

ORIGINAL ARTICLE

Ethnomedicine Analysis Of Bioactive Constituents in Ethanolic Leaf Extract of *Pistia Stratiotes* L. And *Eichhornia Crassipes* (Mart.) Solms By GC-MS

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ABSTRACT

Eichhornia crassipes (water hyacinth) 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. *Pistia stratiotes* 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. and *Eichhornia crassipes* (Mart.) Solms 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. and *Eichhornia crassipes* (Mart.) Solms 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 (1.04%), 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%), Palmitic acid (12.09 %), Phytol (2.12%), 9,12-Octadecadienoic acid, ethyl ester(3.79%), Linolenic acid, ethyl ester (26.26%), Stearic acid, ethyl ester (0.98%), α -Glyceryl linolenate (1.35%), Diisooctyl phthalate (53.84%), Stigmasterol (11.39%), 1-Monolinoleoylglycerol trimethylsilyl ether(1.52%). 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* and *E.crassipes* proves as a potential source of bioactive compounds of pharmacological importance.

Keywords : Hepatoprotective, Antiandrogenic, 5-Alpha reductase inhibitor, Hypocholesterolemic, Antiandrogenic, Anticoronary, Antifouling, phytopharmaceutical.

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INTRODUCTION

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 [1]. *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 antidycentric. In various parts of the world it is also used as anodyne for eyewash. The leaves are used in eczema, leprosy, ulcers and piles [2]. 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[3].

The fresh water aquatic plant *E. crassipes*, commonly known as water hyacinth is a member of the family Pontederiaceae. This fast growing, free-floating, perennial plant is indigenous to Brazil Amazon basin and Ecuador region. In recent years, *E. crassipes* has been studied with a lot of interest because of its effects on habitats, but to be eradicated, a big investment is required [4]. Water hyacinth is a source of many compounds with radical-scavenging activity, such as vitamins, terpenoids, phenolic acids, lignin, stilbens, alcaloids, sterols, and other metabolites with high antioxidant activity [5]. Phytosterols are steroidal molecules that show a similar structure to cholesterol found in many vegetables such as water hyacinth. The most common phytosterol compounds is stigmasterol. Those compounds comprise 98% of all the vegetable sterols identified in plants [6].

The aim of this study was to analyze organic water lettuce and water hyacinth extracts through gas chromatography-mass spectrometry (GC-MS) to elucidate their chemical composition and to determine their potential applications.

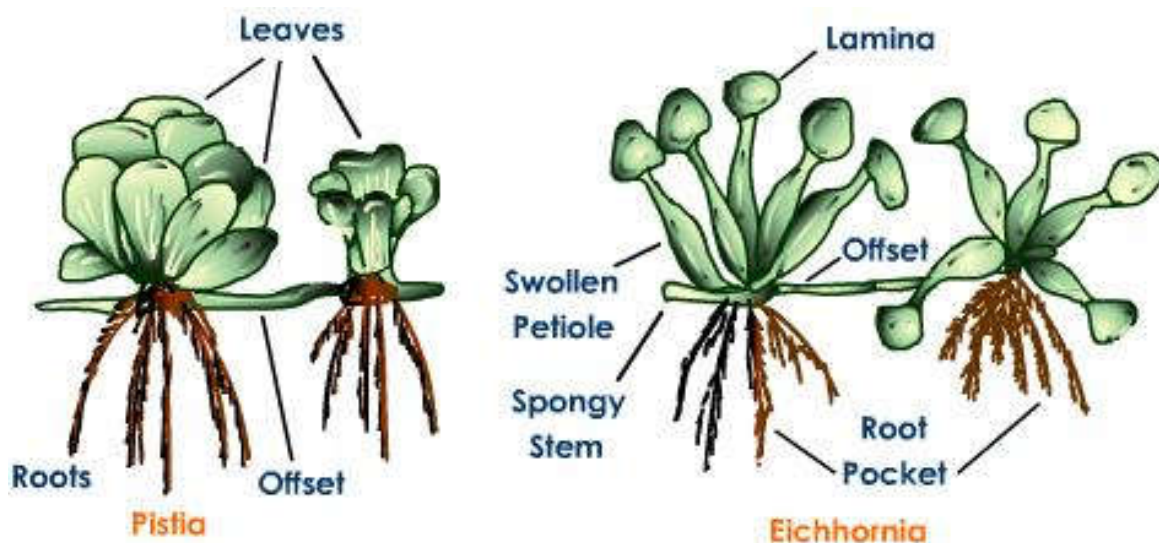


Figure 1: Pictorial View of *Pistia stratiotes* and *E. crassipes*

MATERIAL AND METHODS

Collection of Plant Material

Leaves of *Pistia stratiotes* L. (Araceae) and *Eichhornia crassipes* (Mart.) solms, (Pontederiaceae), were 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.

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 °C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250 °C 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.

RESULT AND DISCUSSION

Gas chromatogram and mass spectra of leaves ethanol extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms are presented in Figures 2 and 3 respectively.

Sample Ref No: 548/C-101/07-16

Sample Name: S4

Abundance

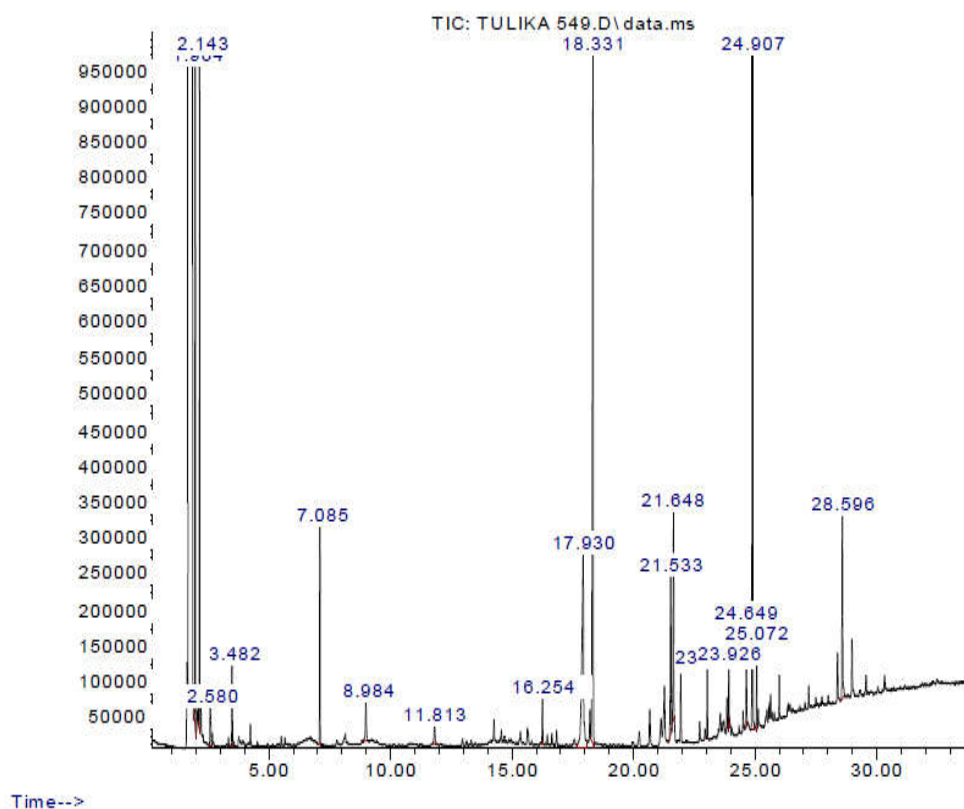
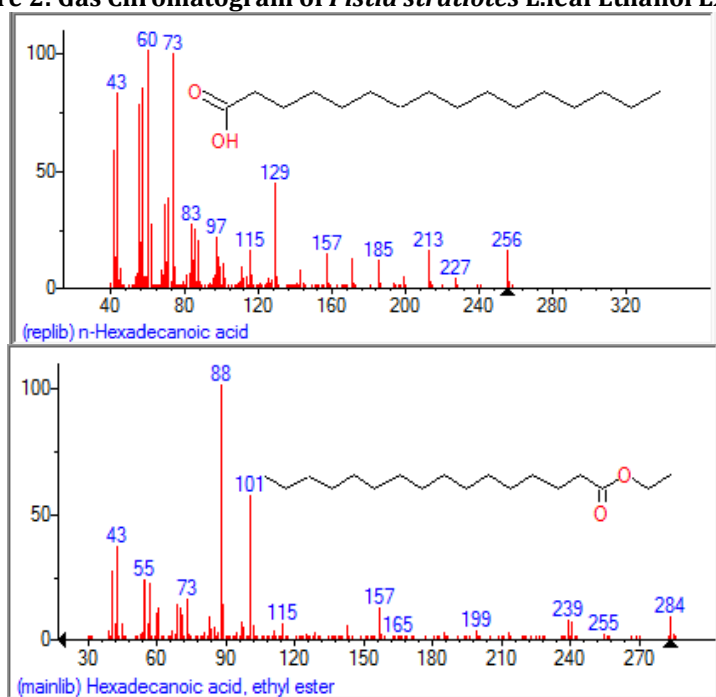


Figure 2: Gas Chromatogram of *Pistia stratiotes* L. leaf Ethanol Extract



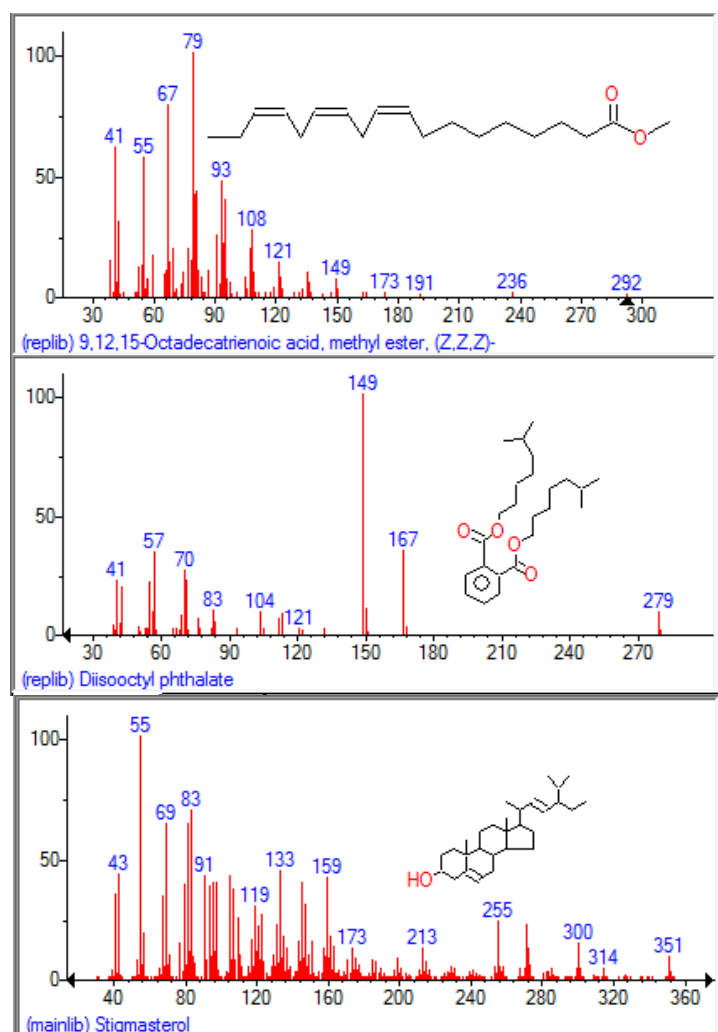


Figure 3: Mass Spectra of *Pistia stratiotes* L. leaf Ethanol Extract

Table 1: Compound present in the Ethanolic Leaves extract of *Pistia stratiotes*

RT	Name of Compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
17.93	n- Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	7.18	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Antiandrogenic, Hemolytic, Pesticide, Lubricant, 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.
21.64	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	C ₁₉ H ₃₂ O ₂	292	2.7	Steroid	Antimicrobial, Anticancer, Hepatoprotective, , Antiarthritic, antiasthama, diuretic.
24.64	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C ₁₉ H ₃₈ O ₄	330	0.96	Amino compound	Hemolytic, pesticide, flavour, antioxidant.
24.90	Diisooctyl phthalate	C ₈ H ₄ (C ₈ H ₁₇ COO) ₂	390	53.84	Plasticizer	Antimicrobial, Antifouling
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.
11.81	L-Glutamine	C ₅ H ₁₀ N ₂ O ₃	146	0.38	Amino acid	Building block of Protein

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

Sample Ref No: 545/C-98/07-16

Sample Name: S1

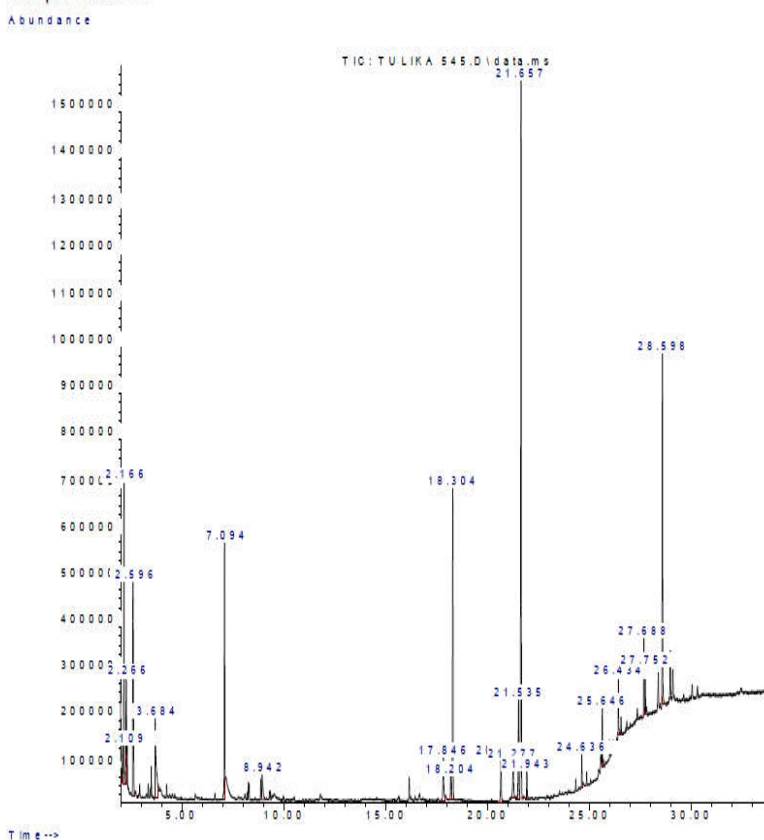
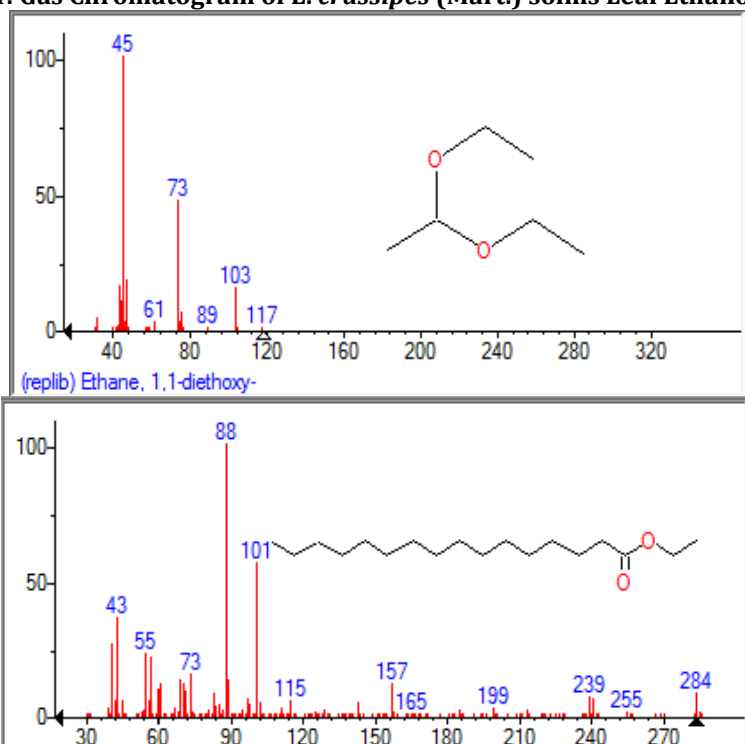


Figure 4: Gas Chromatogram of *E. crassipes* (Mart.) solms Leaf Ethanol Extract



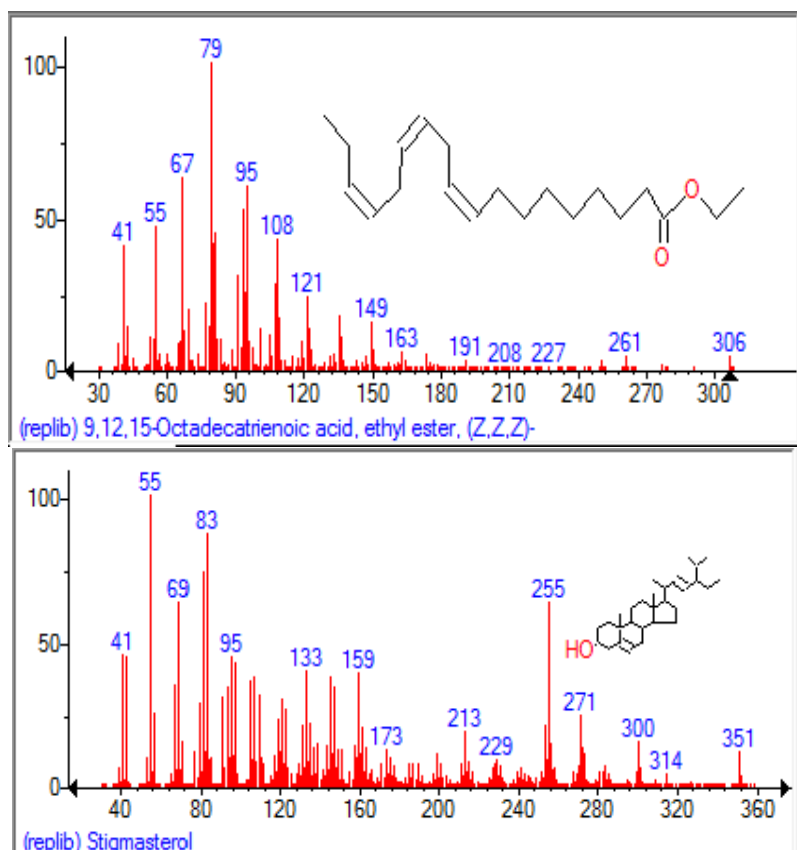


Figure 5: Mass Spectra of *E. crassipes* (Mart.) solms leaf Ethanol Extract

The GC-MS analysis of *P. stratiotes* leaves revealed the presence of 7 major compounds n- Hexadecanoic acid (7.18%), Hexadecanoic acid, ethyl ester (13.29%), 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%), Stigmasterol (2.57%), L-Glutamine (0.38%).

The GC-MS analysis of *E. crassipes* leaves revealed the presence of 11 major compounds n- Hexadecanoic acid (2.34%), E-11-Hexadecanoic acid, ethyl ester (1.04%), Palmitic acid, ethyl ester (12.09%), Phytol (2.12%), 9,12-Octadecadienoic acid, ethyl ester (3.79%), Linolenic acid, ethyl ester (26.26%), Stearic acid, ethyl ester (0.98%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.87%), α -Glyceryl linolenate (1.35%), 1-Monolinoleoylglycerol trimethylsilyl ether (1.52%), Stigmasterol (11.39%). The identified compounds possess many biological properties.

Among the identified phytochemicals, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Palmitic acid have the property of antioxidant, hypocholesterolemic, nematocidal, pesticide, lubricant activities and hemolytic. 5- α is a reductase inhibitor [7, 8]. n-Hexadecanoic acid as the common compound in the leaves of *P. stratiotes* and *E. crassipes*. E-11-Hexadecanoic acid, ethyl ester act as Antifungal, Antitumour, Antibacterial. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester found in the leaves of both plant extract act as Hemolytic, pesticide, flavour, antioxidant. Linolenic acid, ethyl ester found to act as Hypocholesterolemic, Nematocidal, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic, 5- α reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne [9].

Phytol is a diterpene compound and it may act as an antimicrobial, anti-inflammatory, anti-cancer and diuretic. Phytol found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [10]. 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 D₃ [11]. 1-Monolinoleoylglycerol trimethylsilyl ether has many biological activities such as Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic,

antioxidant, anti-inflammatory and anti diabetic [12]. 9,12- Octadecadienoic acid, ethyl ester, to be a polyenoic fatty acid compound and it may be acts as an antihistaminic, hepatoprotective, hypocholesterolemic and antieczemic [13]. 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, nematocide, insectifuge, anti histaminic, anti arthritic, anti coronary, anti eczemic, anti acne, 5-alpha reductase inhibitor and anti androgenic [14]. Diisooctyl phthalate is a plasticizer compound, it may be acts as an antimicrobial and antifouling [15].

Table 2: Compound present in the Ethanolic Leaves extract of *Eichhornia crassipes*

RT	Name of Compound	Molecular Formula	MW	Peak Area %	Compound Nature	Activity
17.93	n- Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	2.34	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematocide, Antiandrogenic, Flavor, Hemolytic
18.2	E-11-Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₄ O ₂	282	1.04	Stearic acid	Antifungal, Antitumour, Antibacterial
18.3	Palmitic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	12.09	Stearic acid	Antifungal, Antitumour, Antibacterial
20.66	Phytol	C ₂₀ H ₄₄ O	296	2.12	Diterpene	Antimicrobial, Anti inflammatory, Anticancer, Diuretic, Antifungal against <i>S. typhi</i> , resistant gonorrhoea, joint dislocation, headache, hernia, stimulant and antimalarial
21.53	9,12-Octadecadienoic acid, ethyl ester	C ₂₀ H ₃₆ O ₂	308	3.79	Polyenoic fatty acid	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic
21.65	Linolenic acid, ethyl ester	C ₂₀ H ₃₄ O ₂	306	26.26	Linoleic acid ethyl ester	Hypocholesterolemic, Nematocide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne
21.94	Stearic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312	0.98	Fatty ester	No activity reported.
24.63	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	C ₁₉ H ₃₈ O ₄	330	0.87	Amino compound	Hemolytic, pesticide, flavour, antioxidant.
25.64	α-Glyceryl linolenate	C ₂₁ H ₃₆ O ₄	352	1.35	Fatty acid Ester	Cosmetic, Coloring agent.
26.43	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	498	1.52	Steroid	Antiarthritic, Hepatoprotective, Antimicrobial, anti inflammatory, antioxidant, anti diabetic, Antiasthma, Diuretic.
28.59	Stigmasterol	C ₂₉ H ₄₈ O	412	11.39	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

Several other compounds were also detected through GC/MS chromatogram having notable medicinal property. It could be concluded that, *P. stratiotes* and *E. crassipes* contains various bioactive compounds. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

CONCLUSION

P. stratiotes and *E. crassipes* 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 leaf of *P. stratiotes* and *E. crassipes* revealed the presence of secondary metabolites of anticancerous, antimicrobial, antioxidant, antidandruff, antiproliferative activities and provides a potential source of industrial application. We concluded that the biological values of *P. stratiotes* and *E. crassipes* contain pharmacological active compounds that may enhance its use as a traditional drug.

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