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°C 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 \( (2.25%) (C_{13}H_{18}O_3) \); 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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Medicinal plants for bioactive compounds against treatment of diseases. However, the screening of the synthesis of antibiotics which are used in the 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].

**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

| Component | Formula | Percentage |
|-----------|---------|------------|
| 1,19-Eicosadiene | C₃₀H₆₀ | 40.94% |
| n-Hexadecanoic acid | C₁₆H₃₃O₂ | 20.13% |
| 2(R)3(S)-1,2,3,4-Butanetetrol | C₈H₁₆O₄ | 6.70% |
| Glycerin | C₃H₈O₃ | 4.24% |
| Benzofuran, 2,3-dihydro- | C₈H₆O | 1.73% |
| n-Hexadecanoic acid methyl ester | C₁₇H₃₄O₂ | 1.15% |
| 10-Octadecenoic acid methyl ester | C₁₈H₃₈O₂ | 1.42% |

*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, hypcholesterolemic 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, Thiruvur 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°C 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°C. GC oven temperature started at 110°C and holding for 2min and it was raised to 200°C at the rate of 10°C/min without holding. Holding was allowed at 280°C for 9min with the program rate of 5°C/min (60°C@8°C/min to 230°C (5min)@6°C/min to 280°C (10min)). GC interface and ion source temperature was maintained at 200°C. 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 (C₃H₈O₃); Benzofuran, 2,3-dihydro- (C₈H₆O); 2-Methoxy-4-vinylphenol (C₁₀H₁₀O); Dodecanoic acid (C₁₂H₂₄O₂); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O); 2-Pentadecanone, 6,10,14-trimethyl- (C₁₈H₃₆O); Hexadecanoic acid, methyl ester (C₁₇H₃₄O₂); n-Hexadecanoic acid (C₁₆H₃₂O₂) and Phytol (C₂₀H₄₀O) were determined in leaf and 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                                                                             | C₆H₁₂O            | 100 | 0.4511      | 3.04 | Aldehyde                 | Antifungal                                      |
| 3-Amino-2-oxazolidinone                                                            | C₃H₆N₂O₂          | 102 | 0.1607      | 3.35 | -                        | Nf                                              |
| 1-Butanamine, N-butylidene-                                                        | C₆H₁₇N            | 127 | 0.0467      | 3.70 | -                        | Nf                                              |
| Pyrazine, methyl-                                                                  | C₃H₆N₂            | 94  | 0.0119      | 3.84 | -                        | Nf                                              |
| Bicyclo[2.1.1]hex-2-ene, 2-ethenyl-                                                | C₈H₁₀             | 106 | 0.1461      | 4.46 | -                        | Nf                                              |
| Hexanoic acid, 2-oxo-, methyl ester                                                | C₅H₁₂O₃           | 144 | 0.0071      | 5.07 | -                        | Nf                                              |
| 1-Butanamine, 3-methyl-N-(3-methylbutylidene)-                                     | C₁₀H₂₁N           | 155 | 0.0300      | 5.25 | -                        | Nf                                              |
| 1-Butanamine, N-butylidene-                                                        | C₆H₁₇N            | 127 | 0.0308      | 5.36 | -                        | Nf                                              |
| Pentylamine, N-isobutyl-N-nitroso-                                                 | C₁₀H₂₂N₂O         | 186 | 0.0404      | 7.01 | -                        | Nf                                              |
| Glycerin                                                                            | C₆H₁₂O₃           | 92  | 0.1108      | 7.98 | Sugar alcohol            | Antimicrobial                                    |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-                                | C₆H₈O₄            | 144 | 0.4866      | 9.89 | Flavonoid compound       | Antioxidant, Antimicrobial, Anti-inflamatory, Antiproliferative |
| 1-[1-(1-Hydroxy-butyl)-cyclopentyl]-2-phenyl-ethanone                              | C₁₇H₂₄O₂          | 260 | 0.2215      | 10.35| -                        | Nf                                              |
| Benzenecarboxylic acid                                                             | C₇H₆O₂            | 122 | 0.2488      | 10.85| Phenolic acid            | Antibacterial, Antimicrobial                     |
| Benzofuran, 2,3-dihydro-                                                           | C₈H₈O             | 120 | 1.1086      | 11.70| Coumaran compound        | Antifungal, Antiproliferative                    |
| 2-Methoxy-4-vinylphenol                                                            | C₉H₁₀O₂           | 150 | 0.2958      | 12.70| Phenolic compound        | Antioxidant                                      |
| 5H-1-Pyridine                                                                     | C₆H₇N             | 117 | 0.2848      | 12.84| -                        | Nf                                              |
| Benzene, 2-(1,3-butadienyl)-1,3,5-trimethyl-6-Methyl-1,2,3,4-tetrahydroquinoline | C₁₃H₁₆            | 172 | 0.0609      | 13.21| -                        | Nf                                              |
| L-Proline, 5-oxo-, methyl ester                                                    | C₆H₄NO₃           | 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_{16}O_{2}  | 206 | 0.1022      | 15.49 | -                    | Nf                                        |
| 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-Dodecanoic acid           | C_{11}H_{16}O_{2}  | 180 | 0.0674      | 16.41 | -                    | Nf                                        |
| Spiro[2.7]dec-4-ene, 1,1,5,6,6,9-heptamethyl-10-methylene-7-(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane 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)-Hexadecanonic acid, methyl ester | C_{13}H_{18}O_{3}  | 222 | 2.2551      | 20.38 | Aroma compound       | Inhibitory effect                         |
| 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-                                            | C_{18}H_{30}O_{2}   | 278 | 2.0509      | 21.94 | Fatty acid           | Antioxidant, Hypcholesterolemic, Nematicide, Pesticide Anti-inflammatory, Hypcholesterolemic, 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_{16}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 Antibacterial |
| (Z)6,(Z)9-Pentadecadien-1-ol                                                       | C_{15}H_{28}O       | 224 | 35.4991     | 25.70 | Fatty acid alcohol   |                                           |

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-aldehyde                           | C_{5}H_{10}O_{2}  | 102 | 0.1403      | 3.05 | Aldehyde           | Antioxidant                                   |
| Acetohydroxamic acid                                          | C_{2}H_{3}NO_{2}  | 75  | 0.1648      | 3.38 | -                  | Nf                                            |
| 2-Octene, (Z)-                                                | C_{8}H_{16}       | 112 | 0.1762      | 6.83 | -                  | Nf                                            |
| Glycerin                                                      | C_{3}H_{8}O_{3}   | 92  | 6.7014      | 7.63 | Sugar alcohol      | Antihyperglycemic                             |
| Heptanoic acid                                                | C_{7}H_{14}O_{2}  | 130 | 0.2621      | 8.56 | Fatty acid         | Analgesic                                     |
| 2,5-Diamino-2-methylpentanoic acid                           | C_{6}H_{14}N_{2}O_{2} | 146 | 0.2606      | 9.50 | -                  | Nf                                            |
| Octanoic Acid                                                 | C_{8}H_{16}O_{2}  | 144 | 0.6287      | 10.29| Fatty acid         | Inhibition of choline acetyl transferase (ChAT) activity, Antibacterial |
| Phenol, 2,4-dichloro-                                          | C_{6}H_{4}Cl_{2}O | 162 | 0.2055      | 10.72| -                  | Nf                                            |
| Dianhydromannitol                                             | C_{6}H_{10}O_{4}  | 146 | 0.0823      | 11.11| -                  | Nf                                            |
| Benzofuran, 2,3-dihydro-                                      | C_{8}H_{8}O       | 120 | 1.7381      | 11.75| Coumaran compound  | Antifungal, Antiproliferative                 |
| 2-Methoxy-4-vinylphenol                                       | C_{9}H_{10}O_{2}  | 150 | 0.1206      | 12.73| -                  | Nf                                            |
| 2(R),3(S)-1,2,3,4-Butanetetrol                                | C_{4}H_{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_{18}H_{32}O     | 228 | 0.0455      | 19.28| -                  | Nf                                            |
| 7-Phenyheptanoic acid                                         | C_{14}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, Hypcholesterolemic, Nematicide,  |
| cis-11-Hexadecenal                         | C_{16}H_{30}O_{2}  | 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_{2}  | 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_{40}       | 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\textsubscript{6}H\textsubscript{12}O), 3-
Amino-2-oxozolidinone (C\textsubscript{6}H\textsubscript{8}N\textsubscript{2}O); 1-
Butanamine; N-butyllidene- (C\textsubscript{9}H\textsubscript{17}N); Pyrazine, methyl-
(C\textsubscript{8}H\textsubscript{14}N); Bicyclo[2,1,1]hex-2-ene,2-
ethenyl- (C\textsubscript{10}H\textsubscript{14}); Hexanoic acid,2-oxo-,methyl
ester (C\textsubscript{7}H\textsubscript{14}O\textsubscript{2}); 1-Butanamine,3-methyl-N (3-
 methylbutylylidene)- (C\textsubscript{10}H\textsubscript{14}N); 1-Butanamine,N-
butyllidene- (C\textsubscript{9}H\textsubscript{17}N); Pentylamine, N-isobutyl-N-
nitroso- (C\textsubscript{10}H\textsubscript{22}N\textsubscript{2}O); 4H-Pyran-4-one,2,3-
Dihydro-3,5-dihydroxy-6-methyl- (C\textsubscript{6}H\textsubscript{8}O\textsubscript{4}); 1-
[1(1-Hydroxy-butyl)-cyclopentyl]-2-
phenylethanoline (C\textsubscript{17}H\textsubscript{34}O\textsubscript{2}); Benzene carboxylic
acid (C\textsubscript{6}H\textsubscript{5}O); 5H-1-Pyrindine (C\textsubscript{9}H\textsubscript{14}N);
Benzene,2-(1,3-butadienyl)-1,3,5-trimethyl-
(C\textsubscript{13}H\textsubscript{16}O); 6-Methyl-1,2,3,4-tetrahydroquinoine
(C\textsubscript{10}H\textsubscript{14}N); L-Proline,5-oxo-methyl ester
(C\textsubscript{7}H\textsubscript{14}NO\textsubscript{2}); 4(2,6,6-
Trimethylcyclohexa-1,3-di enyl)but-3-en-2-one (C\textsubscript{14}H\textsubscript{18}O\textsubscript{2});
2(4H)-Benzofuranone,5,6,7,7a-
trimethyl- (C\textsubscript{11}H\textsubscript{16}O); Spiro(2,7)dec-4-
ene,1,1,5,6,9,9 heptamethyleno-10-methylene-
(C\textsubscript{18}H\textsubscript{20}O); 7(1,3-Dimethylbuta-1,3-dienyl)-1,6,6-
trimethyl-3,8-dioxatricyclo[5.1.0.0(2,4)]octane
(C\textsubscript{15}H\textsubscript{18}O\textsubscript{2}); Tetradecanoic acid (C\textsubscript{14}H\textsubscript{28}O\textsubscript{2}); 2-
Cyclohexen-1-one,4-hydroxy-3,5,6-trimethyl-4 (3-
oxo-1-butenyl)- (C\textsubscript{13}H\textsubscript{16}O\textsubscript{3}); 9,12,15-
Octadecatrienoic acid, (Z,Z,Z)- (C\textsubscript{18}H\textsubscript{30}O\textsubscript{3}); 4-
Octadecanoic acid,4,5-dihydro-2-phenyl-1-
methyl ester ester (C\textsubscript{13}H\textsubscript{15}NO\textsubscript{3}); 11,14,17-
Eicosatrienoic acid, methyl ester (C\textsubscript{21}H\textsubscript{36}O\textsubscript{3}) and
(Z)6,(Z)9-Pentadacadien-1-ol (C\textsubscript{15}H\textsubscript{28}O) were
identified in methanolic extract of leaf of M.
minuta.

The biological activities of phytocompounds of
mechanical 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
everseous 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 ailments. 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 phytocompounds of leaf and stem of *M. minuta* possess antibacterial, antifungal, antimalarial, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic, anticancer, diuretic, anti-hyperglycaemic and analgesic activities. So, the presence of phytocompounds 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, phytocompounds 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 phytocompounds 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 phytocompounds 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.

ACKNOWLEDGEMENT

This research work was financially supported by the University Grants Commission [UGC-MRP-F. No. 42-638/2013 (SR)], New Delhi, India. So, the authors express their sincere thanks to University Grants Commission, New Delhi, India for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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