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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF BIOACTIVE CONSTITUENTS IN THE ETHANOLIC EXTRACT OF *PISTIA STRATIOTES* L.

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ABSTRACT
Pistia stratiotes is an invasive weed that causes serious issues for rivers, lakes, and other reservoirs around the world, although it can be an excellent source for bioactive compounds such as phytosterols and some steroids found in many plants. It belongs to family Araceae. Its leaves are traditionally used against ringworm infection of scalp, boils and syphilitic eruptions. Traditionally, oil extracts is used for treatment of tuberculosis, asthma and dysentery. To isolate and analyze the chemical composition in Ethanolic extract from of *Pistia stratiotes* L. by gas chromatography-mass spectrometry(GC-MS). The shade dried plant powder was extracted with ethanol by using Soxhlet extractor and crude ethanolic extract was obtained. Qualitative analyses of ethanolic crude extract of weed, *Pistia stratiotes* L. by using GC-MS showed that they were different types of high and low molecular weight compounds. n-Hexadecanoic acid (7.18%), E-11-Hexadecenoic acid, ethyl ester (0.24%), Hexadecanoic acid, ethyl ester (13.29%), L- Glutamine (0.38%), Linolelaidic acid, methyl ester (2.41%), 9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z) (2.7%), Diisooctyl phthalate (53.84%), Stigmasterol (2.57%), 1-Monolinoleoylglycerol trimethylsilyl ether(0.22%), Ethyl iso-allocholate (0.92%). Most of the isolated and identified compounds by GC-MS in the crude extracts exhibit following bioactivities. Anticancer, Anti-inflammatory, Antimicrobial, Diuretic, Hepatoprotective, Antiarthritic, Antiasthma, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor, Insectifuge, Antihistaminic, Antieczemic, Antiacne, Anticoronary, Antifouling effects so that they can be recommended as a plant of phytopharmaceutical importance. Therefore, ethanol extract of *Pistia stratiotes* proves as a potential source of bioactive compounds of pharmacological importance.

Keywords: *Hepatoprotective, Antiandrogenic, 5-Alpha Reductase Inhibitor, Hypocholesterolemic, Antiandrogenic, Anticoronary, Antifouling, Phytopharmaceutical*

INTRODUCTION
Herbal plants are being used as medicine from ancient age and usefulness of them are recorded in human history. Herbal plants are reported to be excellent source of several nutrients (Musa, 2005). The use of herbal drugs in treatment of diseases is found among all sections of people in India. The plant *Pistia stratiotes*, commonly known as water cabbage or water lettuce, belongs to the family Araceae, is an edible, aquatic, floating ornamental plant with widely distributed across tropical and sub-tropical areas around the world. The plant leaves are light green, obovate with prominent longitudinal veins at its base (Arber, 1991). *P. stratiotes* is widely distributed and is being loathed in Asia and Africa. This plant and its extracts are potentially believed to have medicinal effects. This plant is proven to be antiseptic, antitubercular and antidysentric. In various parts of the world it is also used as anodyne for eyewash. The leaves are used in eczema, leprosy, ulcers and piles (Kirtikar and Basu, 2000). The plant is bitter, pungent flavor, having cooling, laxative property. It is used in 'Tridosha' fever and diseases of blood. Leaf infusions have been mentioned in the folklore to be used for dropsy, bladder complaints, kidney afflictions, hematuria, dysentery and anemia (Kirtikar and Basu, 2001).
The aim of this study was to analyze organic water lettuce extract through gas chromatography-mass spectrometry (GC-MS) to elucidate their chemical composition and to determine their potential applications.

Figure 1: Pictorial View of *Pistia stratiotes* L.

MATERIALS AND METHODS

Collection of Plant Material

Leaves and Roots of *Pistia stratiotes* L. (Araceae), was collected from Kishor Sagar lake, Kota city, Rajasthan, India. It is situated between 25°11'0"N latitude and 75°50'0"E longitude. Kishor Sagar lake in Kota city is one of the major water bodies enhances the ground water level around this area (Figure 1).

Preparation of Plant Extract

The collected plant materials were air dried and ground into uniform powder. Dry powder of plant sample was extracted with ethanol using soxhlet apparatus for 6 hours. The extract was filtered, followed by concentrated using rotary evaporator.

The concentrated extract was subjected to freeze drying in a lyophilizer till dry powder was obtained. Finally, the extracted powder was suspended with the ethonal at the concentration of 100mg/ml (w/v) followed by filtration through Varian Bond Elute C18 solid phase extraction to remove impurities. 1µl of this solution was employed for GC-MS-MS analysis.

GC-MS Analysis

The GC-MS analysis was carried out using Agilent Technologies GC-MS (GC-7890A, MS 5975C) with Fused silica 15m x 0.2 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 30-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS compounds present in the plants sample were identified.

Identification of Phyto-Compounds

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Gas chromatogram of leaves and root ethanol extract of *Pistia stratiotes* L. is presented in Figures 2 and 3 respectively.

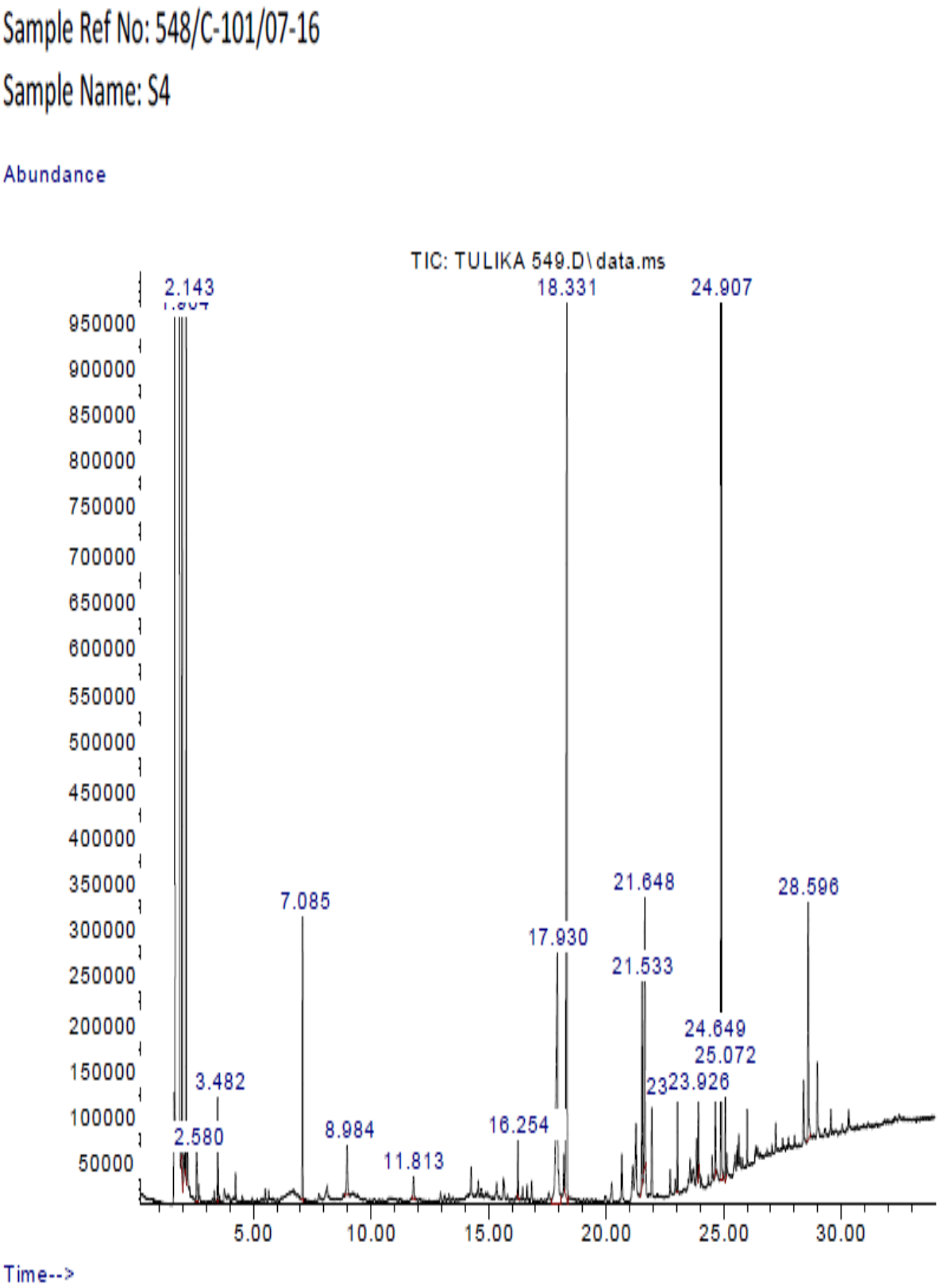

Figure 2: Gas Chromatogram of *Pistia Stratiotes* L. Leaf Ethanol Extract

Table 1: Proposed Retention Time, Compounds, Molecular Formula, Molecular Weight, Peak Area %, Compound Nature and Bioactivity of *Pistia stratiotes* L. Leaf Ethanol Extract

RT	Name of Compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
2.14	Isobutyl alcohol	$C_4H_{10}O$	74	5.42	Alcohol	Biofuel
3.48	Formic acid,1- methylethyl ester	$C_4H_8O_2$	88	0.93	Carboxylic acid	Preservative, antibacterial agent, treatment for warts.
7.08	Propane,1,1-diethoxy-2-methyl	$C_8H_{18}O_2$	146	2.05	Ether	No activity reported.
11.81	L-Glutamine	$C_5H_{10}N_2O_3$	146	0.38	Amino acid	Building block of Protein
17.93	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.18	Palmitric acid (saturated fatty acid)	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor, antipsychotic.
18.33	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	13.29	Palmitic acid ester	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Antiandrogenic.
21.53	Linolelaidic acid, methyl ester	$C_{19}H_{34}O_2$	294	2.41	Fatty acid	No activity reported.
21.64	9,12,15- Octadecacatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292	2.7	Steroid	Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic.
23.92	12,15- Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	0.50	Unsaturated fatty acid ester	No Activity reported.
24.64	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	0.96	Amino compound	Antimicrobial
24.90	Diisooctyl phthalate	$C_8H_4(C_8H_{17}COO)_2$	390	53.84	Plasticizer compound	Antimicrobial, Antifouling.
25.07	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368	0.69	Fatty ester	No Activity reported.
28.59	Stigmasterol	$C_{29}H_{48}O$	412	2.57	Steroid	Antioxidant, hypoglycemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer, antiarthritic, antiasthama, anti inflammatory, diuretic.

Activity Source: - Dr. Duke's Phytochemical and Ethnobotanical Databases

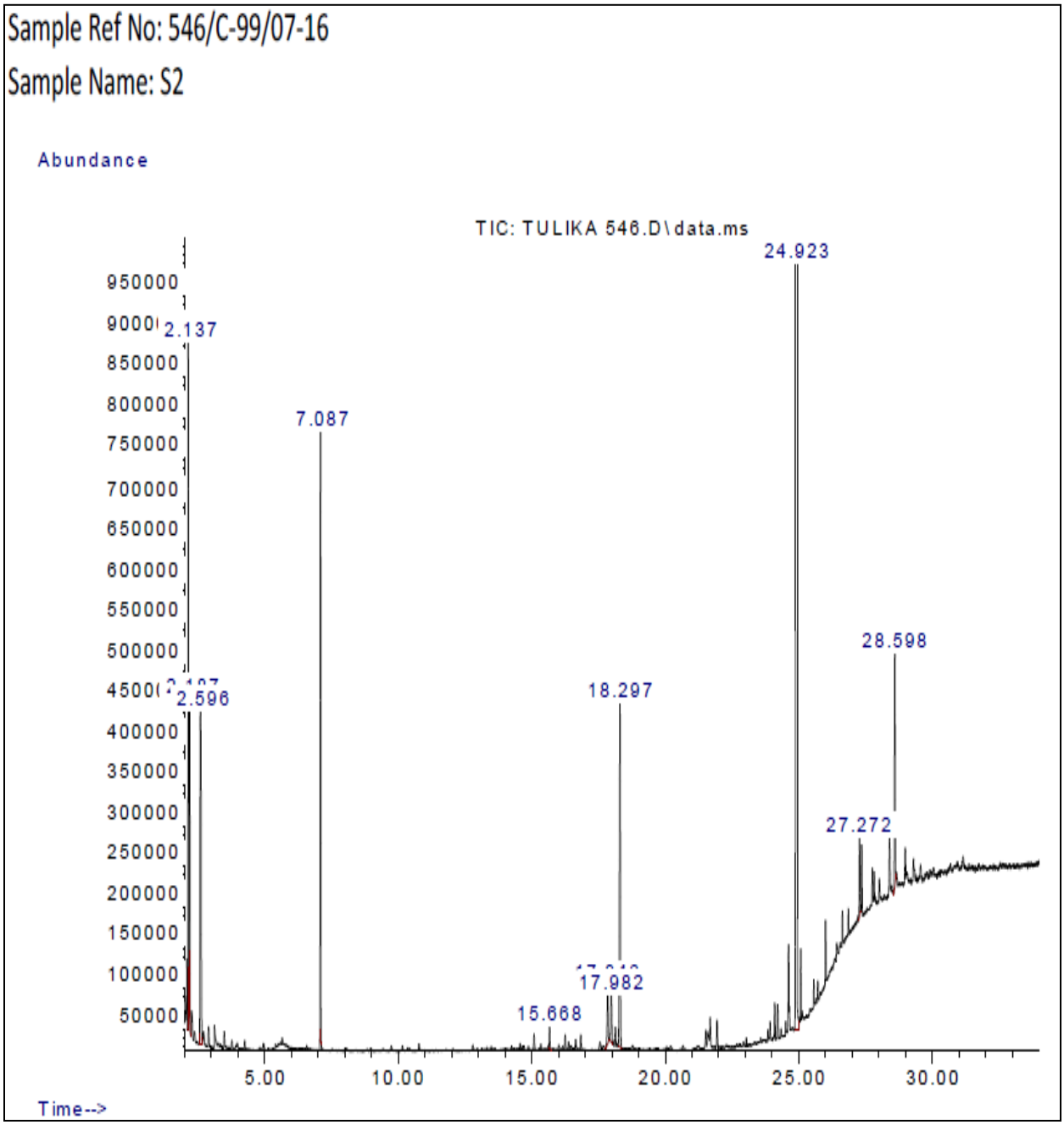

Figure 3: Gas Chromatogram of *Pistia Stratiotes* L. Root Ethanol Extract

The GC-MS analysis of *P. stratiotes* leaves revealed the presence of 10 major compounds L-Glutamine (0.38%), n- Hexadecanoic acid (7.18%), Hexadecanoic acid, ethyl ester (13.29%), Linolelaidic acid (2.41%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (2.7%), Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl) ethyl ester (0.96%), Diisooctyl phthalate (53.84%), Docosanoic acid, ethyl ester (0.69%), Stigmasterol (2.57%).

The GC-MS analysis of *P. stratiotes* roots revealed the presence of 6 major compounds n- Hexadecanoic acid (0.30%), E-11-Hexadecanoic acid, ethyl ester (0.24%), Hexadecanoic acid, ethyl ester (1.63%), Bis (2-ethylhexyl) phthalate (91.49%), 1-Monolinoleoylglycerol trimethylsilyl ether (0.22%), Ethyl iso-allocholate (0.92%).

Table 2: Proposed Retention Time, Compounds, Molecular Formula, Molecular Weight, Peak Area %, Compound Nature and Bioactivity of *Pistia stratiotes* L. Root Ethanol Extract

RT	Name of Compound	Molecular Formula	Molecular Weight	Peak Area %	Compound Nature	Activity
2.13	Isobutyl alcohol	$C_4H_{10}O$	74	1.70	Alcohol	Biofuel
2.59	Ethane, 1,1-diethoxy	$C_6C_{14}O_2$	118	1.79	Ether	Flavoring agent
7.08	Propane,1,1-diethoxy-2-methyl	$C_8H_{18}O_2$	146	1.61	Ether	No activity reported.
15.66	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.07	Fatty ester	No activity reported.
17.84	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.30	Palmitric acid (saturated fatty acid)	Antioxidant, antipsychotic, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-alpha reductase Inhibitor.
17.98	E-11-Hexadecanoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	0.24	Stearic acid	Antifungal, Antitumour, Antibacterial.
18.29	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.63	Palmitic acid ester	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Antiandrogenic
24.92	Bis (2-ethylhexyl) phthalate	$C_8H_4(C_8H_{17}COO)_2$	390	91.49	Plasticizer compound	Solvent in glowstick.
27.27	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	0.22	Steroid	Antiarthritic, Anticancer Hepatoprotective, Antimicrobial, Antiasthma, Diuretic.
28.59	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.92	Steroid	Antimicrobial, Diuretic, Anti-inflammatory, Antiasthma.

Activity Source: - Dr. Duke's Phytochemical and Ethnobotanical Databases

Among the identified phytochemicals, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester have the property of antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors (Jegadeeswari *et al*., 2012; Upgade and Anusha, 2013). n-Hexadecanoic acid as the comman compound in the leaves and root of *P. stratiotes*. E-11-Hexadecanoic acid, ethyl ester acts as Antifungal, Antitumour, Antibacterial. Linolenic acid, methyl ester found to act as Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective Antiandrogenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne. Ethyl iso-allocholate is suggested to be a sterol compound and it may use as an antibacterial, antioxidant, antitumor, cancer preventive, pesticide and chemo preventive agent (Duke, 1992-1996). L-Glutamine is an amino act found in less quantity but is a major component in building Protein.

Stigmasterol is an unsaturated plant sterol and act as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. It is also used as the precursor of Vitamin D_3 (Kametani and Furuyama, 1987).

1-Monolinoleoylglycerol trimethylsilyl ether has many biological activities such as Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic, antioxidant, anti-inflammatory and anti diabetic (Senthil *et al*., 2016). 9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)- is a polyenoic fatty acid compound and it may be acts as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, anti histaminic, anti arthritic, anti coronary, anti eczemic, anti acne, 5-alpha reductase inhibitor and anti androgenic (Vohra and Kaur, 2011). Diisooctyl phthalate is a plasticizer compound, it may be acts as an antimicrobial and antifouling (Sangeetha and Vijayalakshmi, 2011).

Several other compounds were also detected through GC/MS chromatogram having notable medicinal property. The above said compounds found in the ethanol extract of *P. stratiotes* leaves and roots are being used for the pharmacological work.

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results.

Conclusion

P. stratiotes is one of the aquatic weeds pose serious threat to aquatic ecosystem throughout the world, found to possess many medicinal values. Various management procedures have been adapted to control this weed, but no effective strategy has been developed till date. Therefore, commercial use of this plant could be an alternate for its management contributing to solve environmental and economic problems caused by it. GC-MS analysis of ethanol extract of leaves and root of *P. stratiotes* revealed the presence of secondary metabolites of anticancerous, antimicrobial, antioxidant, antidandruff, antiproliferative activities and provides a potential source of industrial application.

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REFERENCES

Arber A (1991). The vegetative morphology of *Pistia* and the Lemnaceae. *Proceedings of the Royal Society* **91** 96- 103.

Duke's Phytochemical and Ethnobotanical Databases (1992 -1996). *U.S. Department of Agriculture, Agricultural Research Service* [Online]. Available: http://phytochem.nal.usda.gov [Accessed 12 July 2016].

Jegadeeswari P, Nishanthini A, Muthukumaraswamy S and Mohan VR (2012). GC-MS analysis of bioactive components of Aristolochia krysagathra (Aristolochiaceae). *Journal of Current Chemical and Pharmaceutical Sciences* **2** 226-236.

Kametani T and Furuyama H (1987). Synthesis of Vitamin D3 and related compounds. *Medicinal Research Reviews* **7**(2) 147-171.

Kirtikar KK and Basu BD (2001). *The Indian Medicinal Plants.* (India, Dehradun: Oriental Enterprises) 3576-3579.

Kirtikar KR and Basu BD (1960). *Indian Medicinal Plant* **3** 1702-1712.

Kirtikar KR and Basu BD (2000). *Indian Medicinal Plants,* (India, New Delhi: Sri Satguru Publications).

Musa O (2005). Mineral composition of different parts of *Capparis onila* Dest Var. Cenescens (Coss) Heywood growing wild in Turkey. *Journal of Medical Food* **8** 405-407.

Sangeetha J and Vijayalakshmi K (2011). Determination of bioactive components of ethyl acetate fraction of *Punica granatum* Rind extract. *International Journal of Pharmaceutical Science and Drug Research* **3**(2) 116-122.

Senthil J, Rameashkannan MV and Mani P (2016). PhytochemicalProfiling of Ethonolic Leaves Extract of Ipomoea sepiaria (Koenig Ex. Roxb). *International Journal of Innovative Research in Science, Engineering and Technology* **5**(3) 3140-3147.

Upgade A and Anusha B (2013). Characterization and medicinal importance of phytoconstiuents of Carica papaya from down south Indian region using gas chromatography and mass spectroscopy. *Asian Journal of Pharmaceutical and Clinical Research* **6**(4) 101-106.

Vohra A and Kaur H (2011). Chemical investigation of medicinal plant *Ajuga bracteosa*. *Journal of Natural Product and Plant Resources* **1**(1) 37-45.