PHYTOCHEMICAL ANALYSIS OF *SALVINIA MOLESTA* D.S. MITCHELL. BY GC-MS ANALYSIS

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ABSTRACT

The present investigation was undertaken to explore the potential bioactive components present in the ethanol, acetic acid and ethyl acetate extract of *S. molesta* by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The GC-MS analysis of ethanol extract showed 10 bioactive compounds. The major compounds identified were 3-Pyrrolidinol, 4-Imidazolidinone,5,5-diphenyl-2-thioxo-, 1,2-Dimethyl-3-nitro-4-nitroso-benzene, Harmaline, Tridecanoic acid, Phenytoin, Benzofuran-2-one, 2,3-dihydro-3, 3-dimethyl-4-nitro-, i-Propyl 9-tetradecenoate, 8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2, 4-quinazolinedione and Benzenemethanimine, .alpha.-phenyl-. Acetic acid extract shows the bioactive compounds were be 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5, 6, 7-tetrahydro-, isopropyl ester, 1H-Indole, 5-methyl-2-phenyl-, Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3, 3-dimethyl-, Pyrido [2, 3-d] pyrimidine, 4-phenyl- and Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(2-fluoroanilino)propionate. The ethyl acetate shows the phytochemical compounds like Cholesta-6, 22, 24-triene, 4,4-dimethyl and 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester. The compounds identified were phenolic compound, alkaloid, saturated fatty acid, steroid and indole derivative.

Keywords: Analysis, compounds, extract, GC-MS, and retention time,

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INTRODUCTION

Phytochemicals are natural bioactive compound found in plants which acts as a defense system against diseases to protect against diseases (James and Anderson, 1983).

GC-MS is normally used for direct analysis of components that in traditional medicines composed of two major building blocks; the gas chromatography and the mass spectrophotometer (Sridharan *et al.,* 2011). GC-MS is the best technique to identify the bioactive components of long chain hydrocarbons, alcohols and acids used in the analysis of the herbal medicines (Sridharan *et al.,* 2011). The two components GC and MS used together allows a much finer degree of substance identification than either unit used separately. The Mass spectrometry capture, ionize, accelerate, deflect, and detect the ionized molecules separately by detecting ionized fragments using their mass to charge ratio (Amirav *et al.,* 2008).

Globally *Salvinia* species are known as one of the most troublesome fresh water weeds because of their negative impacts on the environment. These ferns are spread all over the surface of the water levels by forming a mat-like mesh and affecting the ecosystem of fresh water resources like ponds, rivers, lakes, and rice paddies (Mujaju *et al.,* 2021). Different species of *Salvinia* were reported for the presence of many pharmacological compounds and various activities such as antimicrobial, antioxidant and immunomodulatory activity (Purgato *et al.,* 2021; Naheed *et al.,* 2021; Nithya *et al.,* 2019). So, the aim of the present study is to identify the active compounds present in *S. molesta* by GC-MS analysis and collected from Poovangaparambu, Kanniyakumari Dist.

MATERIALS AND METHODS

Collection, Identification and Preparation of extracts

The fresh samples of *S. molesta* were manually collected and were identified by the experts. It is initially washed with pond water, followed by running tap water and finally with sterile distilled water to remove the adhering microscopic and macroscopic debris. It was shade dried and powdered. The fine powdered samples were stored in an airtight container for further use.

The dried powdered samples (10gm) extracted using various solvent extracts (ethanol, acetic acid, and ethyl acetate). Then the extracts were stored in a refrigerator for further use (Rebecca *et al.,* 2012).

GC-MS (Gas Chromatography-Mass Spectrometry)

A high-resolution mass spectrum equipped with a data system in combination with Gas Chromatography was used for the chemical analysis of aquatic pteridophyte. GC-MS analysis of the extracts was carried out by the following method of Hema *et al*. (2010) using a GC-MS Clarus 500 Perkin Elmer system and gas chromatography interfaced to mass spectrometer (GC-MS). The detection of the compounds employed with the NIST (National Institute of Standards and Technology). The relative % amount was calculated by comparing its peak area to the total areas. Software adopted to handle mass spectra and chromatogram was Turbomass (Version 5.2).

RESULTS AND DISCUSSION

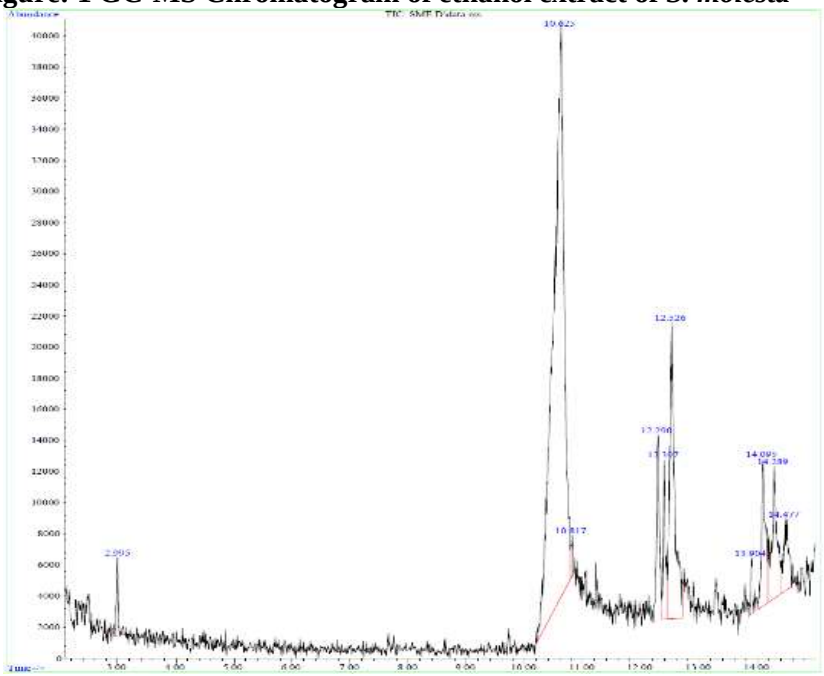
GC-MS analysis was carried out using of GC Clarus 500 Perkin Elmer System and Gas chromatography (GC-MS) instrument. The GC-MS identification of the chemical constituents was based on comparison of their mass spectra with NIST library. Structure was defined by percentage similarity values. They are confirmed by the study of base peaks, retention time (RT) and molecular weight (MW) of the compounds.

The active constituent of ethanol extract of *S. molesta* were analysed by GC-MS analysis, the detail tabulation is given in Table. 1. The ethanol extract showed the presence of ten phytochemical constituents. The highest peak was identified was 3-Pyrrolidinol with the retention time of 2.955. It showed the peak area of 1.38 %. The other peaks identified was 4-Imidazolidinone, 5,5-diphenyl-2-thioxo- with the retention time of 10.625 and the peak area of 58.83 %. 1,2-Dimethyl-3-nitro-4-nitroso-benzene had the retention time of 10.817 with a peak area of 0.69 %. Harmaline showed retention time of 12.290 with a peak area of 5.21%. Tridecanoic acid and Phenytoin with retention time of 12.397 have a peak area of 3.20 % and 12.526 with a peak area of 14.38 % respectively. The compound Benzofuran-2-one, 2,3-dihydro-3,3-dimethyl-4-nitro- showed the sharp peak area of 0.86 % at a retention time (RT) of 13.904 minutes. i-Propyl 9-tetradecenoate is a compound with a peak area of 5.26 % with the retention time of (RT) 14.095 minutes. 8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2, 4-quinazolinedione is a compound had a peak area of 6.80 % and showed its presence at a retention time of 14.289 minutes. The compound Benzenemethanimine, .alpha.-phenyl- had a peak area of 3.39 % with the retention time of 14.477 minutes Table: 1; Fig: 1.

Table: 1 Compounds identified as ethanol extract of *S. molesta*

Peak No.	Compound Name	Area of %	Retention Time (Min.)	Molecular Weight (G/mol.)	Molecular Formula
1	3-Pyrrolidinol	1.38	2.995	87.12	C_4H_9NO
2	4-Imidazolidinone, 5,5-dipenyl-2-thioxo-	58.83	10.625	268.3	$C_{15}H_{12}N_2OS$
3	1,2-Dimethyl-3-nitro-4-nitroso-benzene	0.69	10.817	180	$C_8H_8N_2O_3$
4	Harmaline	5.21	12.290	214.26	$C_{13}H_{14}N_2O$
5	Tridecanoic acid	3.20	12.397	214.34	$C_{13}H_{26}O_2$
6	Phenytoin	14.38	12.526	252.27	$C_{15}H_{12}N_2O_2$
7	Benzofuran-2-one, 2,3-dihydro-3, 3-dimethyl-4-nitro-	0.86	13.904	207.18	$C_{10}H_9NO_4$
8	i-Propyl 9-tetradecenoate	5.26	14.095	268	$C_{17}H_{32}O_2$
9	8-Isopropyl-5-methyl-5,6,7,8-tetrahydro-2, 4-quinazolinedione	6.80	14.289	222.28	$C_{12}H_{18}N_2O_2$
10	Benzenemethanimine,.alpha. -phenyl-	3.39	14.477	181.23	$C_{13}H_{11}N$

Figure: 1 GC-MS Chromatogram of ethanol extract of *S. molesta*

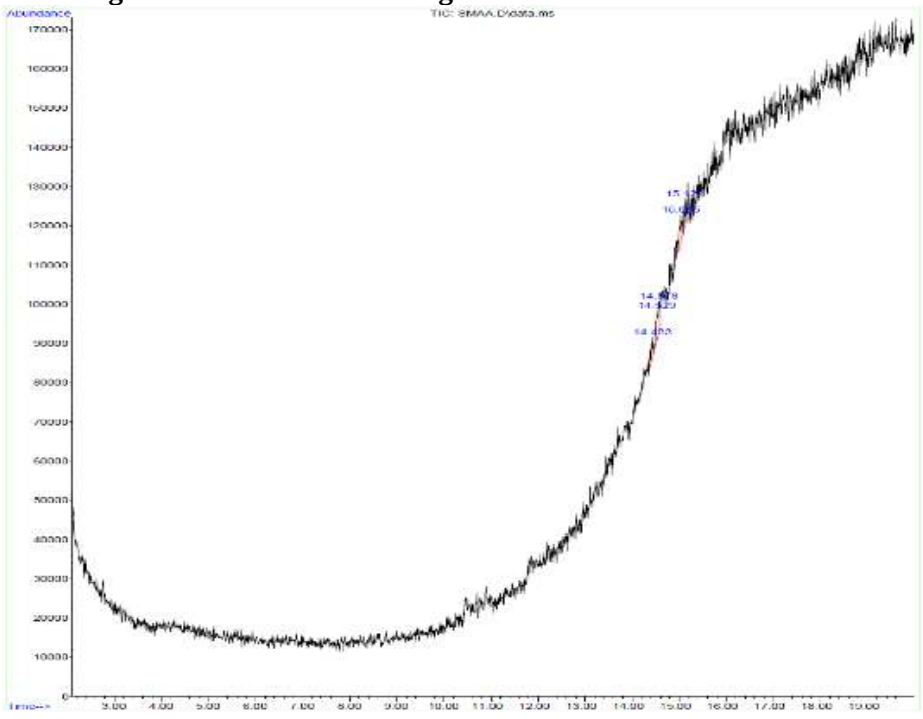


The compounds present in acetic acid extract of *S. molesta* is displayed in Table: 2; Fig: 2. Five bioactive compounds were detected. The bioactive compounds were found to be 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5, 6, 7-tetrahydro-, isopropyl ester showed a highest peak area of 16.17 % and a retention time of (RT) 14.433 minutes. The compound 1H-Indole, 5-methyl-2-phenyl- had a peak area of 26.29 % with the retention time of (RT) 14.529 minutes. Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3, 3-dimethyl- is a compound with a peak area of 15.35 % and a retention time (RT) of 14.578 minutes. Pyrido [2, 3-d] pyrimidine, 4-phenyl- had a peak area of 25.59 % with the retention time of 15.035. A chemical compound identified as Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(2-fluoroanilino) propionate with the peak area of 16.60 % and the retention time was found to be 15.121 minutes.

Table: 2 Compounds identified as acetic acid extract of *S. molesta*

Peak No.	Compound Name	Area of %	Retention Time (Min.)	Molecular Weight (G/mol.)	Molecular Formula
1	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	16.17	14.433	355.43	$C_{21}H_{25}NO_4$
2	1H-Indole, 5-methyl-2-phenyl-	26.29	14.529	207.27	$C_{15}H_{13}N$
3	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3, 3-dimethyl-	15.35	14.578	207.23	$C_{11}H_{13}NO_3$
4	Pyrido [2, 3-d] pyrimidine, 4-phenyl-	25.59	15.035	207.23	$C_{13}H_9N_3$
5	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoro-2-(2-fluoroanilino) propionate	16.60	15.121	356.70	$C_{13}H_{13}ClF_4N_2O_3$

Figure: 2 GC-MS Chromatogram of acetic acid extract of *S. molesta*



The ethyl acetate extract of *S. molesta* showed the presence of two phytochemical constituents. The phytochemicals were found to be Cholesta-6, 22, 24-triene, 4, 4-dimethyl- and 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5, 6, 7-tetrahydro-, isopropyl ester. The compound Cholesta-6, 22, 24-triene, 4, 4-dimethyl- showed the highest sharp peak area of 50.04 % at a retention time of 14.620 minutes and 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5, 6, 7-tetrahydro-, isopropyl ester has a peak area of 49.96 % at a retention time (RT) of 16.348 minutes Table: 3; Fig: 3.

The compounds identified from three extracts were phenolic compound, alkaloid, saturated fatty acid, steroid and indole derivative.

Most of the identified compounds were phenols. Fatty acids were also seen. Natural phytochemical including phenolic compounds and fatty acids are major bioactive compounds known to be beneficial against many diseases and have been reported to possess a wide range of biological effects like anti-oxidant and antibacterial activities (Sofowora, 1996). Phenols have been reported to possess antioxidant (Heima *et al.*, 2002), antibacterial and antifungal activities (Alasalvar *et al.*, 2001; Acamovic and Brooker, 2005; Edreva *et al.*, 2008).

Alkaloids, steroids were also noticed in the extracts. Alkaloids are also essential for the treatment of cardiovascular and kidney disorders (Sweetman, 2005). It was also reported that alkaloids have a wide range of pharmacological activities including antimalarial, antibacterial (Cushnie *et al.,* 2014), anticancer (Kittakoop *et al.,* 2014) and antihyperglycemic activities (Qiu *et al.,* 2014).

Plant steroids possess many interesting medicinal, pharmaceutical and agrochemical activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity (Patel and Savjani, 2015).

The presence of Indole derivatives in the extracts also known to possess various biological activities, i.e., antiviral, anti-inflammatory, anticancer, anti-HIV, antioxidant, antimicrobial, antitubercular, antidiabetic, antimalarial and anticholinesterase activities etc, which created interest among researchers to synthesize a variety of indole derivatives (Kumar and Ritika, 2020).

Ethanol extract revealed the presence of a greater number of compounds. In previous studies also ethanol extract revealed the presence of a greater number of compounds (Tyagi and Agarwal, 2017; Abdelhamid *et al.,* 2015; Sreenath *et al.,* 2016; Kumar *et al.,* 2018).

The ethyl acetate showed least number of compounds. this is because of geographical location, climatic conditions and edaphic factors amongst others are known to affect the chemical composition of plants and their medicinal properties (Gairola *et al.,* 2010).

Table: 3 Compounds identified as ethyl acetate extract of *S. molesta*

Peak No.	Compound Name	Area of %	Retention Time (Min.)	Molecular Weight (G/mol.)	Molecular Formula
1	Cholesta-6, 22, 24-triene, 4,4-dimethyl-	50.04	14.620	394.7	$C_{29}H_{46}$
2	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	49.96	16.348	355.43	$C_{21}H_{25}NO_4$

Figure: 3 GC-MS Chromatogram of ethyl acetate extract of *S. molesta*

CONCLUSION

GC-MS analysis have facilitated to identify bioactive compounds. In the present studies all the studied extracts showed the presence of phyto chemical compounds and ethanol extract of the *S. molesta* has proved to contain more number of phytochemical compounds. Isolation of the individual compound and subjecting it to biological

activity will give fruitful results. Very few works done by GC-MS analysis in this plant so it is the first step towards understanding the nature of active principles in *S. molesta* extracts and this study paves way for further research.

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