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Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms

Tulika Tyagi and Mala Agarwal

Abstract
Pistia stratiotes L. commonly known as water cabbage, water lettuce, Nile cabbage, or shellflower. 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. *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.
The aim to study the phytochemical screening from *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms and analysis of components present in it by gas chromatography-mass spectrometry (GC-MS). The plant were sequentially extracted in different solvents viz., ethanol, methanol, ethyl acetate, petroleum ether, chloroform, acetone, hexane, aqueous, and 1%HCl. The ethanolic crude extract of weed, *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms showed different types of high and low molecular weight compounds by GC-MS analysis. 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, Anti-arthritic, Antiasthma, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anti-androgenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor, Insectifuge, Antihistaminic, Anti-eczemic, Anti-acne, Anti-coronary, 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, anti-androgenic, 5-alpha reductase inhibitor, hypocholesterolemic, anti-androgenic, anti-coronary, 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) [28]. 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) [3]. *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, anti-tubercular and anti-dysentric. 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) [17]. 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) [18].
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. It was introduced as an ornamental species to adorn the water bodies. This invasive weed poses multiple hazards ranging from ecological and

economical to social. It tends to endanger biodiversity, cause eutrophication, shelter pests, clog fresh waterways, affect agriculture and aquaculture, hamper shipping and recreational activities. Existing control methods have been insufficient to contain its aggressive propagation. 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 (Lata and Dubey 2010) [23]. 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 (Jayanthi and Lalitha 2011) [12]. 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 (Nair *et al.* 2006) [29].

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

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

Material and Methods

To isolate the *Pistia stratiotes* and *Eichhornia crassipes* from different sources

The plants were randomly and aseptically collected from different areas of Kota, Rajasthan, India. The plant materials (leaves, root, shoot) were washed with distilled water and dried under shadow then plant material was chopped into small pieces. Plant materials (leaves, root, shoot) extracts were prepared using soxhlet extraction unit, a quantity of 10gm plant materials (leaves, root, shoot) were weighed and suspended with 200 ml of solvent. The extraction for each plant material is carried out by using ethanol solvent. The extracts were dried by using rotor evaporator, which can be store in a refrigerator at 4 ºC until needed for analysis.

Phytochemical Analysis

Various chemical tests were approved for the presence of bioactive constituents in each fraction of both plants by using standard procedures.

Test for Tannins

Braymer's Test: Presence of tannins was determined with the protocol reported by (Sofowara, 1993) [36]. 50 mg of each fraction was boiled in distilled water and was filtered. A few drops of 0.1% $FeCl_3$ was mixed and observed for colour change, the presence of brownish green coloration shows the occurrence of tannins.

Test for phlobatannins

Phlobatannins in various fractions were identified according to (Trease and Evans, 1989) [37]. 80 mg of each plant extract was boiled in 1% HCl; the deposition of a red precipitate indicated the presence of phlobatannins.

Test for Saponins

Foam Test: Determination of saponins in various fractions was carried out according to the standard procedure of (Harborne, 1973). In this method 20 mg fraction was boiled, filtered and combined with few ml of olive oil, formation of emulsion revealed saponin.

Test for steroids

For the presence of steroid add 5 drops of concentrated H_2SO_4 were added to 1 ml of the leaf extract. Development of red colouration was indicative of a positive reaction.

Test for Terpenoids

Salkowski Test: Presence of terpenoids in various fractions was determined according to (Harbrone, 1973). 5 ml (1 mg/ml) of fraction was combined with few drops chloroform, and then 3 ml of concentrated H_2SO_4. Change of reddish brown color revealed terpenoids.

Liebermann – Burchard test: 1 ml of extract was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour.

Test for alkaloids

Alkaloids in various fractions were detected according to (Harbrone, 1973). 0.4 g of every fraction was combined with 8 ml of 1%HCl, warmed and filtered.

Test for Alkaloids

Wagner's test: A fraction of extract was treated with Wagner's test reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's Test: A fraction of extract was treated with Hager's reagent (saturated picric acid solution). Formation of yellow coloured precipitate confirms the presence of alkaloids.

Mayer's Test: A fraction of extract was treated with Mayer's reagent (1.36 g of $HgCl_2$+5g KI in 100 ml of water). Formation of cream coloured precipitate confirms the presence of alkaloids.

Dragendroff's Reagent: A fraction of extract was treated with 1ml Dragendroff's Reagent (0.17g Bismuth nitrate in 2ml alcohol in 8ml of water add 4 g of potassium iodide into another beaker, in 10 ml alcohol and 20 ml water and stir until KI is completely dissolved). Mix the two solutions. Formation of orange or Red coloured precipitate confirms the presence of alkaloids.

Tests for Flavonoids

NaOH Tests: Take 2-3 ml of extract, add few drops of sodium hydroxide (NaOH) solution into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids (Khandewal, 2008) [16].

H_2SO_4 test: A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

Lead acetate test: A small amount of extract was treated with lead acetate and observed for the formation of white precipitate.

NH_3 Solution: 5 ml of ammonium solution were added to the filtrate followed by addition of concentrated H_2SO_4. Observation of yellow colour indicates the presence of flavonoids.

The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to filtrate. A yellow coloration was observed indication presence of flavonoids.

Test for Quinones

The extracts was treated with concentrated HCl appearance of green colouration indicates presence of Quinones.

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia, pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

Test for Cardiac glycosides

Keller-Killani Test: Take 0.5 gm of extract and dissolved in 5 ml water. Two ml of glacial acetic acid containing one drop of 5% ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A reddish brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Kumar *et al.*, 2012) [22].

Test for Sterols

Liebermann-Burchard Test: Take extract and add with 2 ml chloroform. 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 were dropped into the test tube. First red, then blue and finally green colour indicates the presence of sterols (Kokate *et.al*, 2001) [19].

H_2SO_4 test: The fraction of extract was treated with ethanol and H_2SO_4 and observed for the formation of violet blue or green colour.

Test for Terpenoids

Salkowski Test: 2ml chloroform was added to 0.5 gm of the extract. Then 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (Kumar *et al.*, 2012) [22].

Tests for Anthraquinone

0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube followed by addition of 1 ml of 10% ammonia. The resulting solution was observed for color changes to violet indicating presence of anthraquinones (Kumar *et al.*, 2012) [22].

NaOH test: A small amount of extract was treated with 2M NaOH and observed for the formation of blue green colour.

Test for Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Carotenoids: 1g filtrate was extracted with 10 ml chloroform in at test tube with vigorous shaking. The mixture was filtered and 85% sulphuric acid was added. A blue colour at tyhe interface showed the presence of Carotenoids.

Test for Polyphenols: To 2 ml of extract add alcohol and few drops of neutral ferric chloride solution. Formation of greenish blue colour indicates the presence of polyphenol.

Tests for Carbohydrates

Fehling's Test: To the 2 ml of the aliquot, equal volume of freshly prepared Fehling's solution (prepared by mixing solution A: 7.0 gm $CuSO_4$. 7 H_2O in 100 ml distilled water and B: 24.0 gm KOH and 34.6 sodium potassium tartarate in 100 ml distilled water) was added and the mixture was boiled on a water bath. The development of a rusty brown colour or red precipitate indicated the presence of the carbohydrates.

Benedict's Test: To 2 ml of the aliquot, a few drops of Benedict's solution (prepared by dissolving 17.3 gm of sodium citrate, 10.0 gm of Na_2CO_3 in 75 ml of distilled water, which was filtered and to this 17.3 gm of $CuSO_4.7H_2O$ dissolved in 20 ml of distilled water was added with agitation and the volume was raised to 100 ml with distilled water) was added followed by boiling the mixture on a water bath. A sequential change in the colour (blue-green-orange) indicated the presence of carbohydrates.

Molisch's test: Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water, followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Tests for Proteins

Biuret Test: To the 2 ml of the aliquot, 2 ml of 20% KOH solution was added and mixed thoroughly. To this mixture, 1 ml of 0.5% $CuSO_4$ solution was slowly added, which resulted in the development of pale purple colour indicating the presence of proteins.

Ninhydrin Test (aqueous): The extract was treated with aqueous ninhydrin, purple colour indicates the presence of protein.

Ninhydrin Test (acetone): Ninhydrin was dissolved in acetone, the extract was treated with ninhydrin and observed for the formation of purple colour.

Test for Volatile Oils & Resins: Test solution applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of volatile oils and resins.

GC-MS Analysis

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.

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. 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

Table 1: Preliminary Qualitative screening of Primary & Secondary metabolites of *Eichhornia crassipes* in different Solvents

TESTS	ETHANOL			METHANOL			ETHYL ACETATE			PETROLEUM ETHE			CHLOROFORM			ACETONE			HEXANE			AQUOUS			1% HCl		
	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S
TANNIN																											
a) Braymer's Test	+	–	–	+	–	–	+	–	+	–		–	+	–	–	–	–	–	–	–	–	+	–	–	+	–	+
PHLOBATNNIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–
SAPONIN																											
a) From Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
STEROIDS	+	–	+	+	+	+	+	+	–	+		+	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–
TERPENOIDS																											
a) Salkowski	+	–	+	+	–	+	–	–	+	+		+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
b) Libermann-Burchard test	+	–	+	+	–	+	+	–	–	–		–	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–
STEROIDS	+	–	+	–	–	+	–	–	+	+		–	–	–	–	+	–	+	–	–	–	–	–	–	+	–	–
ALKALOIDS																											
a) Mayer's Test	+	–	+	–	–	+	–	–	–	–		–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–
b) Wager's Test	+	–	+	+	–	+	+	–	+	+		+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	+
c) Hager's Test	+	–	+	+	–	+	+	–	+	+		+	+	+	+	–	–	–	+	–	+	–	–	–	+	+	+
d) Dragendroff's Test	+	–	+	+	–	+	+	–	+	+		+	+	+	+	–	+	–	+	–	+	–	–	–	+	–	+
FLAVONOIDS																											
a) NaOH Test	+	–	–	+	–	+	–	–	+	+		+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+
b) H_2SO_4 Test	–	+	–	–	–	+	+	+	+	+		–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
c) Lead acetate	+	+	+	+	+	+	+	–	+	+		+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
d) NH_3 Solution	+	–	+	+	+	+	–	–	–	+		–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–
QUINONES	+	+	+	+	+	+	+	+	+	–		+	+	+	+	+	–	+	+	+	+	–	+	–	+	+	+
ANTHRAQUINONES																											
a) Borntrager's Test	+	–	+	–	+	+	–	–	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARDIC GLYCOSIDES																											
a) Keller-Killani Test	–	–	–	+	–	+	+	+	–	–		–	–	–	–	+	+	+	–	–	–	–	+	–	+	+	+
STEROLS																											
a) Libermann-Burchand test	+	–	–	–	–	+	+	–	+	–		–	–	–	–	+	–	–	+	–	–	–	–	–	+	–	–
b) H_2SO_4 Test	–	–	–	+	+	+	+	–	+	+		–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
c) Acid anhydride	+	–	–	+	–	–	+	–	–	+		–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
TRITERPENES	+	–	–	+	–	–	+	–	–	+		–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
ANTHOCYANIN																											
a) NaOH Test	–	–	–	+	–	–	–	–	–	+		–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–

PHENOLS																									
a) $FeCl_3$ Test	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
CARTENOIDS	+	–	+	+	+	+	–	–	+	+		–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
POLYPHENOLS	–	–	–	+	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	+	–	–	+	–
CARBOHYDRATES																										
a) Molish's Test	+	–	+	+	+	+	+	+	–	–		–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
b) Fehling's Test	–	–	–	–	–	–	+	+	–	–		+	+	+	+	–	+	+	+	+	–	+	–	–	+	–
c) Benedict Test	+	–	+	+	–	–	–	–	–	+		–	+	–	+	–	–	–	+	–	–	+	+	–	+	–
PROTEINS													–	–	–	–	–	–	–	–	–	–	–	–	–	–
a) Ninhydrin (aquous)	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
b) Ninhydrin (Actone)	–	–	+	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
c) Biuret Test	+	–	+	+	–	–	+	–	+	+		–	+	+	+	–	–	–	–	–	–	+	–	+	–	–
VOLTILE OILS	+	–	–	+	–	–	+	–	–	+		–	+	–	–	–	–	–	+	–	–	–	–	–	–	–

Table 2: Preliminary Qualitative screening of Primary & Secondary metabolites of *Pistia stratiotes* L. in different Solvent

Tests	**Ethanol**		**Methanol**		**Ethyl Acetate**		**Petroleum Ether**		**Chloroform**		**Acetone**		**Aquous**	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Tannin														
A) Braymer's Test	+	–	–	–	–	–	–	–	–	–	–	–	+	+
Phlobatannin	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Saponin														
A) Form Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Steroids	+	–	+	–	+	+	–	–	+	+	–	–	–	–
Terpenoids														
A) Salkowski	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B) Libermann-Burchard Test	+	–	+	–	+	–	–	–	+	+	–	–	–	–
Steroids	+	–	+	–	–	–	–	–	+	+	–	–	–	–
Alkaloids														
A) Mayer's Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B) Wager's Test	–	–	+	–	–	–	–	–	–	–	–	–	–	–
C) Hager's Test	–	–	+	–	–	–	–	–	–	–	–	–	–	–
D) Dragendroff's Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Flavonoids														
A) Naoh Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B) H_2SO_4 Test	+	–	–	–	–	–	–	–	–	–	–	–	–	–
C) Lead Acetate	+	+	+	+	–	–	–	–	+	+	–	–	–	+
Quinones	+	+	+	+	+	+	–	–	+	+	–	–	–	+
Anthraquinones														
A) Borntrager's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardic Glycosides														
A) Keller-Killani Test	+	+	+	+	–	–	+	+	–	+	+	+	+	+
Sterols														
A) Libermann-Burchand Test	–	–	–	–	+	–	–	–	+	+	–	–	–	–
B) H_2SO_4 Test	+	–	–	–	+	–	–	–	+	+	–	–	–	–
C) Acid Anhydride	–	–	–	–	+	–	–	–	+	+	–	–	–	–
Anthocyanin														
A) Naoh Test	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Phenols														
A) $Fecl_3$ Test	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Carotenoids	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Polyphenols	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Carbohydrates														
A) Molish's Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B) Fehling's Test	–	–	–	–	–	–	–	–	+	+	–	–	–	–
C) Benedict Test	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Proteins														
A) Ninhydrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C) Biuret Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Voltile Oils & Resins	+	–	+	–	+	–	–	–	+	+	–	–	–	–

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.

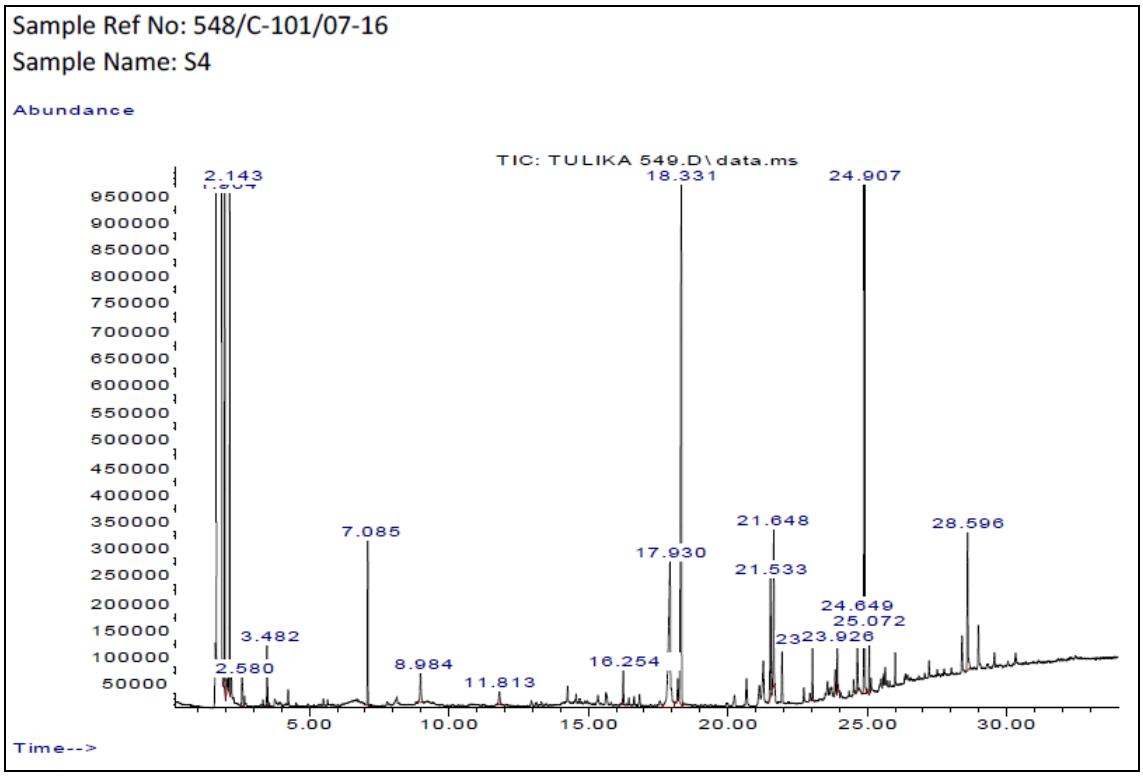
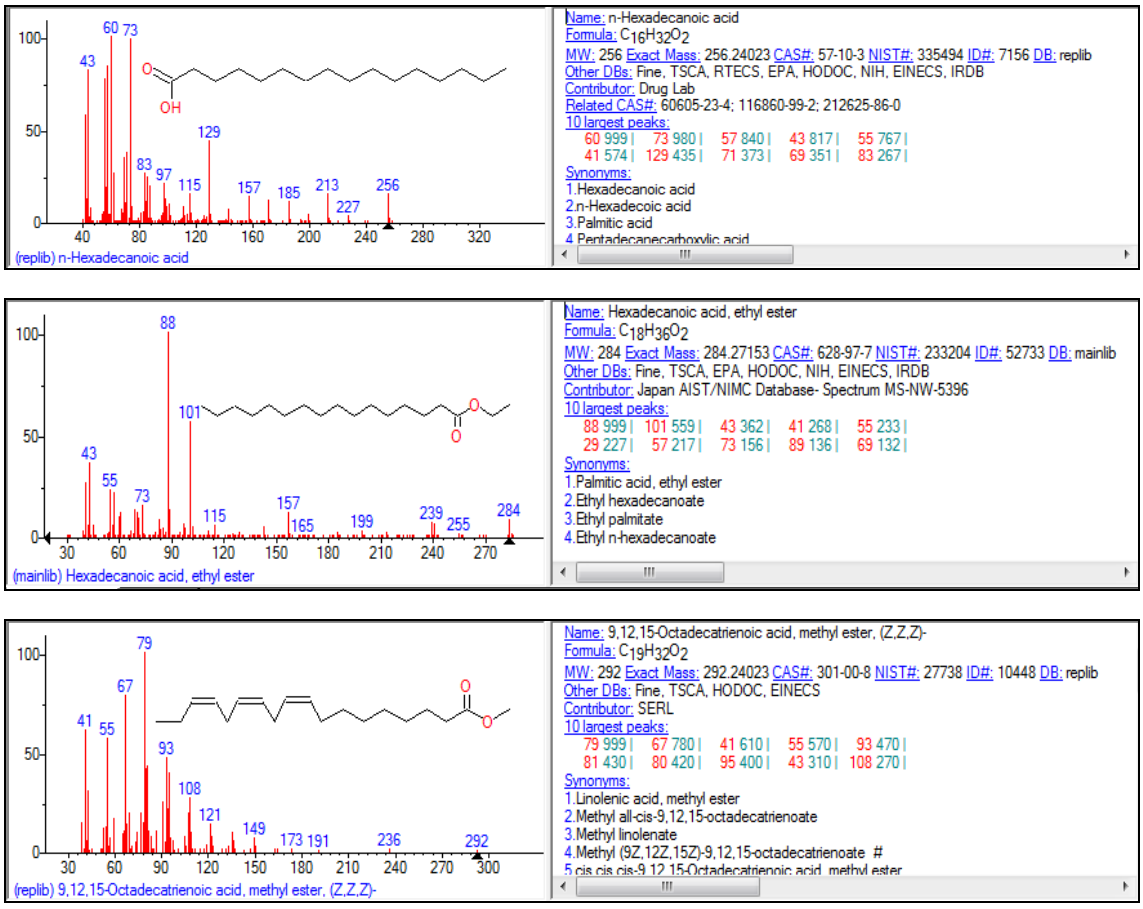


Fig 2: Gas Chromatogram of *Pistia stratiotes* L. leaf Ethanol Extract



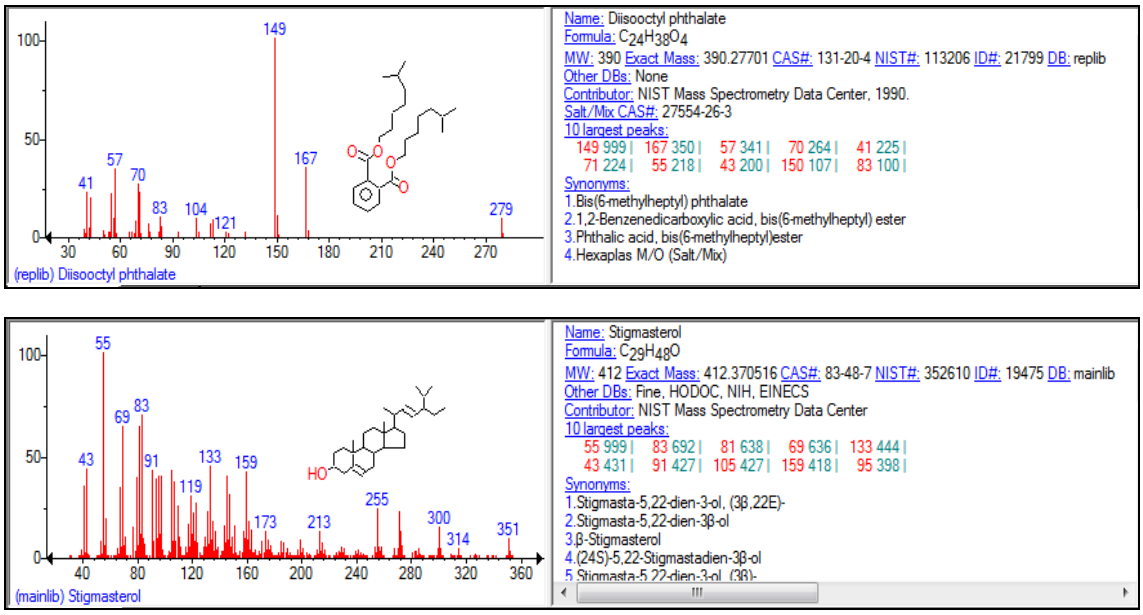


Fig 3: Mass Spectra of *Pistia stratiotes* L. leaf Ethanol Extract

Table 3: 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
17.93	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.18	Palmitic acid ester	Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, 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, Anti-androgenic.
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, Anti-arthritic, anti-asthama, 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	$C_8H_4(C_8H_{17}COO)_2$	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, anti-arthritic, anti-asthama, anti-inflammatory, diuretic.
11.81	L-Glutamine	$C_5H_{10}N_2O_3$	146	0.38	Amino acid	Building block of Protein

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

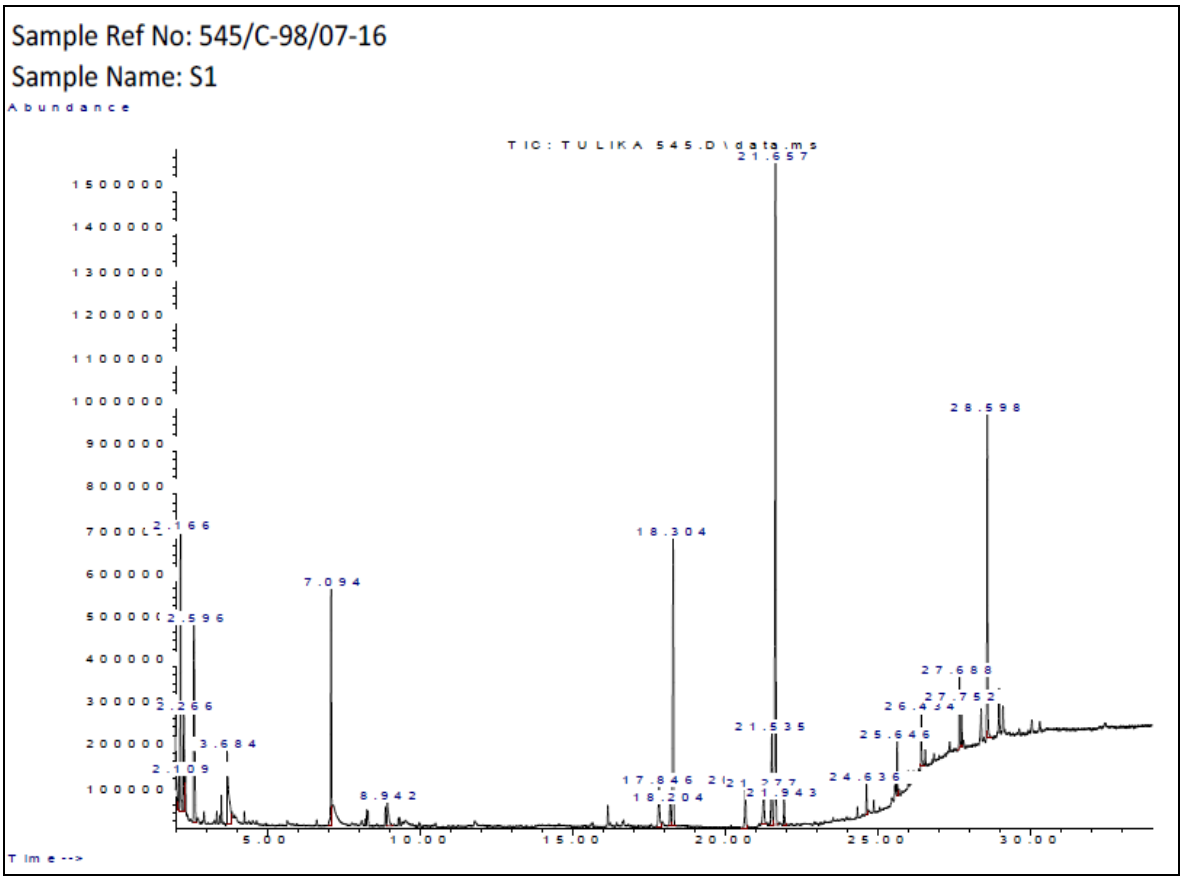
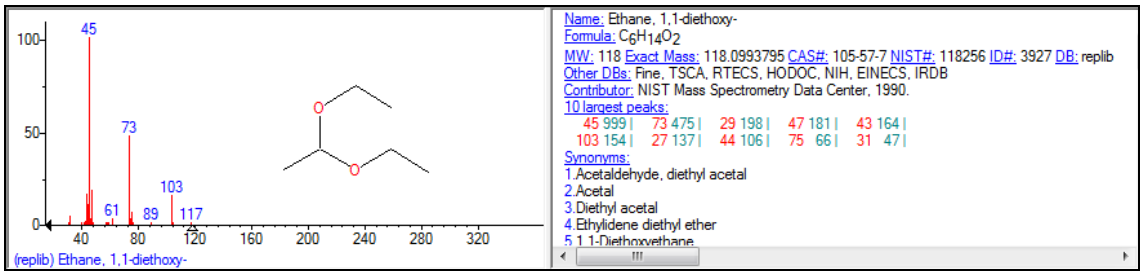
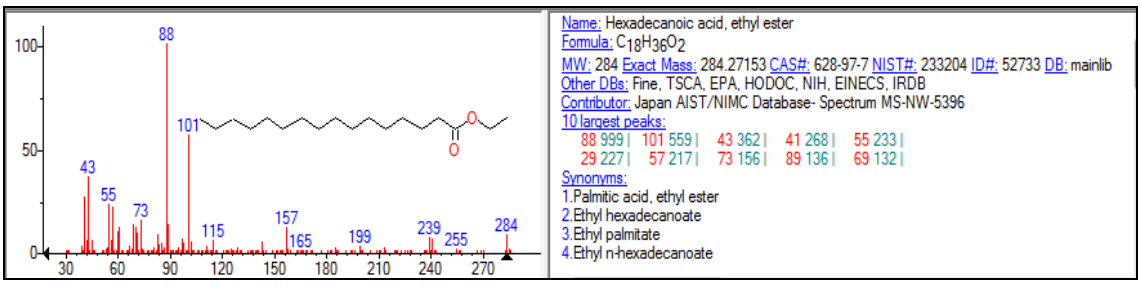
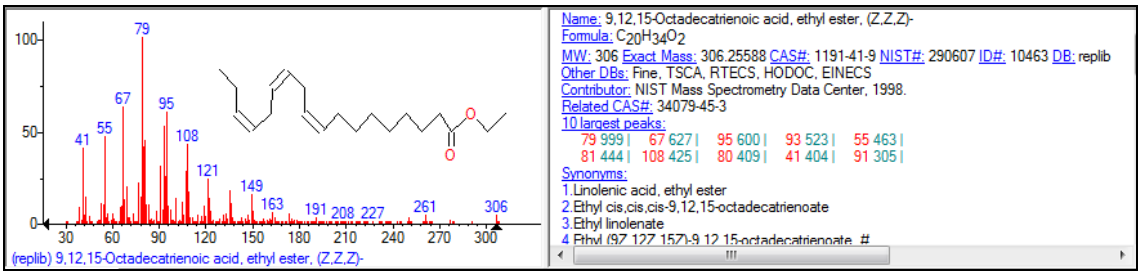


Fig 4: Gas Chromatogram of E. *crassipes* (Mart.) solms Leaf Ethanol Extract







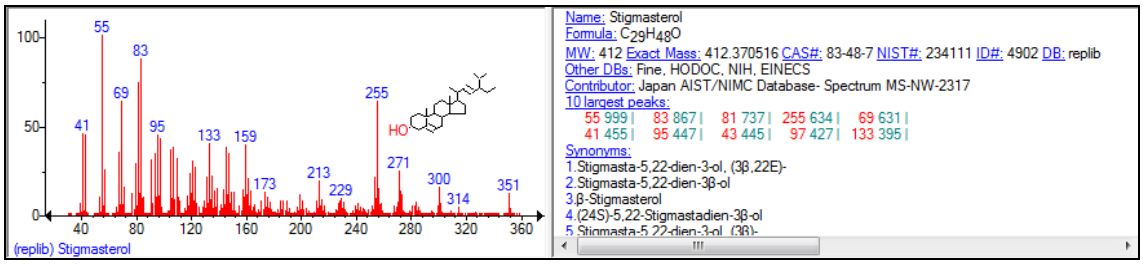


Fig 5: Mass Spectra of E. *crassipes* (Mart.) solms leaf Ethanol Extract

Table 4: Proposed Retention Time, Compounds, Molecular Formula, Molecular Weight, Peak Area%, Compound Nature and Bioactivity of E. *crassipes* (Mart.) solms leaf Ethanol Extract

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, Nematicide, Anti-androgenic, Flavor, Hemolytic
18.2	E-11-Hexadecanoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	1.04	Stearic acid	Antifungal, Anti-tumour, Antibacterial
18.3	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	284	12.09	Stearic acid	Antifungal, Anti-tumour, Antibacterial
20.66	Phytol	$C_{20}H_{44}O$	296	2.12	Diterpene	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against *S. typhi, resistant gonorrhea, 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, anti-eczemic
21.65	Linolenic acid, ethyl ester	$C_{20}H_{34}O_2$	306	26.26	Linoleic acid ethyl ester	Hypocholesterolemic, Nematicide, Anti-arthritic, Hepatoprotective Anti-androgenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Antihistaminic, Anti-coronary, Insectifuge, Anti-eczemic, Anti-acne
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	$C_{27}H_{54}O_4Si_2$	498	1.52	Steroid	Anti-arthritic, 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, anti-arthritic, anti-asthama, anti-inflammatory, diuretic

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

The phytochemical composition of *E. crassipes* determined is summarized in Table 1. The extract of leaves shows the presesnce of alkaloids, glycosides, steroids, flavonoids, tannins, phlobatannin, terpenoids, quinones, anthraquinone, caradic glycosides, sterols, polyphenols, anthrocyanin, Carotenoids, proteins and volatile oils. However, saponin, phlobatannin, were not found.

The extract of root shows the presesnce of alkaloids, glycosides, flavonoids, quinones, anthraquinone, caradic glycosides, sterols, polyphenols, anthrocyanin, Carotenoids, proteins and volatile oils. However, saponin, tannins, phlobatannin, terpenoids, steroids, terpenoids, triterpenes and volatile oils were not found.

The extract of shoot shows the presesnce of alkaloids, glycosides, steroids, flavonoids, tannins, phlobatannin, terpenoids, triterpenes, quinones, anthraquinone, caradic glycosides, sterols, Carotenoids, proteins and volatile oils. However, saponin, phlobatannin, anthrocyanin, polyphenols and volatile oils were not found.

The phytochemical composition of *P. stratiotes* determined is summarized in Table 2. The extract of leaves and roots shows the presesnce of alkaloids, glycosides, steroids, flavonoids, tannins, terpenoids, quinones, anthraquinone, caradic glycosides, sterols, polyphenols, anthrocyanin, and volatile oils and resins. However, saponin, phlobatannin, Carotenoids, and proteins were not found.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Kumar *et al*., 2009) [21]. Different phytochemicals have been found to possess a wide range of activities. The phytochemicals are known to have antimicrobial activity (Ebana, 1995) [6]. Tannin has been found to possess astringent properties hasten the healing of wounds and inflamed mucous membranes (Okwu, 2004; Kozi and Marcia, 1998) [31, 20]. Tannin and flavonoid are thought to be responsible for antidiarrheal activity (Enzo, 2007) [8]. Usman and Osuji reported that tannin has been widely used topically to sprains, bruises and superficial wounds as such. Similarly, Elmarie and Johan reported tannin to have antibacterial. Phytochemicals such as terpenoid, flavonoid, tannin, steroid, and alkaloid have anti-

inflammatory effects (Manach *et al*., 1996; Latha *et al*., 1998; Liu, 2003; Alkindele and Adeyemi, 2007; Iikay Orhan, 2007) [26, 24, 25, 2, 11]. Glycoside, flavonoid, tannin and alkaloid have hypoglycemic activities (Cherian and Augusti 1995) [4]. Alkaloids are heterocyclic indole compounds which have proved to be having pharmacological properties such as hypotensive activity (Ali and Ghatak, 1975) [1], anticonvulsant activity (Singh and Kapoor, 1980) [35], antiprotozoal, antimicrobial and antimalarial activities (Frederich, 2002) [9]. Mensah *et al*. concluded the plants that possessed tannin, Cardiac glycoside & alkaloid are the most effective for managing hypertension and also providing protection for the heart. Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the cell against all stages of carcinogenesis (Okwu,2004) [31]. Flavanoids show anti allergic, anti-inflammatory, anti-microbialand anti-cancer activity (Yamato and Gayor, 2002) [42]. Anthocyanins exhibit important anti-oxidant and anti-inflammatory actions as well as chemotherapeutic effects (Shin Hwa Lee *et al*., 2009) [34].

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, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors (Jegadeeswari *et al*., 2012; Upgade and Anusha, 2013) [13, 38]. n-Hexadecanoic acid as the comman compound in the leaves of *P. stratiotes* and *E. crassipes*. E-11-Hexadecanoic acid, ethyl ester act as Antifungal, Anti-tumour, Anti-bacterial. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester found in the leaves of both plant extract act as Hemolytic, pesticide, flavour, antioxidant. (Duke, 1992-1996) [5]. Linolenic acid, ethyl ester found to act as Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective Antiandrogenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne (Duke, 1992-1996) [5]. L-Glutamine is an amino act found in less quantity but is a major component in building Protein.

Phytol is a diterpene compound and it may be 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 (Ogunlesi *et al*., 2009) [30]. 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) [14].

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- Octadecadienoic acid, ethyl ester, to be a polyenoic fatty acid compound and it may be acts as an antihistaminic, hepatoprotective, hypocholesterolemic and antieczemic (Wu *et al*., 2010) [41]. 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) [40].

Diisooctyl phthalate is a plasticizer compound, it may be acts as an antimicrobial and antifouling (Sangeetha and Vijayalakshmi 2011) [32].

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* and *E. crassipes* leaf 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. 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. Phytochemical screening and 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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