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Analysis of bioactive compounds in *Eichhornia crassipes* leaf extract using GC MS technique

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Abstract
The phytocomponents of *Eichhornia crassipes* leaves evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. The mass spectrum of the compounds found in the *Eichhornia crassipes* leaves extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds via Tridecanoic Acid, 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-Ol, Cis-10-Nonadecenoic Acid, Hexadecanoic Acid, Methyl Ester, Phytol Acetate, 9-Octadecenoic Acid, 9, 12, 15-Octadecatrienoic Acid and Squalene in the methanolic extract of *Eichhornia crassipes*. These findings support the traditional use of *Eichhornia crassipes* for various disorders.

Keyword: Gas chromatography Mass spectroscopy, *Eichhornia crassipes,* Phytocompounds

1. Introduction
Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (De-Fatima *et al*., 2006). Different medicinal plants and their medicinal values are widely used for various ailments throughout the world. Various chemical compounds isolated and characterized from Boraginaceous plant species are described. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi, 2000 and Shahidi, *et al*., 2008) [24, 23]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes (Meurer-Grimes *et al*., 1996; Koduru *et al*., 2006) [13, 9]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998) [12].
Within a decade, there were a number of dramatic advances in analytical techniques including FTIR, UV, NMR and GC- MS that were powerful tools for separation, identification and structural determination of phytochemicals. Gas Chromatography Mass Spectroscopy is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites, 1997) [17]. The aim of this study is to determine the bioactive compounds present in *Eichhornia crassipes* extract with the aid of GC- MS techniques which may provide an insight in its use as traditional medicine. *Eichhornia crassipes* (Mart. Solms is an aquatic perennial herb that belongs to the family Pontederiaceae, an erect free floating herbaceous plant, spread throughout the world.

Materials and Methods
Plant materials
The *Eichhornia crassipes* leaves were collected in January 2015 from Koraiyaru river, Mannargudi, Thiruvarur district, Tamil Nadu. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts

The collected *Eichhornia crassipes* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. Then examined carefully old, infected and fungus damaged portion of the leaves were removed. Healthy leaves were spread out in a plain paper and shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Eichhornia crassipes* leaves extract (ECLE) was stored in refrigerator until used.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013) [28].

Results and Discussion

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, inorganic, biochemistry and identification of unknown samples. Additionally, it can identify trace materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely used as a "gold standard" for forensic substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system (Ronald Hites, 1997) [17].

Identification of components

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 component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The biological activities listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA (Duke's, 2013) [5]. The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound can be identified from NIST library database.

GC-MS Analysis

The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound which can be identified from The National Institute of Standard and Technology (NIST) library database. Twenty compounds were identified in *Eichhornia crassipes* by GC-MS analysis. The active principles with their Retention Time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1and Fig 1). The prevailing compounds were Octadecanoic acid, 2-oxo-, methyl, 2-Hexadecen-1-ol, 3,7,11,15-tetram, Heptadecanoic acid, Ethyl ester, Phytol isomer, Phytol, acetate and Squalene present in the extract. The pharmacological activity of *Eichhornia crassipes* is *represented* in table 2. This study explores the goodness of the leaf of *Eichhornia crassipes* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

The investigation concluded that the stronger extraction capacity of methanol have produced number of active constituents responsible for many biological activities. So these might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer.

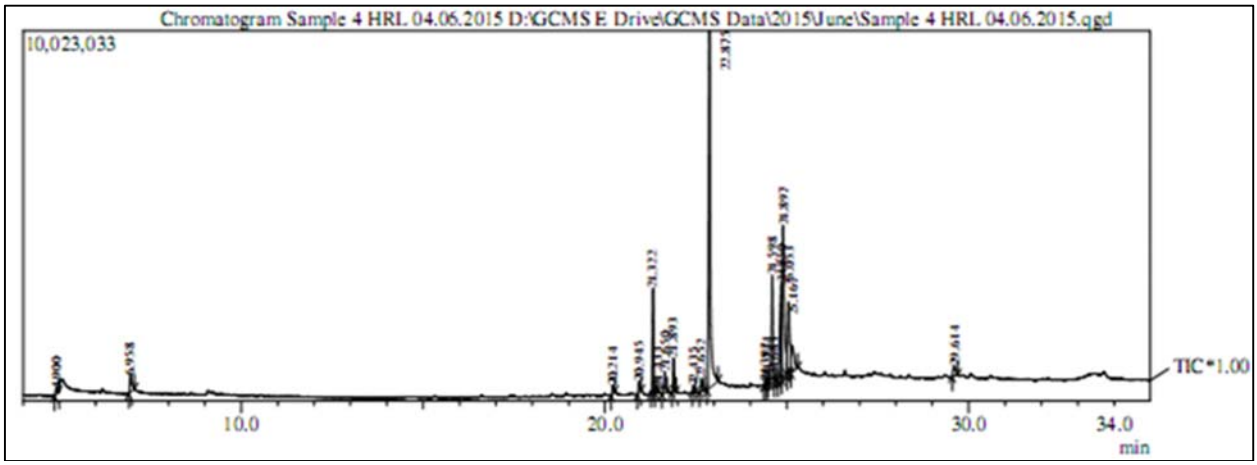

Fig 1: GC MS chromatogram of *Eichhornia crassipes* leaf extract

9-Octadecanoic acid (Z) is recommended to be a saturated fatty acid and it might act as an anti-hypertensive and increases HDL cholesterol decreases LDL level (Teres *et al*., 2008) [29]. Cis-10-Nonadecenoic acid is recommended to be a monosaturated fatty acid and it might act as an Antitumor (Fukazawa *et al*., 2008).

Phytol is reported to have antioxidant, antiallergic (Santos *et al*., 2013) [20] antinociceptive and anti-inflammatory activities (Ryu *et al*., 2011) [18]. Recent studies have revealed that phytol is an excellent immunostimulant. It is superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity (Lim *et al*., 2006) [10]. Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Saikia *et al*., 2010) [19]. Similarly Maria Jancy Rani *et al.* (2011) [11] observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (2011) [27] in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* (Sathish kumar and Manimegalai, 2008). Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al.,* 2005) [8]. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K_1. It is used along with simple sugar or corn syrup as a hardener in candies.

Hexadecanoic acid, ethyl ester is recommended to be a saturated fatty acid and it might act as an Antioxidant, hypocholesterolemic, anti androgenic, hemolytic and alpha reductase inhibitor (Sermakkani, 2012) [22]. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al.,* 2002) [7] and *Melissa officinalis* (Sharafzadeh *et al.,* 2011) [25]. Parasuraman *et al.* (2009) [14] identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid (Siddig Ibrahim *et al.,* 2009). n-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15-Octadecatrienoic cidand Squalene were Identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) [1] and *Vitex negundo* (Praveen kumar *et al.,* 2010) [16]. Squalene has earlier been reported as antimicrobial, antioxidant, anticancer, Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer (Ponnamma and Manjunath, 2012) [15]. Devi *et al.* (2009) [4] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Uraku (2015) [30] investigated the Chemical Compositions of *Cymbopogon citrates* Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) Method. Six compounds were identified in the methanol leaf extract and they include; hexadecanoic acid (8.11%), hepta-9,10,11-trienoic acid (17.43%), octadecenoic acid (8.41%), 2-ethenyltetradecan-1-ol (13.28%), eicosane aldehyde (37.56%) and 1-ethoxyoctadecane (15.20%) as the major chemical constituents.

Das and Sudhakar Swamy (2016) [2] determined the bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia* species from south India. Twenty seven compounds were identified from the mass spectra obtained. 1, 3, 4, 5-Tetrahydroxycyclohexanecar boxylic acid was the major compound identified. In *A. racemosa* also 27 compounds were identified and n-Hexadecanoic acid was the major compound.

Uraku (2016) [31] examined the Bioactive Constituents of Methanol Fraction of *Spilanthes uliginosa* (Sw) Leaves. The major phytocompounds identified in the leaf extract are hexadecanoic acid (8.68%), hepta-9, 10, 11-trienoic acid (19.36%), octadecenoic acid (8.14%), 5-hydroxylmethyl heptadecane (14.02%), docosane aldehyde (41.72%) and 1-ethoxyoctadecane (8.08%).

Table 1: GC-MS analysis revealed the presence of Phytochemical component in leaf of *Eichhornia crassipes*

Peak#	R. Time	Area%	Height%	Molecular formal	Molecular weight	Name of the compounds
1	4.900	0.47	0.50	$C_9H_{22}OSi$	174	1-Butyl (Dimethyl) Silyloxypropane
2	6.958	1.96	1.55	$C_7H_{14}O_2$	130	1-Butanol, 3-Methyl-, Acetate
3	20.214	0.63	0.76	$C_{13}H_{26}O_2$	214	Tridecanoic Acid
4	20.945	0.95	1.09	$C_{13}H_{18}O_3$	222	2-Cyclohexen-1-One, 4-Hydroxy-3,5,5-Trimethyl-4-(3-Oxo-1-Butenyl)
5	21.322	7.08	9.78	$C_{20}H_{38}$	278	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
6	21.433	0.68	0.51	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-Trimethyl-
7	21.650	1.82	1.98	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
8	21.893	2.28	3.22	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
9	22.435	0.40	0.60	$C_{17}H_{34}O_2$	270	Hexadecanoic Acid, Methyl Ester
10	22.652	1.38	1.25	$C_{19}H_{36}O_2$	296	Cis-10-Nonadecenoic Acid
11	22.875	32.64	33.70	$C_{16}H_{32}O_2$	256	N-Hexadecanoic Acid
12	24.392	0.27	0.29	$C_{10}H_{22}O_5Si$	250	Methyl 2,2-Dimethyl-3,6,9-Trioxa-2-Silaundecan-11-Oate
13	24.444	0.40	0.66	$C_{18}H_{34}O_2$	282	9-Octadecenoic Acid
14	24.598	7.76	10.04	$C_{22}H_{42}O_2$	338	Phytol, Acetate
15	24.683	0.41	0.60	$C_{19}H_{38}O_2$	298	Methyl Stearate
16	24.850	10.31	9.09	$C_{15}H_{28}O_2$	240	Cyclopentadecanone,2hydroxy
17	24.897	17.07	14.42	$C_{18}H_{30}O_2$	278	9,12,15-Octadecatrienoic Acid,
18	25.053	7.93	6.78	$C_{18}H_{36}O_2$	284	Octadecanoic Acid
19	25.167	4.28	2.20	$C_{30}H_50$	410	Squalene
20	29.614	1.30	0.99	$C_{21}H_{36}O$	304	3-Pentadecylphenol
		100.00	100.00			

Table 2: GC-MS analysis revealed the presence of phytochemical component in leaf of *Eichhornia crassipes* and their pharmacological activities

S. No.	R. Time	Area%	Name of the compound	Nature of the compound	Pharmacological Activity**
1.	20.214	0.63	Tridecanoic Acid	Fatty acid	No activity reported
2.	21.650	1.82	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	Terpene alcohol	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic Antioxidant
3.	22.652	1.38	Cis-10-Nonadecenoic Acid	Saturated Fatty acid	Antitumor
4.	22.435	0.40	Hexadecanoic Acid, Methyl Ester	Palmittic acid	Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor Antimicrobial,
5.	22.402	1.10	Phytol Acetate	Diterpene	Anti cancer, Anti-inflammatory Hypocholesterolemic, Nematicide, Anticoronary, Antiarthritic, Hepatoprotective, Anti -androgenic,
6.	24.444	0.40	9-Octadecenoic Acid	Oleic acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol.
7.	24.897	17.07	9,12,15-Octadecatrienoic Acid,	Linolenic acid	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Anti androgenic, Nematicide 5-Alpha reductase inhibitor, Antihistaminic Anticoronary, Insectifuge, Antieczemic Anticancer
8.	26.752	5.46	Squalene	Triterpene	Antibacterial, Antioxidant, Pesticide, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

Conclusion

The present study characterized the phytochemical profile of the *Eichhornia crassipes* leaf extract using GC-MS. The mass spectrometer analyses of the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the NIST library. The heights of the different peaks indicates the relative concentration of the compounds exist in the methanolic extract of *Eichhornia crassipes* leaf. The identification of various bioactive compounds (Tridecanoic acid, 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol, Cis-10-Nonadecenoic Acid, Hexadecanoic acid, Phytol Acetate, 9-Octadecenoic Acid, 9,12,15-Octadecatrienoic Acid and Squalene) confirms the therapeutic application of *Eichhornia crassipes* leaf for a variety of diseases.

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