International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

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Bioseparation of phytochemical constituents from leaf and stem extracts of *Mimosa pudica* L

Ujjwal Kumar Mondol and W. Islam *

Institute of Biological Sciences, University of Rajshahi, Bangladesh.

International Journal of Biological and Pharmaceutical Sciences Archive, 2023, 06(01), 023–032

Publication history: Received on 27 June 2023; revised on 05 August 2023; accepted on 08 August 2023

Article DOI: https://doi.org/10.53771/ijbpsa.2023.6.1.0075

Abstract

The isolation procedure of chemical constituents was mainly based on fractionation by solvents of varying polarity. After cold extraction, solvent-solvent partitioning of extract was done with different solvents to yield different extracts of leaf and stem of *Mimosa pudica* L. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out for petroleum ether, chloroform and ethyl acetate extracts of leaf as well as chloroform and ethyl acetate extracts of stem and 16, 11, 3, 26 and 3 compounds were identified respectively. The major compounds were benzene,1-ethyl-3-methyl- (14.83%) for petroleum ether extract of leaf, fumaric acid, ethyl 2-methylallyl ester (16.96%) for chloroform extract of leaf, glycerin (74.71%) for ethyl acetate extract of leaf, ticlopidine (80.90%) for chloroform extract of stem and hexadecenoic acid, methyl ester (69.95%) for ethyl acetate extract of stem.

Keywords: *Mimosa pudica;* Phytoconstituents; Quantitative; Gas Chromatography-Mass Spectrometry (GC-MS); Extraction; Aqueous; Retention indices (RI)

1. Introduction

Around 50% of the modern drugs are of plant origin. But only small fractions of the biologically active medicinal plant molecules have been assayed and much phytochemical investigations of higher plants are going on. After isolation of the phytoconstituents, they are screened for different types of biological activities. Crude plant extracts of particular activities are assayed and then the active fractions are analyzed phytochemically (Harborne, 1998). Plants have been using as food as well as medicine and the medicinal plants are using as raw materials for manufacturing drugs as well as synthesizing phytochemicals that are beneficial for human health which are not synthesized in human body (Martinez *et al.,* 2008). The present study aims to investigate the biochemical activities of different crude extracts of leaf and stem of *M. pudica*, followed by further fractionation by column chromatography, and analyze the fraction by Gas Chromatography-Mass Spectrometry (GC-MS) coupled system.

2. Material and methods

2.1. Collection of sample plants

M. pudica was collected from the Botanical Garden of Rajshahi University Campus, and the leaf and stem were separated. The petioles were separated from the rachis and the stem was chopped into small pieces. The leaf and stem were dried in normal room temperature keeping them into wooden trays. Drying was carried out under shed to prevent the changes of the constituents in it due to drying. After drying they were grinded into dust Figure 1.

* Corresponding author: W. Islam

Figure 1 Leaf of pudica *M. pudica*

Figure 2 Stem of *M. pudica*

Figure 3 Dust of leaf

Figure 4 Dust of stem

2.2. Cold extraction

The extraction procedure was adopted from Alam *et al.* (2002). The total weight of powdered leaf material was 650 g. That amount was taken in an amber colored extraction bottle and soaked in 100% methanol (2.0L x 3 times). The bottle was kept first time for 7 days with occasional shaking and stirring and also submitted to ultrasonic agitation for an hour daily in a sonicator (Power Sonic 510). The organic phase or the supernatant was then filtered separately through cotton followed by Double Rings Filter paper No. 102 and collected in a beaker. Second time the bottle was kept for 5 days and third time for 3 days with occasional shaking and stirring and also ultrasonic agitation daily in a sonicator and the filtered mixture was collected in the same beaker. Then the extraction was kept open in room temperature, aeration by an aerator for evaporation of the solvent to afford crude extract (32 g).

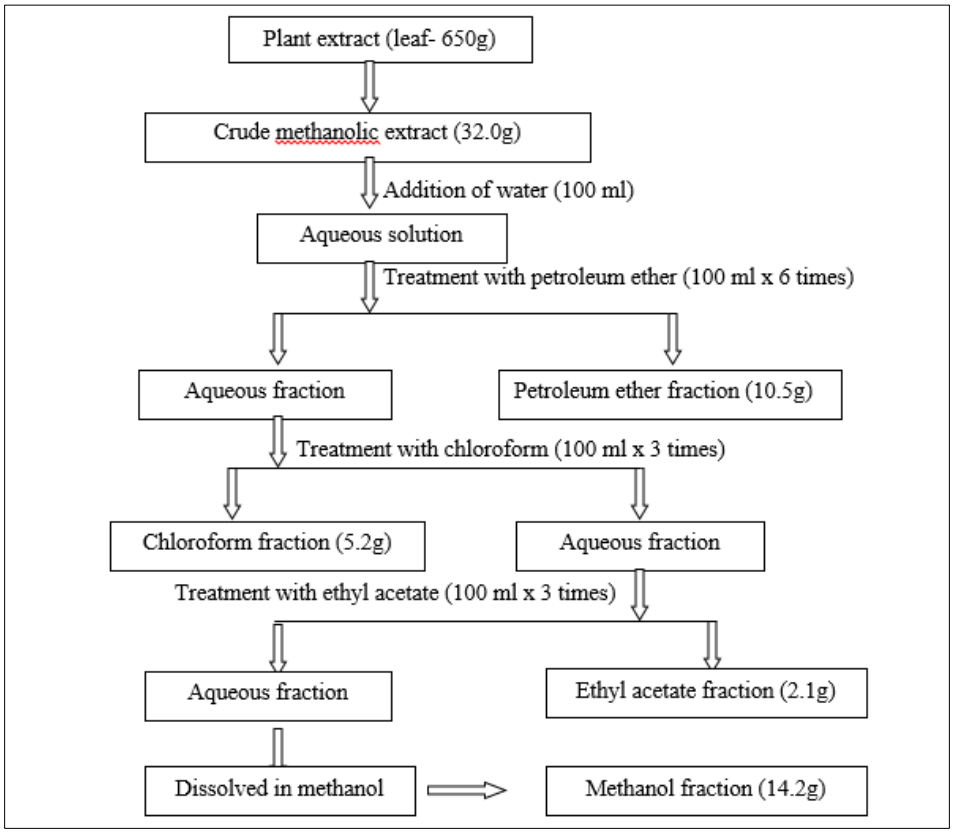

Figure 5 Schematic representation of solvent-solvent partitioning of crude methanolic extract

2.3. Solvent-solvent partitioning of crude extracts

Solvent-solvent partitioning of extracts was done using the protocol designed by Kupchan and modified by Wagenen *et al.* (1993).

2.4. Extraction with petroleum ether

The crude methanolic extract was made slurry with water (100 ml) and taken in a separating funnel of 500 ml. Petroleum ether (100 ml) was added with the aqueous methanolic solution and shaken well. The mixture was kept untouched until the layers were separated. The upper organic layer was then collected and repeated the process for five times. The collected combined petroleum ether extract was filtered and allowed the solvent to evaporate off in room temperature. The dried extract afforded a bluish-black colored oily mass (10.5 g).

2.5. Extraction with chloroform

The aqueous fraction was then added with chloroform (100 ml) and shaken well. When the layers were separated, the lower organic layer was collected in a beaker. The process was repeated twice. The combined chloroform extract was filtered and evaporated off in normal room temperature. The dried, concentrated extract obtained a coffee-colored mass (5.2 g).

Figure 6 Filtering of the extractions

Figure 7 Solvent-solvent partitioning of extracts

2.6. Extraction with ethyl acetate

After extraction of chloroform fraction, the aqueous fraction was extracted with ethyl acetate (100 ml x 3 times) and shaken well. The combined ethyl acetate extract was collected, filtered and evaporated off the solvent. The dried extract obtained a coffee-colored mass (2.1 g).

2.7. Extraction with methanol

Finally, the left aqueous fraction was dissolved in methanol and the combined methanol extract was evaporated off, filtered and dried to obtain a blackish mass (14.2g).

The same procedure was followed to yield the extracts of stem of *M. pudica.* All the output extracts were removed to glass vials and preserved in refrigerator at 4 ^{0}C with proper labeling.

2.8. Identification of bioactive compounds by GC-MS

The GC-MS analysis of the plant extract was made in a Shimadzu QP 2020 (Japan) instrument. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 55 minutes.

GC-MS analysis is a common confirmation test. It is best used to make an effective chemical analysis. This analysis provides a representative spectral output of all the compounds that get separated from the sample. The injection of the sample to the injected port of the GC device is the first step of GC-MS method. The GC-MS instrument vaporizes the

sample and then separates and analyzes of the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed between elution and injection is called retention time. Differentiation among the compounds is identified using the retention time. The peak is measured from the base to the tip of the peak.

Retention indices (RI) of the compounds are determined by comparing the retention time of a series, and identification of each component is confirmed by comparison of its RI with data in the literature. Interpretation of mass spectrum is carried out by using the database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of unknown components is compared with the spectrum of known components which is stored in the NIST library.

3. Results and discussion

3.1. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the samples

GC-MS analysis was carried out for crude extracts of leaf and stem of the sample plant *M. pudica.*

3.2. Petroleum ether extract of leaf

Petroleum ether extract of leaf was analyzed by GC-MS method and was identified sixteen compounds. The major compounds were benzene,1-ethyl-3-methyl- (14.830%), mesitylene (13.267%), vitamin E (13.117%), nonadecane (7.248%), tetratetracontane (6.486%), benzene,1-ethyl-2-methyl- (6.305%) and benzene,1,2,4-trimethyl (5.649%) (Table 1) (Fig 8).

3.3. Chloroform extract of leaf

GC-MS analysis of chloroform extract of leaf revealed eleven compounds of which the major compounds were fumaric acid, ethyl 2-methylallyl ester (16.959%), 1-(2-[3-(2-acetyloxiran-2-yl)-1,1-dimethyl)propyl]cycloprop-2-enyl)ethanone (15.829%), 1H-pyrrole-2,5-dione, 3-ethyl-4-methyl- (14.966%), 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- (12.960%) and 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (7.907%) (Table 2) (Fig 9).

3.4. Ethyl acetate extract of leaf

Three compounds were identified from ethyl acetate extract of leaf through GC-MS analysis *viz.* glycerin (74.711%), 9-octadecenamide (15.297%) and hexadecenoic acid, methyl ester (9.989%) (Table 3) (Fig 10).

3.5. Chloroform extract of stem

GC-MS analysis of chloroform extract of stem showed twenty six compounds. Among them tigloidine (80.902%) was the major compound (Table 4) (Fig 11).

3.6. Ethyl acetate extract of stem

Three compounds were identified by GC-MS analysis of ethyl acetate extract of stem *viz.* hexadecanoic acid, methyl ester (69.947%), 9-octadecenamide (15.620%) and 9-octadecenoic acid (Z)-, methyl ester (14.433%) (Table 5) (Fig 12).

Table 1 Quantitative result of petroleum ether extract of leaf

ID#	Name	R.Time	Area	Height	Conc.	Conc.Unit
1	Benzene, 1-ethyl-3-methyl-	3.305	22980	12893	14.830	%
2	Benzene, 1-ethyl-2-methyl-	3.353	9770	5601	6.305	%
3	Benzene, 1,2,4-trimethyl-	3.423	8754	5202	5.649	%
4	Mesitylene	3.817	20558	11526	13.267	%
5	Aniline, N-methyl-	5.127	7733	3746	4.990	%
6	Hexadecane	13.513	5307	2303	3.425	%

7	Nonadecane	19.150	11231	3694	7.248	%
8	Tetratetracontane	26.494	10051	3531	6.486	%
9	10-Methylnonadecane	31.676	5625	2272	3.630	%
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	34.022	5159	1755	3.329	%
11	13-Docosenamide, (Z)-	45.514	7337	2945	4.735	%
12	Squalene	45.999	5709	2310	3.684	%
13	.gamma.-Tocopherol	49.664	4394	1291	2.836	%
14	Vitamin E	51.200	20325	5154	13.117	%
15	.beta.-Sitosterol	53.346	3498	531	2.257	%
16	Stigmasterol	54.006	6524	1154	4.210	%

Table 2 Quantitative result of chloroform extract of leaf

ID#	Name	R. Time	Area	Height	Conc.	Conc.Unit
1	Allyl(methoxy)dimethylsilane	4.247	3091	1551	3.808	%
2	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	9.190	12149	4314	14.966	%
3	5-exo-Vinyl-5-endo-norbornenol	9.878	4502	1338	5.546	%
4	2(4H)-Benzofuranone,5,6,7,7\a-tetrahydro-4,	16.846	10521	3514	12.960	%
5	Fumaric acid, ethyl 2-methylallyl ester	18.058	13767	3745	16.959	%
6	3-Hydroxy-.alpha.-ionene	20.324	4519	1085	5.567	%
7	1-(2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpr	22.523	12850	3174	15.829	%
8	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahy	25.320	6419	1630	7.907	%
9	Phenethyl alcohol, 2,5-dihydroxy-. alpha.-me	25.968	4654	1018	5.733	%
10	Hexadecanoic acid, methyl ester	29.885	3862	1407	4.757	%
11	9-Octadecenamide	45.502	4844	1819	5.967	%

Table 3 Quantitative result of ethyl acetate extract of leaf

ID#	Name	R.Time	Area	Height	Conc.	Conc.Unit
1	Glycerin	3.615	39005	5321	74.714	%
2	Hexadecanoic acid, methyl ester	29.884	5215	2016	9.989	%
3	9-Octadecenamide	45.507	7986	3031	15.297	%

Table 4 Quantitative result of chloroform extract of stem

ID#	Name	R.Time	Area	Height	Conc.	Conc. Unit
1	Hexanoic acid	3.745	13626	2949	1.028	%
2	Dehydromevalonic lactone	7.392	16578	7079	1.250	%
3	L-Proline, 1-acetyl-	8.958	9913	4073	0.748	%
4	Isoneral	9.224	7509	2997	0.566	%
5	Bicyclo[3.1.1]hept-3-en-2-ol,4,6,6-trimethyl	10.066	16277	6753	1.228	%
6	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	10.869	4745	2024	0.358	%
7	cis-Verbenol	12.721	1353	345	0.102	%
8	2-Cyclohexen-1-ol, 1-methyl-4-(1-methyleth	12.818	5098	1883	0.384	%
9	Vanillin	13.451	10852	2395	0.818	%
10	Tigloidine	22.800	1072712	132995	80.902	%
11	4-[3,4-Dimethoxycyclohexyl]-n-butanol	23.097	4705	1220	0.355	%
12	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp	24.324	6863	1522	0.518	%
13	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahy	25.309	3663	951	0.276	%
14	Acetic acid,10,11-dihydroxy-3,7,11-trimethy	28.011	2626	673	0.198	%
15	Bicyclo[4.2.0]octa-1,3,5-triene, 7-(3-butenyl	29.948	7441	2277	0.561	%
16	(5-Nitrohex-1-enyl)benzene	30.954	15037	3869	1.134	%
17	Acetohydrazide, 2-(2-isopropyl-5-methylphe	31.315	2722	763	0.205	%
18	Bicyclo[4.2.0]octa-1,3,5-triene, 7-(3-butenyl	32.095	43668	14490	3.293	%
19	1,5-Diphenyl-1,5-hexadiene	32.646	19515	6491	1.472	%
20	Benzeneethanol, .beta.-ethenyl-	32.763	18086	5682	1.364	%
21	3-Buten-2-ol,4-(2,6,6-trimethyl-2-cyclohexe	35.422	4904	1605	0.370	%
22	[1R-(1.alpha.,7a.beta.)]-[1-((Z)-2-Methyl-1-o	35.644	7749	2722	0.584	%
23	Heliosupine	36.171	2937	815	0.222	%
24	1,2-Benzenedicarboxylic acid, diisooctyleste	41.671	13935	5420	1.051	%
25	7-Hydroxy-3-(4-methoxyphenyl)chromen-2-	43.889	7533	2316	0.568	%
26	9-Octadecenamide	45.506	5892	2114	0.444	%

Table 5 Quantitative result of ethyl acetate extract of stem

ID#	Name	R.Time	Area	Height	Conc.	Conc. Unit
1	Hexadecanoic acid, methyl ester	29.886	27760	10385	69.947	%
2	9-Octadecenoic acid (Z)-, methyl ester	33.813	5728	2153	14.433	%
3	9-Octadecenamide	45.505	6199	1991	15.620	%

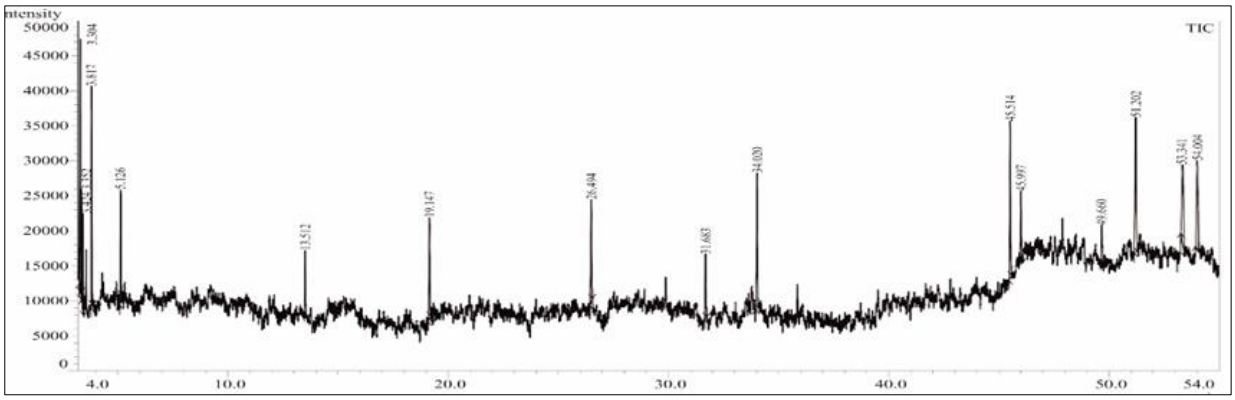

Figure 8 Mass spectrum graph of petroleum ether extract of leaf

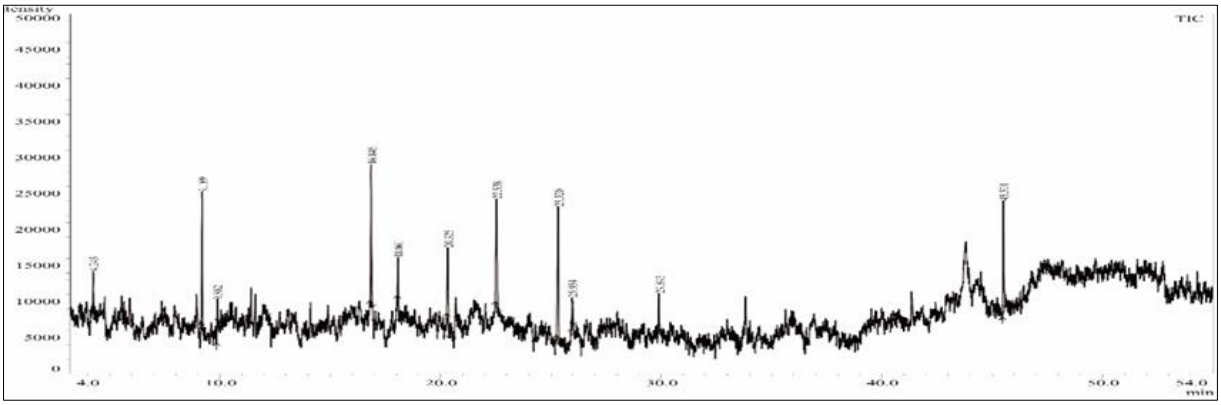

Figure 9 Mass spectrum graph of chloroform extract of leaf

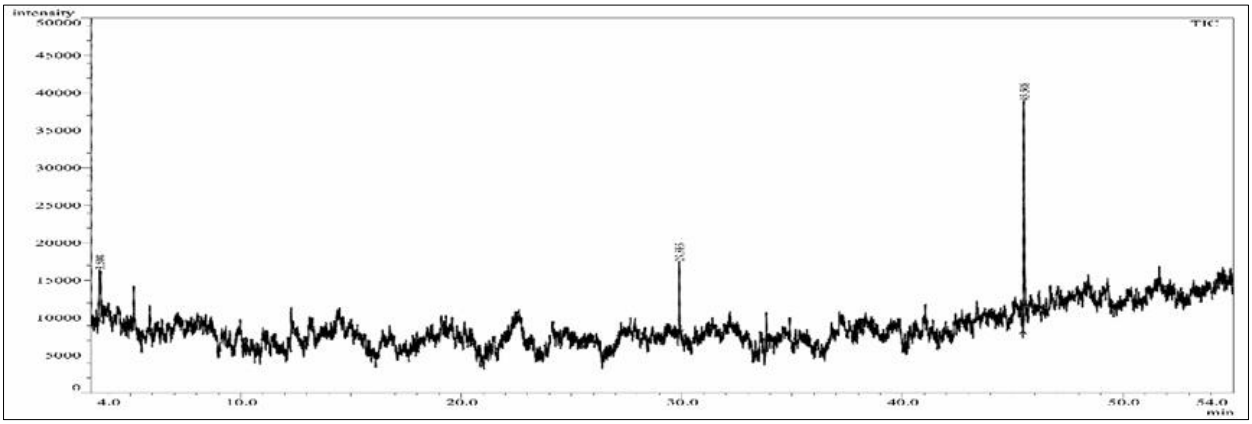

Figure 10 Mass spectrum graph of ethyl acetate extract of leaf

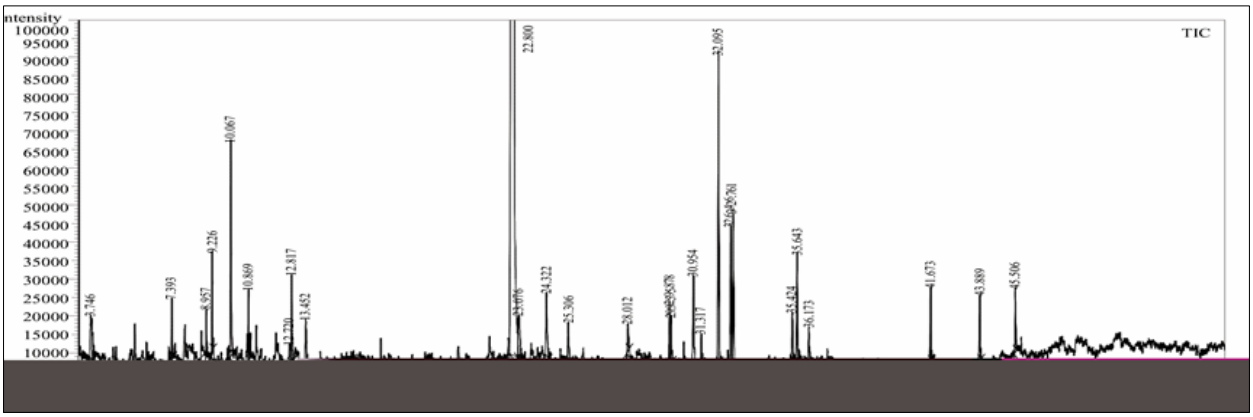

Figure 11 Mass spectrum graph of chloroform extract of stem

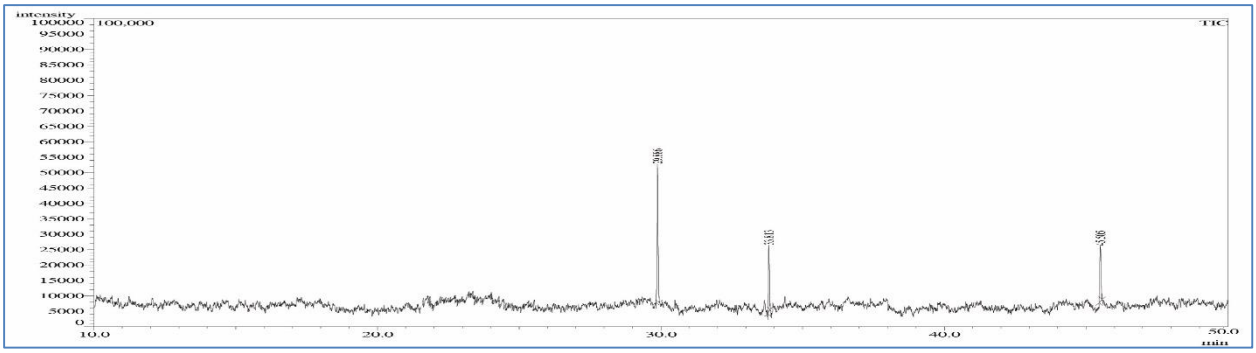

Figure 12 Mass spectrum graph of ethyl acetate extract of stem

Araujo (2010) identified the compound β-sitosterol in hexane fraction of leaves, fruits, branches and barks of the same species. Galic acid was identified from the aerial parts of another species of the same genus, *M. hamata* by Hussain *et al.* (1979). As well as lupeol was isolated from the leaves of *M. artemisiana* (Nascimento *et al.* 2012), aerial parts of *M. hostiles* (Ohsaki *et al.* 2006) and flowers of *M. caesalpiniifolia* (Araujo 2010). In the present investigation, more or less similar compounds were isolated through GC-MS analysis. For example, β-sitosterol (2.26%), stigmasterol (4.21%), γ-tocopherol (2.84%), mesitylene (13.27%), etc.

Ramesh *et al.* (2014) analyzed the methanolic extract leaf of *M. pudica* by gas chromatography coupled to mass-selective detector and 52 compounds were separated from peak value. 3,7,11,15-tetramethyl-2-hexadecen-1-ol possessed the highest peak area (18.80%) and the main compounds obtained from the sample were 1,3,5-cycloheptatriene-4-pentenal, 2-methyl, 1-propene, 1-(2-propenyloxy)-E, p-xylene, 2-cyclopentene-1, 4-dione, 3-hydroxybutanamade, w-phenylmethoxy, tetrahydro-4H-pyran-4-ol, carbamic acid, phenyl ester 2(3H)-furanone, benzenacetaldehyde, heptanal, 1-butanol, 3-methyl-acetate, 4H-pyran-4-one, 2,3-dihydroxymethyl, hydroxyethyl-hydroxymethyl benzene, benzene, coumaranone, 3-oxo-4-phenyl butyranitrile nonanoic acid, 5H-1-pyrindine, 2-methoxy-4-vinylphenol naphthalene, decanoic acid, 3-isopropoxybenzaldehyde, octose, vanillin tyrosine, benzofuranone, octadiene, 3-hydroxy-7,8-dihydro-a-ionol, trimethyl-tetraclopenta, 3-buten 3-O-methyl-d-glucose, acetic acid, buten, cyclohexane, cyclopentanol, phytol, etc. Tunna *et al.* (2015) analysed the sample by gas chromatography time of flight mass spectra (GC-Q-TOF-MS) for identification of the chemical compounds from the aerial part of *M. pudica* and matched to the NIST library. The result showed that the methanol extract and its fractions of hexane, ethyl acetate and methanol contained 46, 34, 22 and 33 different compounds respectively based on the mass spectra peaks. Harborne (1984) revealed that a non-polar solvent, hexane, brought out the fatty acid and wax components from the initial methanol extract that were basically long chain carbohydrates. Oboh *et al.* (2012) showed that the hexane fraction contained methyl sulfide, dioxolane, carotene and acids like aspartic, malonic and mannopyranosyl dodecaborane. Ethyl acetate was a semi-polar solvent mainly used for the extraction of polyphenolic compounds due to its particular affinity mostly to phenolic compounds. In the present study, the result showed that the petroleum ether extract of leaf contained 16 compounds based on the mass spectra peaks and the compounds identified were benzene, 1-ethyl-3-methyl- (14.83%), aniline, N-methyl- (4.99%),

hexadecane (3.43%), nonadecane (7.25%), 13-docosenamide, (Z)- (4.74%), squalene (3.68%), vitamine E (13.12%), etc. The ethyl acetate extract of leaf contained glycerin (74.71%), hexadecanoic acid, methyl ester (9.99%) and 9-octadecenamide (15.30%) and ethyl acetate extract of stem contained hexadecanoic acid, methyl ester (69.95%), 9-octadecenoic acid (Z)-, methyl ester (14.43%) and 9-octadecenamide (15.62%).

4. Conclusion

GC-MS analysis was carried out for petroleum ether, chloroform and ethyl acetate extracts of leaf; chloroform and ethyl acetate extracts of stem. Sixteen compounds were identified from petroleum ether extract of leaf. The major compounds were benzene,1-ethyl-3-methyl- (14.830%), mesitylene (13.267%), vitamin E (13.117%), nonadecane (7.248%), tetratetracontane (6.486%), benzene,1-ethyl-2-methyl- (6.305%) and benzene,1,2,4-trimethyl (5.649%). Eleven compounds were isolated from chloroform extract of leaf where the major compounds were fumaric acid, ethyl 2-methylallyl ester (16.959%), 1-(2-[3-(2-acetyloxiran-2-yl)-1,1-dimethyl)propyl]cycloprop-2-enyl)ethanone (15.829%), 1H-pyrrole-2,5-dione, 3-ethyl-4-methyl- (14.966%), 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- (12.960%) and 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (7.907%). Three compounds were identified from ethyl acetate extract of leaf and those were glycerin (74.714%), 9-octadecenamide (15.297%) and hexadecenoic acid, methyl ester (9.989%). Twenty six compounds were found from chloroform extract of stem and tigloidine (80.902%) was the major compound. Three compounds were identified from ethyl acetate extract of stem that were hexadecanoic acid, methyl ester (69.947%), 9-octadecenamide (15.620%) and 9-octadecenoic acid (Z)-, methyl ester (14.433%).

Compliance with ethical standards

Acknowledgments

The researchers are grateful to University Grants Commission for financial support and the Director, Institute of Biological Sciences, University of Rajshahi for providing necessary laboratory facilities for the completion of the research.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest exists.

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