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GC-MS Analysis of Methanolic Extracts of Leaf and Stem of *Marsilea minuta* (Linn.)

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Authors' contributions

This work was carried out in collaboration between both authors. The corresponding author RU designed the research problem and wrote the protocol. The first author GS performed the research work and wrote the initial draft of manuscript. The corresponding author RU corrected the final format of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To analyze the chemicals composition of methanolic extracts of leaf and stem of *M. minuta* collected from Uppur Village, Tamilnadu, India by GC-MS.
Study Design: Experimental study.
Methodology: The Methanolic extracts were prepared and concentrated at 40℃ using hot air oven. The concentrated methanolic extracts were subjected to GC-MS analysis using the instrument Perkin Elmer Clarus 500.
Results: The methanolic extract of leaf of *M. minuta* showed the presence of 36 phyto compounds including n-Hexadecanoic acid (44.41%) ($C_{16}H_{32}O_2$); (Z)6,(Z)9-Pentadecadien-1-ol (35.49%) ($C_{15}H_{28}O$); Phytol (5.10%) ($C_{20}H_{40}O$); 2-Cyclohexane-1-one,4-hydroxy-3,5,6-trimethyl-4 (3-oxo-1-butenyl) (2.25%) ($C_{13}H_{18}O_3$); 9,12,15-Octadecatrienoic acid (Z,Z,Z) (2.0%) ($C_{18}H_{30}O_2$); 3,7,11,15-Tetramethyl-2-hexadecan-1-ol (1.99%) ($C_{20}H_{40}O$) and Benzofuran, 2,3-dihydro- (1.1%) (C_8H_8O). The methanolic extract of stem of *M. minuta* showed the presence of 27 bioactive compounds

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including 1,19-Eicosadiene (40.94%) ($C_{20}H_{38}$); n-Hexadecanoic acid (20.13%) ($C_{16}H_{32}O_2$); 2(R)3(S)-1,2,3,4-Butanetetrol (16.31%) ($C_4H_{10}O_4$); Glycerin (6.70%) ($C_3H_8O_3$); Octadecanoic acid (4.24%) ($C_{18}H_{36}O_2$); Benzofuran, 2,3-dihydro- (1.73%) (C_8H_8O); 3,7,11,15 – Tetramethyl-2 hexane decen-1-ol (1.55%) ($C_{20}H_{40}O$); n-Hexadecanoic acid methyl ester (1.15%) ($C_{17}H_{34}O_2$) and 10-Octadecenoic acid methyl ester (1.42%) ($C_{19}H_{36}O_2$).

Conclusion: The results of the present study concluded that the presence of various phytocompounds in leaf and stem of *M. minuta*. Therefore, plants are the rich sources of chemicals but largely unknown and unexplored. So, it is our hope that this study will encourage further research on isolation and purification of therapeutically important phytocompounds from *M. minuta*

Keywords: Marsilea minuta; phytocompounds; chromatogram; GC-MS analysis; leaf; stem.

1. INTRODUCTION

Plant is a source of medicinal agents for a long time. Medicinal plants have been used for years in daily life to treat various diseases [1]. India has the richest and most diverse cultural traditions especially with the use of traditional systems of medicine. Nutraceuticals are the alternative forms of conventional medicine with a view of accomplishing desirable therapeutic outcomes and reduction in side effects compared with synthetic therapeutic agents [2]. People have been using medicinal plants based on their acclaimed therapeutic values and till date over 85,000 medicinal plants with various therapeutic benefits have been identified and documented globally [3]. The traditional medicine in India consists of different components such as Ayurveda, Siddha, Unani, Homeopathy and Naturopathy [4]. Medicinal plants are the source of drugs in traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for drug synthesis [5]. Medicinal plants have been used in the folkloric medicine for the treatment of number of diseases [6]. Medicinal plants and their purified compounds have shown pharmacological properties [7]. The analysis of plant extracts has been a great interest for researchers to identify new pharmacologically important molecules of new drugs that will be useful to treat diseases [8].

The identification of bioactive chemical compounds from medicinal plants is an important task in the pharmaceutical industry for drug development and preparation of medicines [9]. Some microorganisms are the primary source for the synthesis of antibiotics which are used in the treatment of diseases. However, the screening of medicinal plants for bioactive compounds against diseases is an important field and it may be serve as an alternatives means for the synthesis of antibiotics [10,11].

Marsilea minuta is a common aquatic or sub-aquatic fern. It is a member of *Marsileaceae* family [12,13]. *M. minuta* is an important medicinal plant and it is distributed worldwide. It comprises 53 well defined living and 10 fossil species, among these 9 species are found in India [14,15]. *M. minuta* leaves and shoots are commonly used as vegetables. It is used in the treatment of cough and respiratory disorders. Juice of fresh shoots and decoction of leaves of *M. minuta* are used to treat cough [16,17]. Traditionally, *M. minuta* is used to treat indigestion, nose bleeding, kidney infection, toxicity and hepatitis [18]. *M. minuta* is also used to treat psychopathic conditions, diarrhoeal, respiratory and skin diseases [19,20]. The whole plant of *M. minuta* is used as astringent, expectorant, digestive and diuretic drug [21].

The medicinal plant *M. minuta* is used to treat insomnia and mental problems. The regular usage of the plant *M. minuta* as green vegetables is believed to exert favorable effects on hypertension, sleeping disorder, bronchitis, fever and headache [12]. It is also recommended for the treatment of spastic condition of leg muscle, epilepsy and migraine [12,19,22]. Leaves of *M. minuta* are prescribed by folk medical practitioners to treat diabetes and gastrointestinal disorders [23,24]. In some mental clinics, the decoction of *M. minuta* could be prescribed to patients with psychological disorders along with their meal [25]. *M. minuta* is reported to possess CNS active principles, so it is recommended for the treatment of various neurological disorders [26]. It has also been reported to possess hepatoprotective and anti-aggressive properties [27,28].

In vitro antibacterial activity of leaf extract of *M. minuta* against human pathogens has been reported [29]. Marsiline is an important active compound and its chemical name is ester of 1-triacontanol and hexacosanoic acid isolated from *M. minuta*. The researchers reported that the marsiline possess sedative, anticonvulsant, antidepressant, hypocholesterolemic and antifertility properties [30,31,32]. The phytochemical constituents of leaf of *M. crenata* using GC-MS have been reported [33]. The anti-stress activity of *M. minuta* may be potentially valuable for the treatment of stress and stress related disorders [34]. *M. minuta* has also been reported to possess antihepatotoxic properties [35].

The antimicrobial activity of rhizome of *M. minuta* against some human bacterial and fungal pathogens has been reported [36] and the extract of *M. minuta* has been shown to possess antioxidant [37] and antidiabetic [38] properties. Several studies have been conducted using *M. minuta*, but no study has characterized the active phytocompounds present in the leaf and stem of *M. minuta*. So, the present study was designed to characterize the phytocompounds present in the leaf and stem of *M. minuta* using GC-MS.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The fresh plants of *M. minuta* were collected from natural habitats of Uppur Village, Thiruvarur District, Tamilnadu, India. The collected plant was identified by Rev. Dr. S. John Britto, Director, Rabinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and deposited in the herbarium (Voucher specimen number: KG 002). The collected plants were brought into the laboratory and washed thoroughly in running tap water to remove the soil particles and adhered debris and then finally washed with sterile distilled water. The leaf and stem of *M. minuta* were separated and dried under shade for 10 days at room temperature. Then the plant materials were pulverized into 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. minuta* extracts were prepared according to previously reported procedure [39]. For this, 50 g of leaf and stem powder of *M. minuta* was soaked in 500 ml of methanol and kept in orbital shaker for 48h. After 48h, it was filtered through Whatman no. 1 filter paper (125 mm) and then the supernatant was concentrated at 40℃ till the solvent evaporated completely using hot air oven. The concentrated methanolic extracts of leaf and stem of *M. minuta* were subjected to GC-MS analysis.

2.3 GC- MS Analysis

The GC–MS analysis was performed to identify the chemical compounds present in the leaf and stem of *M. minuta* by using an instrument Perkin Elmer Clarus 500 [39]. 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 maintained at 270℃. GC oven temperature started at 110℃ and holding for 2min and it was raised to 200℃ at the rate of 10℃/min without holding. Holding was allowed at 280℃ for 9min with the program rate of 5℃/min (60℃@8℃/min to 230℃ (5min)@6℃/min to 280℃ (10min)). GC interface and ion source temperature was maintained at 200℃. The mass spectrum of compounds in the sample was obtained by electron ionization at 70eV 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 450Da were maintained. The total running time was 36 minutes.

2.4 Identification of Chemical Compounds

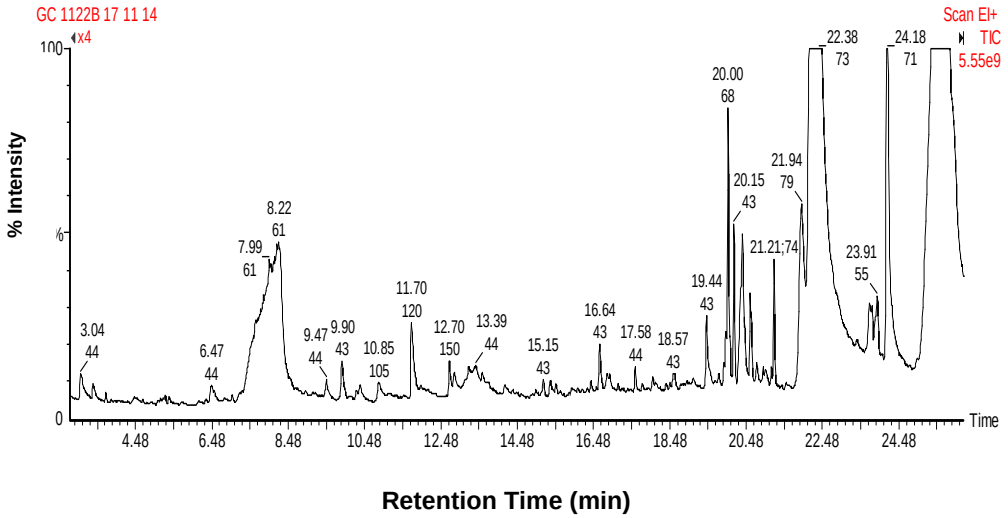
Interpretation of mass spectra of the extracts of leaf and stem of *M. minuta* was conducted using the database of National Institute of Standard and Technology [NIST] library. The library has more than 62,000 spectral patterns. The spectrum of the compound was compared with the spectrum of NIST library database. The identity of the spectra above 95% was needed for the identification of compounds. The name, molecular weight and structure of the compounds identified and characterized from the extracts of leaf and stem of *M. minuta* 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 using the Turbomass version 5.2.0.

3. RESULTS

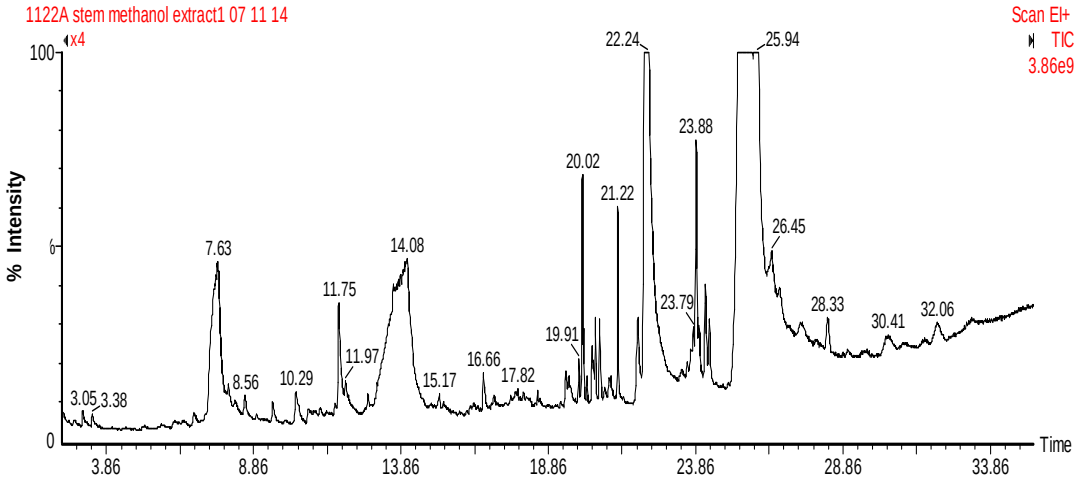
The GC-MS chromatogram of methanolic extracts of leaf and stem of *M. minuta* revealed the presence of various compounds with corresponding peaks at different retention time [Figs. 1 and 2]. The molecular formula, molecular weight, peak area %, retention time, nature and biological activities of compounds of methanolic extracts of leaf and stem of *M. minuta* were represented in Tables 1 and 2.

The phytocompounds such as Glycerin ($C_3H_8O_3$); Benzofuran,2,3-dihydro- (C_8H_8O); 2-Methoxy-4-vinylphenol ($C_9H_{10}O_2$); Dodecanoic acid ($C_{12}H_{24}O_2$); 3,7,11,15 – Tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$); 2-Pentadecanone,6,10,14-trimethyl- ($C_{18}H_{36}O$); Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$); n-Hexadecanoic acid ($C_{16},H_{32}O_2$) and Phytol ($C_{20}H_{40}O$) were determined in leaf and stem of *M. minuta.*



Retention Time (min)

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



Retention Time (min)

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

Table 1. List of compounds identified and characterized from the methanolic extract of leaf of *M. minuta*

Name of the compound	Molecular formula	MW	Peak area %	RT	Nature of compound	Activity *
Hexanal	$C_6H_{12}O$	100	0.4511	3.04	Aldehyde	Antifungal
3-Amino-2-oxazolidinone	$C_3H_6N_2O_2$	102	0.1607	3.35	-	Nf
1-Butanamine, N-butylidene-	$C_8H_{17}N$	127	0.0467	3.70	-	Nf
Pyrazine, methyl-	$C_5H_6N_2$	94	0.0119	3.84	-	Nf
Bicyclo[2.1.1]hex-2-ene, 2-ethenyl-	C_8H_{10}	106	0.1461	4.46	-	Nf
Hexanoic acid, 2-oxo-, methyl ester	$C_7H_{12}O_3$	144	0.0071	5.07	-	Nf
1-Butanamine, 3-methyl-N-(3-methylbutylidene)-	$C_{10}H_{21}N$	155	0.0300	5.25	-	Nf
1-Butanamine, N-butylidene-	$C_8H_{17}N$	127	0.0308	5.36	-	Nf
Pentylamine, N-isobutyl-N-nitroso-	$C_{10}H_{22}N_2O$	186	0.0404	7.01	-	Nf
Glycerin	$C_3H_8O_3$	92	0.1108	7.98	Sugar alcohol	Antimicrobial
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.4866	9.89	Flavonoid compound	Antioxidant, Antimicrobial, Anti-inflammatory, Antiproliferative
1-[1-(1-Hydroxy-butyl)-cyclopentyl]-2-phenyl-ethanone	$C_{17}H_{24}O_2$	260	0.2215	10.35	-	Nf
Benzenecarboxylic acid	$C_7H_6O_2$	122	0.2488	10.85	Phenolic acid	Antibacterial, Antimicrobial
Benzofuran, 2,3-dihydro-	C_8H_8O	120	1.1086	11.70	Coumaran compound	Antifungal, Antiproliferative
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.2958	12.70	Phenolic compound	Antioxidant
5H-1-Pyrindine	C_8H_7N	117	0.2848	12.84	-	Nf
Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-	$C_{13}H_{16}$	172	0.0609	13.21	-	Nf
6-Methyl-1,2,3,4-tetrahydroquinoline	$C_{10}H_{13}N$	147	0.0572	13.57	-	Antioxidant
L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	0.1521	14.15	Amino acid	Nf

Name of the compound	Molecular formula	MW	Peak area %	RT	Nature of compound	Activity *
4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	$C_{13}H_{18}O$	190	0.1249	15.15	Flavour	Antimicrobial, Antioxidant, Antitumor
3-Oxo-à-ionone	$C_{13}H_{18}O_2$	206	0.1022	15.49	-	Nf
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180	0.0674	16.41	-	Nf
Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.4303	16.64	Fatty acid	Antimicrobial
Spiro[2.7]dec-4-ene, 1,1,5,6,6,9,9-heptamethyl-10-methylene-	$C_{18}H_{30}$	246	0.1682	17.58	-	Antimicrobial
7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane	$C_{15}H_{22}O_2$	234	0.0429	17.77	-	Nf
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.6984	19.44	Fatty acid	Larvicidal, Repellent, Antibacterial, Antifungal
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.9987	20.00	Terpenol	Antioxidant, Antimicrobial
2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.8047	20.15	Terpene ketone	Antimicrobial, Antiosteoporotic
2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222	2.2551	20.38	Aroma compound	Inhibitory effect
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.5867	21.21	Fatty acid methyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	2.0509	21.94	Fatty acid	Anti- inflammatory, Hypocholesterolemic, Hepatoprotective
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	44.4127	22.38	Fatty acid	Antioxidant, Anticancer
4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	$C_{13}H_{15}NO_3$	233	0.9049	23.71	-	Nf
11,14,17-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320	0.7981	23.91	-	Nf
Phytol	$C_{20}H_{40}O$	296	5.1028	24.18	Diterpene	Antimicrobial, Anticancer, Diuretic, Anti-inflammatory
(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	35.4991	25.70	Fatty acid alcohol	Antibacterial

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

Table 2. List of compounds identified and characterized from the methanolic extracts of stem of *M. minuta*

Name of the compound	Molecular formula	MW	Peak area %	RT	Nature of compound	Activity*
Butanal,4-hydroxy-3-methyl-	$C_5H_{10}O_2$	102	0.1403	3.05	Aldehyde	Antioxidant
Acetohydroxamic acid	$C_2H_5NO_2$	75	0.1648	3.38	-	Nf
2-Octene, (Z)-	C_8H_{16}	112	0.1762	6.83	-	Nf
Glycerin	$C_3H_8O_3$	92	6.7014	7.63	Sugar alcohol	Antihyperglycemic
Heptanoic acid	$C_7H_{14}O_2$	130	0.2621	8.56	Fatty acid	Analgesic
2,5-Diamino-2-methylpentanoic acid	$C_6H_{14}N_2O_2$	146	0.2606	9.50	-	Nf
Octanoic Acid	$C_8H_{16}O_2$	144	0.6287	10.29	Fatty acid	Inhibition of choline acetyl transferase (ChAT) activity, Antibacterial
Phenol, 2,4-dichloro-	$C_6H_4Cl_2O$	162	0.2055	10.72	-	Nf
Dianhydromannitol	$C_6H_{10}O_4$	146	0.0823	11.11	-	Nf
Benzofuran, 2,3-dihydro-	C_8H_8O	120	1.7381	11.75	Coumaran compound	Antifungal, Antiproliferative
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.1206	12.73	-	Nf
2(R),3(S)-1,2,3,4-Butanetetrol	$C_4H_{10}O_4$	122	16.3143	14.08	Sugar alcohol	Biological activity, Biosurfactants
Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.3538	16.66	-	Nf
1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	0.0455	19.28	-	Nf
7-Phenylheptanoic acid	$C_{13}H_{18}O_2$	206	0.0616	19.55	-	Nf
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280	0.1977	19.91	Aromatic hydrocarbon	Synergistic activity
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.5556	20.02	Diterpene alcohol	Antinociceptive, Antioxidant
2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.1203	20.17	-	Nf
8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252	0.4886	20.45	-	Nf

Name of the compound	Molecular formula	MW	Peak area %	RT	Nature of compound	Activity*
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.1571	21.22	Fatty acid methyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide
cis-11-Hexadecenal	$C_{16}H_{30}O$	238	0.9269	21.90	-	Nf
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.1331	22.24	Fatty acid	Antitumor, Antioxidant, Anti-inflammatory
10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.4214	23.88	Fatty acid methyl ester	Antimicrobial
Phytol	$C_{20}H_{40}O$	296	0.9604	24.20	Diterpene alcohol	Antinociceptive, Antioxidant
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	0.5936	24.32	-	Nf
1,19-Eicosadiene	$C_{20}H_{38}$	278	40.9464	25.65	-	Nf
Octadecanoic acid	$C_{18}H_{36}O_2$	284	4.2428	25.94	Fatty acid	Cancer preventive, Insectifuge

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

Compounds such as Hexanal ($C_6H_{12}O$); 3-Amino-2-oxozolidinone ($C_3H_6N_2O_2$); 1-Butanamine; N-butylidene- ($C_8H_{17}N$); Pyrazine, methyl- ($C_5H_6N_2$); Bicyclo[2,1,1]hex-2-ene,2-ethenyl- (C_8H_{10}); Hexanoic acid,2-oxo-,methyl ester ($C_7H_{12}O_3$); 1-Butanamine,3-methyl-N (3-methylbutylidene)- ($C_{10}H_{21}N$); 1-Butanamine,N-butylidene- ($C_8H_{17}N$); Pentylamine, N-isobutyl-N-nitroso- ($C_{10}H_{22}N_2O$); 4H-Pyran-4-one,2,3-Dihydro-3,5-dihydroxy-6-methyl- ($C_6H_8O_4$); 1-[1(1-Hydroxy-butyl)-cyclopentyl]-2-phenylethanone ($C_{17}H_{24}O_2$); Benzenecarboxylic acid ($C_7H_6O_2$); 5H-1-Pyrindine (C_8H_7N); Benzene,2 (1,3-butadienyl)-1,3,5-trimethyl- ($C_{13}H_{16}$); 6-Methyl-1,2,3,4-tetrahydroquinoline ($C_{10}H_{13}N$); L-Proline,5-oxo-methyl ester ($C_6H_9NO_3$); 4(2,6,6- Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one ($C_{13}H_{18}O$); 3-Oxo-a-ionone ($C_{13}H_{18}O_2$); 2(4H)-Benzofuranone,5,6,7,7a-trimethyl- ($C_{11}H_{16}O_2$); Spiro(2,7)dec-4-ene,1,1,5,6,6,9,9 heptamethyl-10-methylene- ($C_{18}H_{30}$); 7(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane ($C_{15}H_{22}O_2$); Tetradecanoic acid ($C_{14}H_{28}O_2$); 2-Cyclohexen-1-one,4-hydroxy-3,5,6-trimethyl-4 (3-oxo-1-butenyl)- ($C_{13}H_{18}O_3$); 9,12,15-Octadecatrienoic acid,(Z,Z,Z)- ($C_{18}H_{30}O_2$); 4-Oxozolecarboxylic acid,4,5-dihydro-2-phenyl-,1-methylethyl ester ($C_{13}H_{15}NO_3$); 11,14,17-Eicosatrienoic acid, methyl ester ($C_{21}H_{36}O_2$) and (Z)6,(Z)9-Pentadecadien 1-ol ($C_{15}H_{28}$ O) were identified in methanolic extract of leaf of *M. minuta.*

The methanolic extract of stem of *M. minuta* showed that the presence of phytocompounds like Butanal,4-hydroxy-3-methyl- ($C_5H_{10}O_2$); Acetohydroxamic acid ($C_2H_5NO_2$); 2-Octene,(Z)- (C_8H_{16}); Heptanoic acid ($C_7H_{14}O_2$); 2,5-Diamino-2-methylpentanoic acid ($C_6H_{14}N_2O_2$); Octanoic acid ($C_8H_{16}O_2$); Phenol,2,4-dichloro- ($C_6H_4Cl_2O$); Dianhydromannitol ($C_6H_{10}O_4$); 2(R),3(S)-1,2,3,4-Butanetetrol ($C_4H_{10}O_4$); 1-Dodecanol,3,7,11-trimethyl- ($C_{15}H_{32}O$); 7-Phenylheptanoicacid ($C_{13}H_{18}O_2$); 2-Hexadecane,3,7,11,15,-tetramethyl-,[R-[R*R*(E)]]- ($C_{20}H_{40}$); 8 Hexadecen -al,14-methyl (Z) ($C_{17}H_{32}O$); cis-11-Hexadecenal($C_{16}H_{30}O$); 10-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$); Octadecanoic acid, methyl ester ($C_{19}H_{38}O_2$); 1,19-Eicosadiene ($C_{20}H_{38}$) and Octadecanoic acid ($C_{18}H_{36}O_2$).

The biological activities of phytocompounds of methanolic extracts of leaf and stem of *M. minuta* were mentioned in the Tables 1 and 2 based on the Phytochemical and Ethnobotanical Databases created by Dr. Duke's of the Agricultural Research Service / USDA [40].

4. DISCUSSION

Plants are an important part of our everyday diet, and the plant constituents and their nutritional value have been intensively studied for decades. Secondary metabolites are characterized by enormous chemical diversity and every plant has its own characteristic set of secondary metabolites. So, plants synthesize an extensive array of secondary metabolites often highly complex structures. The chemical investigations of medicinal plants have largely been driven to find new drugs to treat human disease. The secondary metabolites have been of interest to humans as flavors, fragrances, dyes, pesticides and pharmaceuticals.

Currently, a number of modern drugs have been isolated from natural sources. Plant derived pharmacological compounds have recently become a great interest owing to their versatile applications. Ethnobotanical research has increased considerably in the last few years and is presently considered a subject of great interest. Medicinal plants have been good source for the synthesis of many drugs. There is a growing awareness in correlating the active principles from the medicinal plants with their biological activities [5]. Phytocompounds are possessing biological properties and demonstrated to exert beneficial effects. Nature is and will still serve as the man's primary source for the cure of his aliments. However the potential of higher plants are as sources for new drugs. There are many thousand plant species in the world, but only a small proportion has been investigated both phytochemically and pharmacologically. In this study, the selected medicinal plant *M. minuta* is an important and used in the treatment of many diseases by tribal people. So, there is need to identify and characterize the active phytochemicals from *M. minuta.*

The bioactive compounds of leaf and stem of *M. minuta* were determined by GC-MS analysis and the identified bioactive compounds were documented in Tables 1 and 2. The gas chromatogram of the leaf and stem of *M. minuta*

showed that the relative concentration of various compounds getting eluted at different retention time. The height of the peak indicates that the relative concentration of the compound present in the extract of leaf and stem of *M. minuta*. The mass spectrometry analysis was carried out to identify the compounds in the leaf and stem of *M. minuta* eluted at different retention time. The mass spectra are the fingerprints of phytocompounds of leaf and stem of *M. minuta,* which were identified by NIST library. Similarly, the bioactive compounds of stem and leaf of *Tiliacora acuminata* [41], leaf of *Cassia italica* [42] leaf, stem and seed of *Cajanus cajan* [43], leaf of *Allamanda cathartica* [44] and stem bark of *Dolichandrone atrovirens* [45] were determined by GC-MS analysis.

The biological activities of phytocompounds of leaf and stem of *M. minuta* were predicted by Duke's Ethnobotanical Databases [40]. As per Duke's Ethnobotanical Database, the identified phytochemicals of leaf and stem of *M. minuta* possess antibacterial, antifungal, antimalarial, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic, anticancer, diuretic, antihyperglycaemic and analgesic activities. So, the presence of phytochemicals in leaf and stem of *M. minuta* may be responsible for controlling diseases. Similarly, the presence of many phytocompounds in leaf and stem of *M. quadrifolia* with their biological activities were reported [39]. Due to their large pharmacological activities, phytochemicals have been used for centuries in traditional medicine.

Bioactive compounds from plants belong to various chemical groups such as phenolic compounds, flavonoids, alkaloids, tannins, saponins, glycosides, lignans, terpenoids, etc. The methanol is generally used as a first solvent for extraction of bioactive compounds in medicinal plants, because lots of polar compounds and certain group of non-polar compounds are dissolving in it. So in this study, the methanol was used for extraction of bioactive compounds. The results of GC-MS profile of leaf and stem of *M. minuta* were also showed that the presence of phenolic compounds and flavonoids. No other studies are available on phytochemicals of leaf and stem of *M. minuta* using GC-MS. So, up to date of our knowledge the present study may be the first report on phytochemicals of leaf and stem of *M. minuta* using GC-MS. In this study, the identified bioactive compounds of leaf and stem of *M. minuta* were reported as new compounds.

The uses of medicinal plants and phytomedicines have led to need for the analysis of plant compounds. In this study, we used the GC-MS technique for the analysis of secondary metabolites in leaf and stem of *M. minuta.* Similarly in our previous studies, we used the GC-MS technique for the analysis of the phytocompounds in leaf, flower and stem of *Aerva lanata* [46] and leaf, fruit and latex of *Croton bonplandianum* [47]. Continuing our research on the chemical composition of the different parts of medicinal plants, we now reported the results of phytocompounds from leaf and stem of *M. minuta*. We are particularly hopeful that this study will help guide research on further analysis of chemical compounds of leaf and stem of *M. minuta.*

5. CONCLUSION

The results of this study confirmed that the methanolic extracts of leaf and stem of *M. minuta* possess many bioactive constituents and which may be responsible for the pharmacological activities. Further studies are needed to isolate and purify the phytocompounds possess pharmacological properties. So, the present study may be useful in the identification of novel drugs from leaf and stem of *M. minuta.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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