydro-
102 isobenzofuran-3a-ol; Glycyl-L-proline; Bicyclo[4.3.0]nonane, 2,2,6,7-tetramethyl-7-hydroxy-;
103 Hexadecanoic acid, methyl ester; 9,12,15-Octadecatrien-1-ol (Z,Z,Z)-; 6,11-Eicosadienoic
104 acid, methyl ester; 9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)-; Phytol; Z,E-2-
105 Methyl-3,13-octadecadien-1-ol; 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)-
106 and Eicosanoic acid were present in leaf extract of *M. quadrifolia*.

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108 The stem extract of *M. quadrifolia* revealed that the presence of Furfural; N,N-
109 Dinitropiperazine; 1,2-Cyclopentanedione; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one;
110 Pantolactone; 2,5-Furandicarboxaldehyde; 2,5-Furandicarboxaldehyde; Acetyl valeryl;
111 2(3H)-Furanone, dihydro-4-hydroxy-; 4-Ethyl-2-hydroxycyclopent-2-en-1-one; 2,5-
112 Pyrrolidione, N-[2-(thienyl)acetyloxy]-; Methyl 4-O-acetyl-2,3-di-O-methyl-à-D-
113 xylopyranoside; Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-; Ethyl N-(o-anisyl)formimidate;
114 Phenol 4-methoxy-2-nitro-; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Tetradecanoic acid,

115 12-methyl-, methyl ester, (S)-; Isophytol; 6-Octadecenoic acid, (Z)- and Octadecanoic
 116 acid. The GC-MS chromatogram of methanolic extracts of leaf and stem of *M. quadrifolia*
 117 revealed the presence of various compounds with corresponding peaks at different retention
 118 time (Figures 1 and 2). The molecular formula, molecular weight, peak area %, retention
 119 time and biological activity of compounds, which are present in the leaf and stem of
 120 *M. quadrifolia*, were represented in Table 1 and 2. The biological activities of the
 121 phytocompounds of *M. quadrifolia* mentioned in the Table 1 and 2 were given based on the
 122 phytochemical and ethnobotanical databases by Dr. Jim Duke of the Agricultural Research
 123 Service/USDA.



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

132 (X axis - Retention time and Y axis - % intensity / % abundance)

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

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Name of the compound	Molecular Formula	MW	Peak Area %	RT	Activity
2-Furanmethanol	C ₅ H ₆ O ₂	98	1.84	5.24	Antioxidant
Butanoic acid, 2-methyl-	C ₅ H ₁₀ O ₂	102	0.89	5.38	Nf
Butanoic acid, 4-hydroxy-	C ₄ H ₈ O ₃	104	1.24	6.27	Nf
2-Furancarboxaldehyde, 5-methyl-	C ₆ H ₆ O ₂	110	2.08	7.13	Antimicrobial, Preservative
Ethyl(dimethyl)ethoxysilane	C ₆ H ₁₆ OSi	132	1.16	7.54	Nf
Phenol	C ₆ H ₆ O	94	0.47	8.01	Antioxidant, antibacterial
1-Piperazinecarboxaldehyde	C ₅ H ₁₀ N ₂ O	114	0.19	8.64	Nf
Benzeneacetaldehyde	C ₈ H ₈ O	120	1.20	8.77	Nf
3,5-Octadien-2-one	C ₈ H ₁₂ O	124	1.17	9.64	Nf
2-Pyrrolidinone	C ₄ H ₇ NO	85	0.62	9.83	Nf
1,2-Cyclopentanediol, 3-methyl-	C ₆ H ₁₂ O ₂	116	0.20	10.46	Nf
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144	21.41	11.01	Flavonoid fraction, 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	Nf
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C ₆ H ₆ O ₃	126	9.39	12.66	Aldehyde, Antimicrobial, Preservative Clastogenic activity

1,2,3-Propanetriol, monoacetate	C ₅ H ₁₀ O ₄	134	0.10	13.03	Nf
2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	1.24	14.10	Antioxidant
Piperidin-2-one-5-carboxylic acid	C ₆ H ₉ NO ₃	143	2.38	14.45	Nf
Naphthalene, 1,2-dihydro-1,5,8-trimethyl-	C ₁₃ H ₁₆	172	0.14	15.01	Nf
DL-Proline, 5-oxo-, methyl ester	C ₆ H ₉ NO ₃	143	2.04	16.10	Nf
1,3;2,5-Dimethylene-4-methyl-d-rhamnitol	C ₉ H ₁₆ O ₅	204	0.21	18.14	Nf
à,à-Dimethyl-á-methylsuccinimide	C ₇ H ₁₁ NO ₂	141	0.26	19.08	Nf
Methyl-2,4-di-O-methylàd-glucopyranoside	C ₉ H ₁₈ O ₆	222	0.96	20.22	Nf
D-Allose	C ₆ H ₁₂ O ₆	180	3.44	20.60	Anticancer, antioxidant
Megastigmatrienone	C ₁₃ H ₁₈ O	190	0.25	22.54	Nf
1,6;3,4-Dianhydro-2-O-acetyl-á-d-allopyranose	C ₈ H ₁₀ O ₅	186	0.10	23.22	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	Nf
1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	C ₁₄ H ₂₆ O ₂	226	1.36	24.61	Nf
Glycyl-L-proline	C ₇ H ₁₂ N ₂ O ₃	172	2.17	25.78	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	Nf
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	C ₁₈ H ₃₂ O	264	3.54	27.82	Sugar, Antioxidant, antibacterial
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	17.47	28.09	Palmitic acid, Antitumor, Antioxidant anti-inflammatory
6,11-Eicosadienoic acid, methyl	C ₂₁ H ₃₈ O ₂	322	0.06	29.56	Nf

9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	C ₂₀ H ₃₄ O ₂	306	0.26	29.67	Ester
Phytol	C ₂₀ H ₄₀ O	296	1.38	29.82	Nf
Z,E-2-Methyl-3,13-octadecadien-1-ol	C ₁₉ H ₃₆ O	280	0.69	30.32	Nf
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292	12.96	30.42	Antimalarial, Antioxidant
Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312	0.35	30.63	Ester

RT – Retention Time, MW – Molecular Weight, Nf – Not found

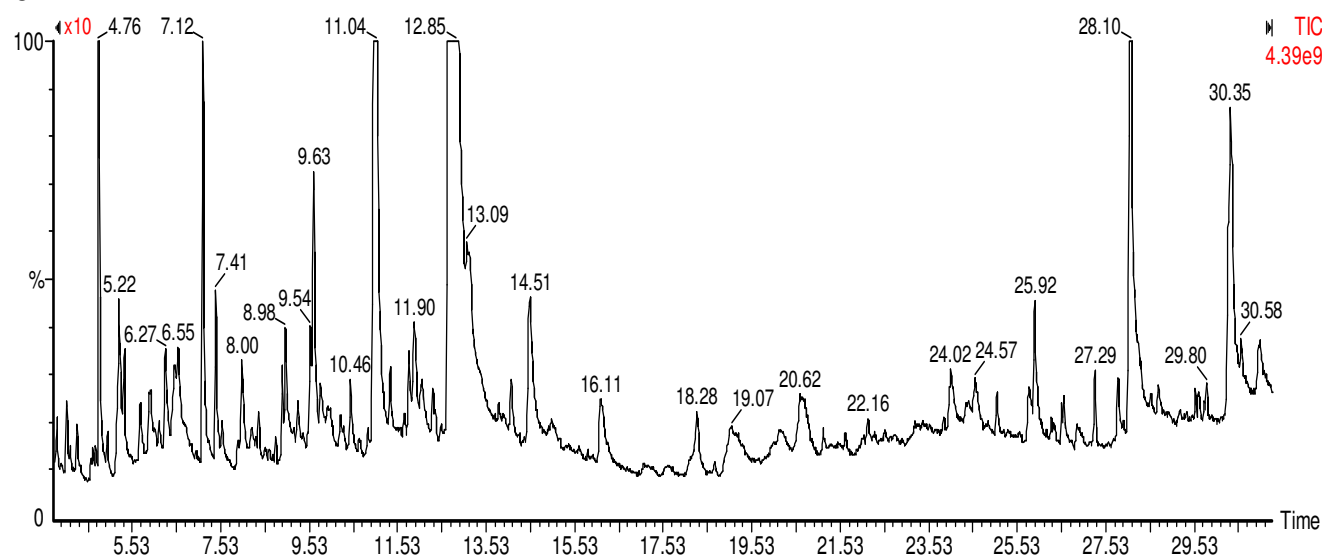


Fig 2. GC-MS Chromatogram of methanolic extract of stem of *M. quadrifolia*.

(X axis - Retention time and Y axis - % intensity / % abundance)

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

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Name of the compound	Molecular Formula	MW	Peak Area %	RT	Activity
Furfural	C ₅ H ₄ O ₂	96	2.23	4.76	Nf
2-Furanmethanol	C ₅ H ₆ O ₂	98	1.69	5.22	Antioxidant
N,N-Dinitropiperazine	C ₄ H ₈ N ₄ O ₄	176	0.40	6.27	Antimicrobial
1,2-Cyclopentanedione	C ₅ H ₆ O ₂	98	0.89	6.55	Nf
2-Furancarboxaldehyde, 5-methyl-	C ₆ H ₆ O ₂	110	1.54	7.12	Antimicrobial, Preservative
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144	0.60	7.41	Antioxidant
Phenol	C ₆ H ₆ O	94	0.64	8.00	Antioxidant, antibacterial
Pantolactone	C ₆ H ₁₀ O ₃	130	0.20	8.98	Nf
2,5-Furandicarboxaldehyde	C ₆ H ₄ O ₃	124	0.08	9.54	Nf
2,5-Furandicarboxaldehyde	C ₆ H ₄ O ₃	124	1.47	9.63	Antifungal
Acetyl valeryl	C ₇ H ₁₂ O ₂	128	0.31	10.46	Antibacterial
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C ₆ H ₈ O ₄	144	13.88	11.04	Flavonoid fraction, antimicrobial, anti-inflammatory, antiproliferative Antioxidant, Automatic nerve activity
2(3H)-Furanone, dihydro-4-hydroxy-	C ₄ H ₆ O ₃	102	0.37	11.90	Nf
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C ₄ H ₆ O ₃	126	60.42	12.85	Aldehyde, Antimicrobial, Preservative Clastogenic activity

4-Ethyl-2-hydroxycyclopent-2-en-1-one	C ₇ H ₁₀ O ₂	126	0.26	13.09	Nf
2,5-Pyrrolidione, N-[2-(thienyl)acetyloxy]-	C ₁₀ H ₉ NO ₄ S	239	1.54	14.51	Nf
DL-Proline, 5-oxo-, methyl ester	C ₆ H ₉ NO ₃	143	0.85	16.11	Nf
Methyl 4-O-acetyl-2,3-di-O-methyl-à-D-xylopyranoside	C ₁₀ H ₁₈ O ₆	234	0.05	18.28	Antiproliferative activity
Butanamide, 2-hydroxy-N,2,3,3-tetramethyl-	C ₈ H ₁₇ NO ₂	159	0.47	19.07	Nf
D-Allose	C ₆ H ₁₂ O ₆	180	1.21	20.62	Anticancer, antioxidant
Ethyl N-(o-anisyl)formimidate	C ₁₀ H ₁₃ NO ₂	179	0.14	22.16	Nf
Phenol, 4-methoxy-2-nitro-	C ₇ H ₇ NO 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	Nf
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	0.44	25.92	Nf
Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	C ₁₆ H ₃₂ O ₂	256	0.29	27.29	Nf
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	6.00	28.10	Palmitic acid, Antitumor, Antioxidant, anti-inflammatory
Isophytol	C ₂₀ H ₄₀ O	296	0.10	29.80	Antimicrobial, antidiabetic, arthritis
6-Octadecenoic acid, (Z)-	C ₁₈ H ₃₄ O ₂	282	2.69	30.35	Nf
Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	0.09	30.58	Antibacterial, Antifungal

RT – Retention Time, MW – Molecular Weight, Nf – Not found

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156 4. DISCUSSION

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158 Plants have been used in the traditional systems of medicine for thousands of years.

159 Traditional knowledge of medicinal plants has always guided the search for new drugs. In

160 spite of the advantage of modern high throughput drug discovery and screening techniques,

161 traditional medicinal knowledge have also given clues to the discovery of valuable drugs

162 [10, 11]. There is growing awareness in correlating the phytochemical compounds with their

163 biological activities [12, 13, 14, 15].

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165 In the present study, we characterized the phytochemical profile of leaf and stem of

166 *M. quadrifolia* using GC- MS. The gas chromatogram showed that the relative concentration

167 of various compounds getting eluted at different retention times. The heights of the peak

168 indicate that the relative concentrations of the components present in the plant extract. The

169 mass spectrometer analysis used to identify the nature and structure of the compounds

170 eluted at different retention times. The large fragments into small compounds giving rise to

171 appearance of peaks at different m/z ratios. The mass spectra of plant extract are a

172 fingerprint of phytocompounds of methanolic extracts of *M. quadrifolia* which were identified

173 from the NIST data library. Some of the Identified phytocompounds by GC-MS analysis has

174 been proved their antioxidant, antimicrobial, antimalarial, anticancer, antidiabetic, anti-

175 inflammatory, antitumor, and antiproliferative activities [16]. Hence, the results of the GC-MS

176 profile of *M. quadrifolia* showed the presence of many phytocompounds including phenolic

177 compounds and flavonoids. The result of the present study has supported by the previous

178 observations in other plant species [11, 17].

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182 5. CONCLUSION

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

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196 instrumental facilities.

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199 CONFLICT OF INTEREST STATEMENT

200 We declare that we have no conflict of interest.

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202 AUTHORS' CONTRIBUTIONS

203 The first author KG carried out the research work and wrote the manuscript under the
204 supervision of author RU and the second author RU designed the research problem and
205 corrected the manuscript. Both authors read and approved the final manuscript.

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