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PHYTOCHEMICAL SCREENING OF LEAF AND ROOT OF *MIMOSA PUDICA* L. BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC-MS)

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ABSTRACT
The aim of the present study is to analyze the chemical composition of ethanolic extracts of leaf and root of *M. pudica* by GC-MS. The ethanolic extracts of leaf and root were prepared and concentrated at 40°C using hot air oven. The concentrated ethanolic extracts were subjected to GC-MS analysis using an instrument Perkin Elmer Clarus 500. The GC-MS analyes showed that the presence of 23 phytocompounds in the ethanolic extract of leaf including n-Hexadecanoic acid (32.46%); 9,17-Octadecadienal, (Z)- (17.96%); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (12.00%) and Octadecanoic acid (8.31%) and the presence of 17 bioactive compounds in root of *M. pudica* including Z, E-2-Methyl-3,13-octadecadien-1-ol (40.90%); n-Hexadecanoic acid (32.09%); Octadecanoic acid (9.30%) and Cyclohexanecarboxylic acid, 4-methoxyphenyl ester (2.62%). So, the present study confirmed that the presence of bioactive compounds in leaf and root of *M. pudica.* In future, the isolation of bioactive compounds from the leaf and root of *M. pudica* would be useful for the determination of novel drugs.

KEYWORDS: Phytocompounds; ethanolic extract; *Mimosa pudica*; leaf; root; GC-MS analysis.

INTRODUCTION

Plants have a significant role in maintaining human health and improving quality of human life for thousands of years (Lubna Azmi *et al*., 2011). In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter (Grover *et al*., 2002). Nature has been a source of medicinal agents for thousands of years (Nair *et al*., 2005). The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in developing countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000). In fact, around 80% of the population from developing countries still uses medicinal plants for their primary health care (Arokiyaraj, 2012). Medicinal plant is an important element of indigenous medical systems in all over the world. The ethnobotany provides a rich resource for natural drug research and development (Farnsworth, 1990). Natural remedies from medicinal plants proved as safe and effective. Many plant species have been used in folklore medicine to treat various ailments (Balamurugan, 2012). The most important biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds (Kiruba, 2011). Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Boots *et al*., 2008). Phytochemical analyses are of paramount importance for the identification of new sources of therapeutically and industrially valuable compounds with medicinal significance and for the best and most judicious use of naturally available materials (Hossain *et al*., 2011).

Mimosa pudica L. (Mimosaceae) is a common plant in moist waste ground, lawns, open plantations and weedy thickets. It is native from Middle America and now widely distributed in all tropical areas (Sastri, 1962). The parts of the plant such as leaves, flowers, stem, root, and fruits were used as medicines in the traditional healthcare systems (Chowdhury, 2008). The roots and leaves of this plant were commonly used by tribal people for the treatment of headache, migraine, dysentery, fever, piles, insomnia, epilepsy, *etc* (Merlin, 2009; Joy, 2001). The plant is used as bitter, astringent, acrid, cooling vulnerary, febrifuge, alexipharmic, diuretic, emetic and tonic (Vaidyaratanm, 2001). A variety of pharmacological functions of this plant such as antihyperglycemic (Umamaheswari, 2007), antidiarrhoeal (Balakrishnan, 2006), anticonvulsant (Bum, 2007), cytotoxic (Chowdhury, 2008) and hepatoprotective (Rajendran, 2009) properties were reported. The preliminary phytochemical studies were conducted and revealed that the presence of various bioactive compounds. The presence of phytocompounds in GC-MS analysis of ethyl acetate extract of leaves (Ramesh *et al.,* 2014) and methanolic extract of leaves of *M. pudica* (Sriram Sridharan *et al.,* 2011) were reported. But, there is no phytochemical study on ethanol extract of leaf and root of *M. pudica*. So, the present study was aimed to analyze the phytocompounds of ethanol extract of leaf and root of *M. pudica* using gas chromatography-mass spectrometry (GC-MS). Up to our knowledge, this study may be the first

report on phytochemical analysis of ethanolic extract of leaf and root of *M. pudica*.

MATERIALS & METHODS

Collection of Plant Material

The fresh plants of *M. pudica* L. were collected from natural habitats of Thirupanipet Village, Thanjavur 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: KV 001). 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 root of *M. pudica* 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.

Preparation of Plant Extracts

The leaf and root of *M. pudica* extracts were prepared according to previously reported procedure (Gopala krishnan and Udayakumar, 2014). For this, 50g of leaf and root powder of *M. pudica* was soaked in 500 ml of ethanol 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 ethanolic extracts of leaf and root of *M. pudica* were subjected to GC-MS analysis.

GC- MS Analysis

The GC–MS analysis was performed to identify the chemical compounds present in the leaf and root of *M. pudica* by using an instrument Perkin Elmer Clarus 500 (Gopalakrishnan and Udayakumar 2014). 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.

Identification of Chemical Compounds

Interpretation of mass spectra of the extracts of leaf and root of *M. pudica* was conducted using the database of National Institute of Standard and Technology [NIST] library. The NIST library has more than 62,000 spectral patterns for chemical compounds. The spectrum of the identified 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 root of *M. pudica* 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.

RESULTS

The GC-MS chromatogram of ethanolic extracts of leaf and root of *M. pudica* revealed the presence of various compounds with corresponding peaks at different retention time (Figures 1 and 2). The molecular formula, molecular weight, peak area %, retention time, nature and biological activities of identified compounds in ethanolic extracts of leaf and root of *M. pudica were* represented in Tables 1 and 2. The biological activities of compounds were predicted based on the Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke's of the Agricultural Research Service/USDA. The compounds such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-($C_6H_8O_4$); Phenol, 2,4-bis (1,1-dimethylethyl)-($C_{14}H_{22}O$); Dodecanoic acid ($C_{12}H_{24}O_2$); 3-O-Methyl-d-glucose ($C_7H_{14}O_6$); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$); n-Hexadecanoic acid ($C_{16}H_{32}O_2$) and Octadecanoic acid ($C_{18}H_{36}O_2$) were commonly present in both leaf and root of *M. pudica*. There are phyto compounds such as D-Arabinitol ($C_5H_{12}O_5$) 1-Methyl-2, 4, 5- trioxoimidazolidine ($C_4H_4N_2O_3$); Piperidine, 3-phenyl-($C_{11}H_{15}N$); 3-Hexadecene, (Z)-($C_{16}H_{32}$); Sucrose ($C_{12}H_{22}O_{11}$); 5-Dodecanol ($C_{12}H_{26}O$); Myo-Inositol, 4-C-methyl-($C_7H_{14}O_6$); 2-Pentadecanone-6,10,14-trimethyl ($C_{18}H_{36}O$); Methyl α-d-Mannofuranoside, ($C_7H_{14}O_6$); Phytol ($C_{20}H_{40}O$); 9, 17-Octadecadienal, (Z)- ($C_{18}H_{32}O$); E-11-Hexa decenal ($C_{16}H_{30}O$); Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester ($C_{35}H_{68}O_5$); 7,11-Hexadecadienal ($C_{16}H_{28}O$); 16-Heptadecenal ($C_{17}H_{32}O$) and 2,6,10,14,18, 22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-($C_{30}H_{50}$) were found in ethanolic leaf extract of *M. pudica*. The screening of ethanolic root extract of *M. pudica* were showed that the presence of compounds such as Ethane, 1,1-diethoxy- ($C_6H_{14}O_2$); Furan, 2,5-dimethyl- (C_6H_8O); 2-Pyrazoline, 1,3,4-trimethyl- ($C_6H_{12}N_2$); 2,5-Furandicarboxaldehyde ($C_6H_4O_3$); 2-Furancarboxaldehyde, 5-(hydroxylmethyl)- ($C_6H_6O_3$); Cyclohexanecarboxylic acid, 4-methoxyphenyl ester ($C_{14}H_{18}O_3$); Pentadecanal- ($C_{15}H_{30}O$); Heptadecanoic acid ($C_{17}H_{34}O_2$); Z,E-2-Methyl-3,13-octadecadien-1-ol ($C_{19}H_{36}O$) & 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester ($C_{16}H_{22}O_4$).

TABLE 1. List of identified phytocompounds of ethanolic extract of leaf of *M. pudica* by GC-MS analysis

Name of the compound	Molecular Formula	MW	Peak area %	RT	Nature of compound	Activity*
D-Arabinitol	$C_5H_{12}O_5$	152	2.2489	6.90	-	Nf
1-Methyl-2,4,5-trioxoimidazolidine	$C_4H_4N_2O_3$	128	1.1320	8.46	-	Nf
4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.9462	10.15	Flavonoid	Antimicrobial, Anti-inflammatory, Antiproliferative Antioxidant, Automatic nerve activity
Piperidine, 3-phenyl-	$C_{11}H_{15}N$	161	0.0921	13.10	-	Nf
3-Hexadecene, (Z)-	$C_{16}H_{32}$	224	0.3391	14.96	-	Nf
Sucrose	$C_{12}H_{22}O_{11}$	342	2.0935	18.19	Carbohydrate	Nf
Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	1.2056	18.90	Antioxidant compound	Antifungal, Antioxidant
Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.0739	20.47	Fatty acid	Antimicrobial
5-Dodecanol	$C_{12}H_{26}O$	186	0.3501	23.32	-	Nf
Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194	0.3137	25.27	Inositol compound	Antimicrobial
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	1.4998	25.88	Sugar moiety	Nf
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	12.0002	27.10	Diterpene	Antibacterial, Antidiabetic, Antiinflammatory
2-Pentadecanone-6,10,14-trimethyl	$C_{18}H_{36}O$	268	2.0570	28.23	-	Nf
Methyl α-d-Mannofuranoside	$C_7H_{14}O_6$	194	0.0595	28.47	Sugar moiety	Nf
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	32.4614	31.12	Fatty acid	Anti-inflammatory, Antimicrobial, Antioxidant
Phytol	$C_{20}H_{40}O$	296	5.7593	34.06	Diterpene alcohol	Antimicrobial, Antiinflammatory, Anticancer, Antidiabetic, Diuretic
9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	17.9628	35.32	Aldehyde	Antimicrobial
Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.3167	35.76	Fatty acid	Antibacterial, Antiviral
E-11-Hexadecenal	$C_{16}H_{30}O$	238	1.7645	37.22	-	Nf
Hexadecanoic acid,2hydroxy1,3propanediyl ester	$C_{35}H_{68}O_5$	568	0.7948	38.13	-	Nf
7,11-Hexadecadienal	$C_{16}H_{28}O$	236	1.5039	41.88	-	Nf
16-Heptadecenal	$C_{17}H_{32}O$	252	0.9594	42.90	-	Nf
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	$C_{30}H_{50}$	410	5.0656	48.54	Hydrocarbon and triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive

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

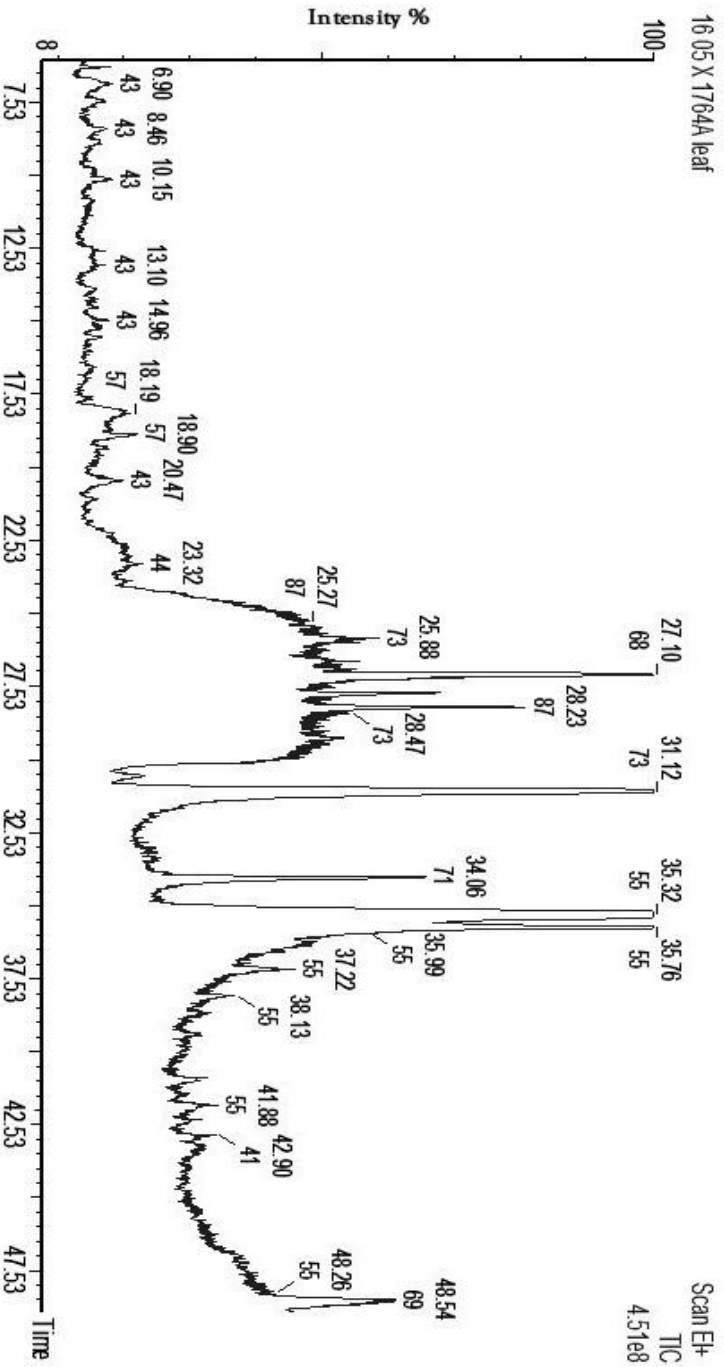

FIGURE 1. GC-MS Chromatogram of ethanolic extract of leaf of *M. pudica*

TABLE 2. List of identified phytocompounds of ethanolic extract of root of *M. pudica* by GC-MS analysis

Name of the compound	Molecular Formula	MW	Peak area %	RT	Nature of compound	Activity *
Ethane, 1,1-diethoxy-	$C_6H_{14}O_2$	118	0.2239	2.38	-	Nf
Furan, 2,5-dimethyl-	C_6H_8O	96	0.3437	3.57	Furan group	-
2-Pyrazoline, 1,3,4-trimethyl-	$C_6H_{12}N_2$	112	0.2542	4.69	Alkaloid	Anti-inflammatory
2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124	1.3003	8.37	Furan aldehyde	Antifungal
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.3898	10.19	Flavonoids	Anti-diabetic, Antioxidant
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	2.7951	12.59	Furan aldehyde	Antimicrobial, Preservative Clastogenic activity, Uterotonic activity
Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.7823	18.94	Antioxidant compound	Antifungal, Antioxidant
Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.9179	20.54	Fatty acid	Antimicrobial
Cyclohexanecarboxylic acid, 4-methoxyphenyl ester	$C_{14}H_{18}O_3$	234	2.6207	24.36	-	Photooxidation
3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	1.7316	25.97	Sugar moiety	Reducing toxicity
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.0340	27.12	Diterpene	Antibacterial, Antidiabetic, Anti-inflammatory
Pentadecanal-	$C_{15}H_{30}O$	226	0.6596	28.25	-	Nf
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	32.0933	31.16	Fatty acid	Anti-inflammatory, Antimicrobial, Antioxidant
Heptadecanoic acid	$C_{17}H_{34}O_2$	270	1.8399	33.50	Fatty acid	Nf
Z,E-2-Methyl-3,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	40.9013	35.34	Fatty alcohol	Antibacterial, Cytotoxic, Antifungal
Octadecanoic acid	$C_{18}H_{36}O_2$	284	9.3062	35.84	Fatty acid	Antibacterial, Antiviral
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	0.8064	43.42	-	Nf

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

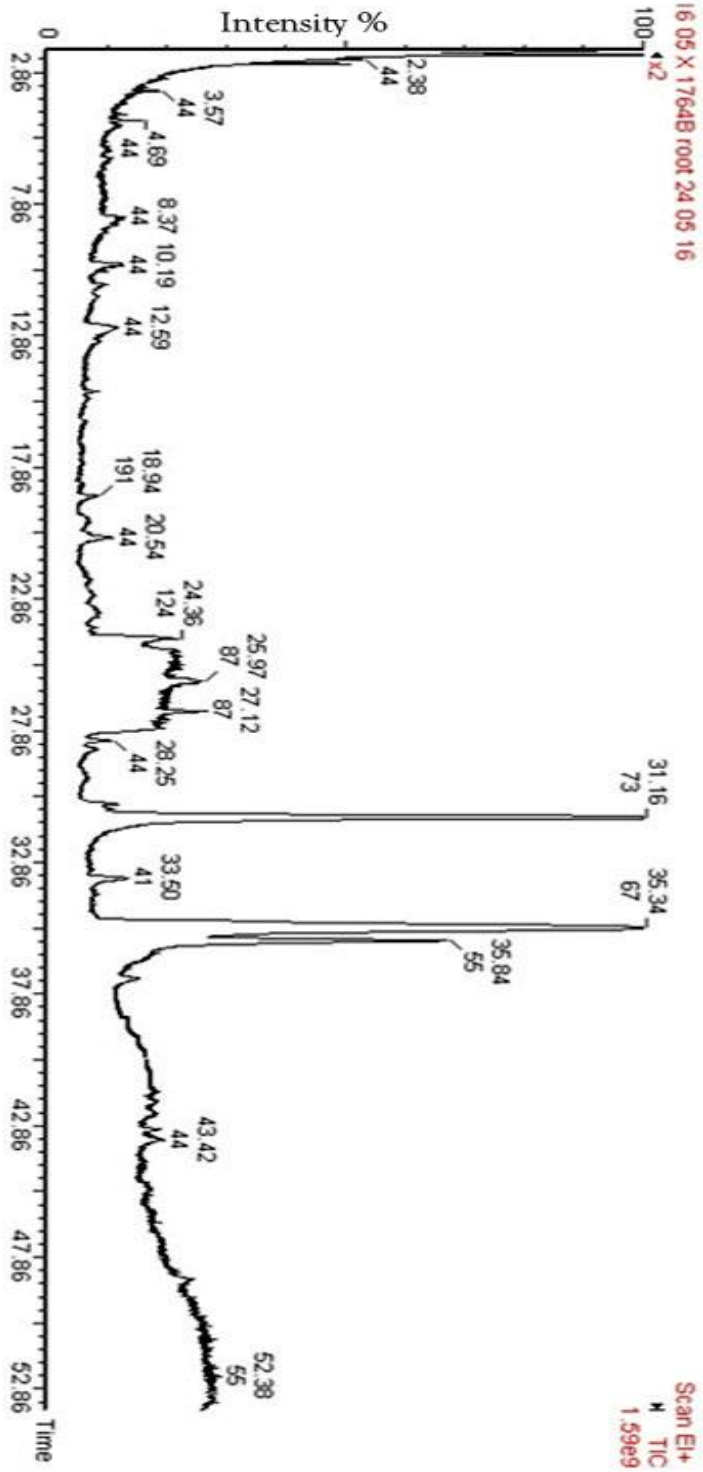

FIGURE 2. GC-MS Chromatogram of ethanolic extract of root of *M. pudica*

DISCUSSION

For a long period of time, plants have been a valuable source of natural products for maintaining human health. Especially, in recent years, plants with various biological properties have been introduced and the investigation has increased in pharmaceutical and food industries due to the medicine derived from plant sources is free from side effects on human health compared to synthetic substances (Nascimento *et al.,* 2000). In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Goldfrank *et al.,* 1982; Vulto and Smet, 1988). Plants are used as food and medicine and more likely to yield pharmacologically active compounds. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent therapeutic efficacy, antioxidant activity, no side effects and economic viability. Medicinal plants are serving as raw material for drugs and synthesize phytochemicals, which are beneficial for health and they cannot be synthesized by the human body (Martinez *et al*., 2008). The most important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss, 2009). There is growing awareness in correlating the phytochemical with their biological activities (Fernie *et al.,* 2004; Summer *et al*., 2003; Robertson, 2005).

Mass spectrometry, coupled with chromatographic separations such as gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional by used medicinal plants as medicines. In recent years GC-MS studies have increased for the analysis of phytocompounds in medicinal plants and this technique has proved to be a valuable method for the analysis of non polar components, volatile essential oil, fatty acids, lipids, alkaloids, terpenoids and steroids by using a few grams of plant material (Jie and Choi, 1991; Betz, 1997; Sermakkani and Thangapandian, 2012).

In the present study, twenty three compounds in ethanolic leaf extract and seventeen compounds in ethanolic root extract of *M. pudica* were identified through GC-MS analyses. The nature and biological activities of identified compounds in *M. pudica* were determined based on Dr. Duke`s Phytochemical and Ethnobotanical Databases. The ethanol extracts of leaf and root of *M. pudica* posses furans, flavonoids, fatty acids, sugar derivatives, terpenes and fatty alcohol. The above mentioned nature of phytocompounds reserves their antibacterial, antifungal, antioxidant, antidiabetic, anti-inflammatory, antiviral and anticancer activities. Based on the GC-MS chromatogram, the peak area percentage of n-Hexadecanoic acid was predominant in ethanolic leaf extract and Z, E-2-Methyl-3; 13-octadecadien-1-ol was predominant in ethanolic root extract of *M. pudica*. Among the identified compounds of ethanolic leaf extract, the 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl- posses antimicrobial, anti-inflamamatory and antiproliferative activities. Phenol, 2,4-bis (1,1-dimethylethyl)- has antifungal and antioxidant activities, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and phytol posses antibacterial, antidiabetic and anti-inflammatory activities (Table 1). The root extract showed that the compound 2-Pyrazoline, 1,3,4-trimethyl- posses anti-inflammatory activity, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- posses antidiabetic activity and Octadecanoic acid has antibacterial and antiviral activities (Table 2).

In this study the GC-MS analysis of ethanolic extract of leaf and root of *M. pudica* showed the presence of many phyto compounds. Similarly in the previous studies, the GC-MS analyses of different parts of medicinal plants like leaf, flower and stem of *Aerva lanata* (Vidhya and Udayakumar, 2015), leaf and stem of *Marsilea minuta* (Sabithira and Udayakumar, 2017), leaf and stem of *Marsilea quadrifolia* (Gopalakrishnan and Udayakumar, 2014) and leaf, fruit and latex of *Croton bonplandianum* (Vennila and Udayakumar, 2015) were carried out and reported many phytocompounds. Based on the Dr. Duke's Phytochemical and Ethnobotanical Databases, the bioactive compounds of ethanolic extract of leaf and root of *M. pudica* posses several pharmacological activities. The isolation of bio-active compounds in leaf and root of *M. pudica* will be useful for drug development to control diseases.

CONCLUSION

The results of this study confirmed that the presence of bioactive compounds such as phenolic compounds, flavonoids, alkaloids, fatty acids, ditrepenes and triterpenes in leaf and root of *M. pudica.* The identified phytocompounds may be responsible for the antimicrobial, antidiabetic, anticancer, cytotoxic and antioxidant properties of *M. pudica*.

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