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Extraction and molecular characterization of biological compounds from water hyacinth

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Abstract
Eichhornia crassipes is an invasive aquatic plant whose individual morphological parts like roots and shoots were studied using different solvent extracts namely, benzene water and ethanol. All the solvent extracts of the root and shoot portions of the fresh *Eichhornia crassipes* were screened for the presence of various phytochemicals such as alkaloids, flavonoids, tannins, phenols, sterols, terpenoids, anthraquinones, and anthocyanins. The isolation and purification of the major bioactive compounds such as tannins and flavonoids were done by thin layer chromatography using the extract purified from column chromatography. The results from the Gas Chromatography showed the retention of many important phenolic and sterol based compounds, denoting their richness of bioactive compounds useful for pharmaceutical industries as drugs and environmental sectors thereby protecting the aquatic micro ecosystem.

Keywords: *Eichhornia crassipes*, phytochemical, mass spectrometry, gas chromatography, thin layer chromatography, water hyacinth

1. Introduction
Eichhornia crassipes, commonly known as Water Hyacinth, is a free floating perennial aquatic plant native to tropical and subtropical South America. The plant has glossy, ovate shaped thick leaves, which makes them float above the water. They have long bulbous stalks which are spongy. The feathery, freely hanging roots are purple-black (Lata & Dubey, 2010) [6]. *Eichhornia crassipes* were extracted using different fractions of petroleum ether, acetone, ethyl acetate, aqueous, acid, chloroform and ethanol and were screened by standard procedures for detecting the presence of phytochemicals such as sterols, flavonoids, terpenoids, alkaloids, quinones and anthocyanins was noted in aqueous and acid extract (Jayanthi, Lalitha, & Shubashini, 2011) [4]. The antioxidant potentials studied using reducing power, free radical scavenging potential and metal ion chelating ability showed their activity. Further, cytotoxicity against lung cancer cells and protection was rendered towards rat tissue (liver, kidney, and brain) homogenate were established. They showed potent antibacterial activity against three bacterial species, *Proteus vulgaris*, *Salmonella typhi*, and *Bordetella bronchiseptica* (Kumar, Kumar, Dwivedi, & Pandey, 2014) [5]. Alkaloids are involved in the protective functions of any metabolism, particularly the steroidal alkaloids. Anthraquinone, an aromatic organic compound possesses many isomers of which each can be seen as a quinone derivative. Tannins exhibit strong antifungal properties while the flavonoids show antioxidant and antitumor activities against tumors at all the three stages (Perchellet *et al*., 2000) [9]. Anthocyanins, a member of the flavonoids class, predominantly occur in higher plants, including leaves, stems, roots, flowers, and fruits in the tissue region (Lila, 2004) [7]. Soxhlet based extraction is one of the effective methods used for extracting the active constituents in the form of liquid using temperature and optimized solvent from powdered dry samples (Tiwari, Kumar, Kaur, Kaur, & Kaur, 2011) [5]. Ethanolic extracts of *Eichhornia crassipes* showed the presence of gallic, protocatechuic, gentisic, and p-hydroxy benzoic acid with lesser amounts of phenolic acids in water extracts (Surendraraj, Farvin, & Anandan, 2013) [14]. The fraction of *Eichhornia crassipes* was eluted with n-hexane: chloroform (1:1) and tested positive for sterols, mainly stigmasterol using Liebermann-Burchard test (Siripong *et al*., 1992) [12]. The whole plant of *Eichhornia crassipes* was studied using polar and lipophilic extracts to identify them as the richest source of stigmasterol, with about 4437 mg kg^{-1} biomass.

The stalk parts showed the maximum content of stigmasterol, which is a strong antioxidant (Silva, de Melo, Silvestre, & Silva, 2015) [11] Stigmasterol is an unsaturated phytosterol used as a precursor for the manufacture of semi-synthetic progesterone, as a regulator of tissue rebuilding mechanism, as an intermediate in the biosynthesis of estrogens, androgens, and corticoids. It is an anticancer agent which acts as a precursor in vitamin D_3 synthesis and also inhibits the cholesterol absorption by lowering the serum cholesterol levels (Panda, Jafri, Kar, & Meheta, 2009) [8]. Similarly, another compound, n-hexadecanoic acid is a common saturated fatty acid present in almost all the plant, animal and microorganisms. Their presence makes water hyacinth a potential source of antioxidant, anti-inflammatory, antimicrobial and anticancer activity (Aparna *et al*., 2012) [1].
Column chromatography and thin layer chromatography (TLC) are one of the basic chromatographic techniques used for their economic, convenience and availability in various stationary phases. They are contemporarily utilized for highly valued separations, increasing polarity using multiple mobile phases is useful. For separation of bioactive molecules, Silica gel column chromatography has been used with some analytical tools (Zhang, Pang, Xuewu, Ji, & Jiang, 2005) [16]. Thin Layer chromatography (TLC) is usually performed to detect the presence of phytochemicals using different solvent extracts. The identification of the compound in the mixture is supported by the R_f of an unknown compound compared with the R_f value of a known compound, usually the standard. Further the spraying of phytochemical screening reagents, binds to the compound and provides color in case of colorless compounds (Panda *et al*., 2009; Sasidharan, Chen, Saravanan, Sundram, & Latha, 2011) [8, 10]. Gas Chromatography– Mass Spectrometry (GC-MS) is an analytical technique which combines the separation and detection of volatile compounds. For analysis using GC, the analyte must have significant vapor pressure between 30 and 300°C. Here in this method the electron ionization (EI) of the sample was performed (Chauhan, Goyal, & Chauhan, 2014) [2]. This work mainly aims to identify the phytochemical constituents present in the best selected extract using different chromatographic techniques such as Thin Layer chromatography and Gas Chromatography - Mass Spectrometry studies.

2. Materials and Methods

2.1 Sample collection and Extraction

Eichhornia crassipes were collected from a lake near Bhavani, Erode. The plant was washed thoroughly and chopped into leaves, root, and shoot portions separately. The root and shoot parts were shade dried completely for 20 days. The dried plant material was finely powdered and stored for further use. 5g of the powdered plant material was extracted with 350ml of ethanol and 350ml of hexane solvents in the Soxhlet apparatus for about 10 cycles. Post extraction, the solvent was removed, concentrated using a rotary evaporator and stored (Tiwari *et al*., 2011) [15].

2.2 Phytochemical screening

Identification of the phytoconstituents namely, tannins, flavonoids, terpenoids, anthraquinones, alkaloids, sterol, terpenoids, anthocyanin and saponins in the *Eichhornia crassipes* plant extracts was done using standard protocols (Jayanthi *et al*., 2011) [4].

Table 1: List of Qualitative phytochemical screening assays performed on the shoot and root extracts of *Eichhornia crassipes*

Phyto chemical	Test	Sample	Reagent added	End Point
Anthocyanin	NaOH test	Extract	2M NaOH	Blue green color
Anthraquinones	Borntrager's test	Powdered extract	Heat with 10% Ferric chloride, 1ml conc. HCl. Diethyl ether extraction with ammonia	Pink or deep red color
Flavonoids	Alkaline reagent test	Extract	Aqueous NaOH and HCl	Yellow Orange Color
	Ferric Chloride test		Ferric Chloride	Intense Green color
	Ammonia test		10% Ammonia	Yellow color
Saponin	Test for saponin	10% boiled extract	5ml distilled water with 3 drops of olive oil- shaken vigorously	Emulsion formation
Sterol	Leibermann-Burchard test	Extract	Chloroform, Acetic anhydride and drops of H_2SO_4	Dark pink or Red color
Tannins	Braymer's test	2.5% boiled extract	0.1% Ferric chloride	Brownish green color

2.3 Column Chromatography

Packing the Column for Gel filtration chromatography was done by adding the slurry containing 25g of fresh silica gel (for column chromatography 60-120 mesh) dissolved in 100mL of hexane. The excess solvent that flows through the plugged in cotton was collected and the silica is allowed to settle by closing the stop clock, once the solvent settles above the silica gel. The sample was then transferred to the solvent layer. The elution process begins by continuous filling of the column with ethanol until the colored compound gets eluted through the column. Each fraction was collected separately and numbered consecutively for further analysis on thin layer chromatography.

2.4 Thin Layer Chromatography (TLC)

Each fraction was applied on the activated TLC plates using capillary tubes at a certain height from the bottom edge. The plate was then placed in the developing chamber containing the solvent system until the developing solvent reaches the top to around 80% of the TLC plate (Gowthama Prabu, Baskar, Kumaresan, & Poorani, 2020) [3]. The plate was then removed, dried and the solvent front was marked. Visualization of the compounds using the bands/ spots formed on the TLC plate was performed using UV light (254nm), Iodine and by spraying a suitable revealing reagent, say, Vanillin-Sulphuric acid for the presence of specific compounds. The visualized spots of the separated components in the chromatoplate were marked and the Retention Factor (R_f) value of each spot is calculated by the formula,

$$Retention\ Factor\ (R_f) = Distance\ travelled\ by\ the\ sample\ (cm) \Big/ Solvent\ Front\ (cm)$$

2.5 Gas Chromatography - Mass Spectrometry (GC-MS)

The ethanolic extracts of shoot and root parts were analysed using Perkin Elmer - GC: Clarus 680 and Mass Spectrometer (MS) - Clarus 600 (EI) using the software - TurboMass (v 5.4.2). The column used was fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m × 0.25mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min at an injector temperature of 260°C. 1µL of extract was injected at different temperatures say, 60°C for 2 minutes, then at 300°C at the rate of 10°C min^{-1} and at 300°C for 6 minutes. After the column, the mass detector conditions like, transfer line and ion source temperature (240°C), ionization mode electron impact (70 eV), scan time (0.2 second) and scan interval (0.1 second) with a wide range over the fragments between 40 and 600 Da were measured. The spectrums of the components were compared with the database of spectrum of known components in the library.

3. Results and Discussion

The plant samples were successfully collected, washed, chopped, dried and extracted using different solvents using Soxhlet extraction. The extracts were concentrated and stored for the various analyses to be performed.

3.1 Phytochemical Screening

The Phytochemical tests were performed on extracts of ethanol, hexane and water solvent and the results were tabulated in the Table 2 (Jayanthi *et al*., 2011) [4].

Table 2: Qualitative Phytochemical analysis of the different extracts of the shoot and root part of *Eichhornia crassipes*

Phytochemical	Test name	Solvent					
		Ethanol		Water		Benzene	
		Shoot	Root	Shoot	Root	Shoot	Root
Anthocyanins	NaOH test	-	-	-	-	-	-
Anthraquinones	Borntrager's test	+	+	+	+	+	+
Flavonoids	Ferric chloride test	+	+	-	-	+	+
	Ammonia test	+	+	-	-	*	*
	Alkaline reagent test	+	+	-	-	*	*
Saponins	Test for Saponin	+	+	+	+	*	*
Sterols	Liebermann-Burchard test	+	-	+	+	+	-
Tannins	Braymer's test	-	+	-	-	-	+

+ Presence; - absence; * immiscible

3.2 Analysis of compounds

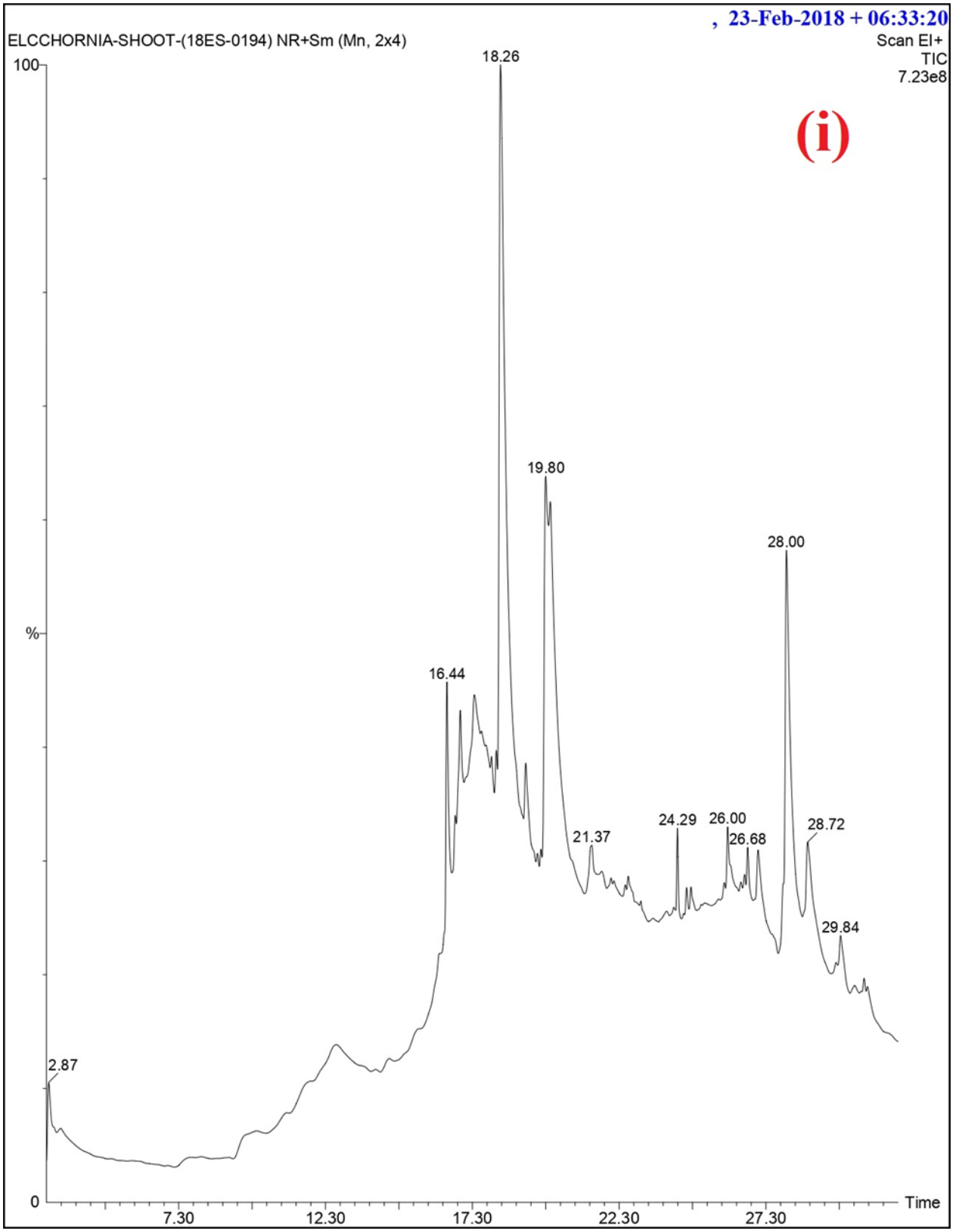
The ethanol extracts of both the shoot and root parts of *Eichhornia crassipes* were found to retain a maximum number of phytoconstituents such as anthraquinones, flavonoids, saponins and sterols. Hence the ethanol extract was purified using gel filtration chromatography with silica gel as the packed column. The pure extract was eluted out using ethanol, i.e., the elution solvent used for the extraction. The collected colored liquid containing the active phytochemical compounds were then used for compound elucidation studies using other chromatographic techniques.

3.2.1 Thin Layer Chromatography

The Thin Layer Chromatography plates were run successfully using the solvent standardized to be n-butanol: Ethyl acetate: Water in the ratio of 1:2:3 for flavonoids and Chloroform: Water in the ratio of 3:2 for tannins. The visualization was performed using Vanillin- Sulphuric acid as the revealing agent. The separation of the compounds along the activated TLC plate could be seen and the Retention factors (R_f) obtained were, 0.83 and 0.9. The obtained R_f values were found to be in accordance with the results provided by Mehta Sonam *et al*., proving the presence of the possible compounds such as tannins and flavonoids could be investigated for the obtained R_f values respectively (Sonam, Singh, & Pooja, 2017) [13].

3.2.2 Gas Chromatography- Mass Spectrometry

The ethanolic extract of the shoot and root parts of the water hyacinth were performed and analysed for its peaks. The mass spectrometry was employed to determine the compounds based on the peak height and its area covered in comparison with the GC-MS NIST (2008) library. Since, GC majorly determines the vaporizable compounds, the Helium gas which acts as the carrier gas was found to elute out many of the volatile organic phytoconstituents. The shoot part of the water hyacinth was found to indicate the separation and identification of sterol compounds such as Stigmasterol (Unsaturated phytosterol) and 16-Heptadecenal (Monounsaturated fatty aldehyde). The root part showed the presence of 14-Heptadecenal (heptadecene aldehyde) with other ester and ether based compounds. Both the shoot and root parts were identified to retain the most common saturated fatty acid, n-Hexadecanoic Acid which is also known as the palmitic acid (Aparna *et al*., 2012) [1].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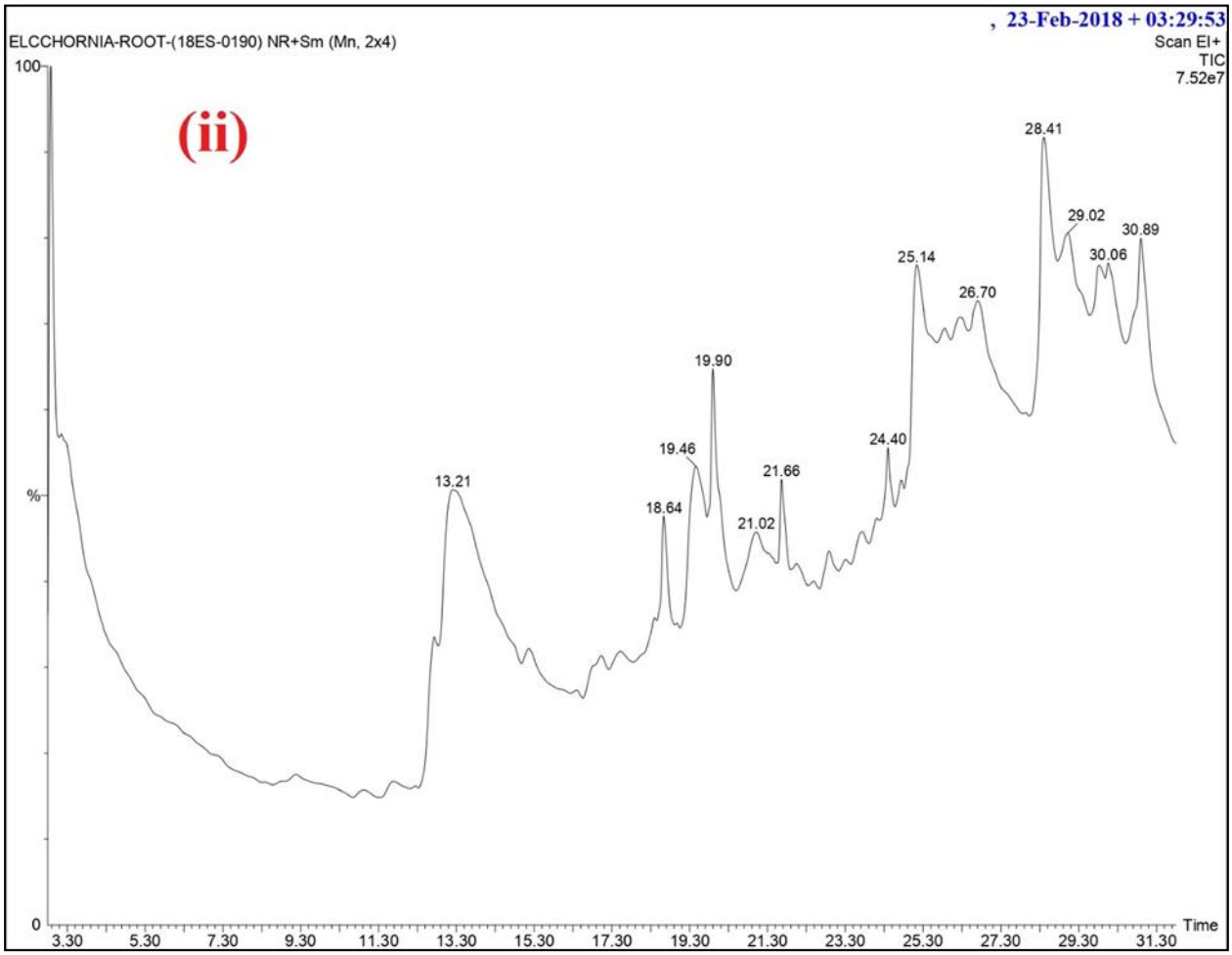

Fig 1: GC-MS Chromatogram of the ethanolic extract of the (i) shoot and (ii) root portions of *Eichhornia crassipes*

Table 3. GC-MS graph analysis with the compounds and their molecular weights of the shoot and root extract of *Eichhornia crassipes*

Sample	Peak reading (%)	Compound name	Molecular weight (g/mol)	Chemical formula
Shoot	16.44	16- Heptadecenal	252	$C_{17}H_{32}O$
	18.26	n-Hexadecanoic Acid	256	$C_{16}H_{30}O$
	19.80	1-Hexyl-2-Nitrocyclohexane	213	$C_{12}H_{23}O_2N$
	28.00	Stigmasterol	412	$C_{29}H_{48}O$
	28.72	22,23-Dibromo Stigmasterol Acetate	612	$C_{31}H_{50}O_2Br_2$
Root	13.21	1,4-dioxane-2,5-dione,3,6-dimethyl	144	$C_6H_8O_4$
	18.64	Nonanoic acid, 5-methyl-,ethyl ester	200	$C_{12}H_{24}O_2$
	19.46	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
	19.90	14-Heptadecenal	252	$C_{17}H_{32}O$
	25.14			
	28.41	4-Dimethyl(Phenyl)Silyloxypentadecane	362	$C_{23}H_{42}O_{81}$
	26.70			
	29.02	3,6 Methano-8H-1,5,7-trioxacyclopenta[1J] cyclopro[A]azulene-4,8(3H)	294	$C_{15}H_{18}O_6$
	30.06	1-Monolinoleoylglycerol Trimethylsilyl ether	498	$C_{27}H_{54}O_4Si_2$
	30.89			

4. Conclusion

Eicchornia crassipes is one of the common invasive aquatic plants which act as rich sources of many medicinal values with their abundant bioactive phytochemicals. Qualitative phytochemical analysis was performed using chosen reagents such as water, benzene and ethanol based on its polarity. Soxhlet based hot extraction was performed to identify the phytoconstituents in the roots and shoot parts of the selected plant. Phytochemical analysis showed the presence of flavonoids and saponins in the non-aqueous extracts, while the sterols and tannins were majorly present in the shoot and root parts respectively. Anthraquinones were present in all the extracts meanwhile anthocyanin was merely present in the extracts. The presence of secondary metabolites strongly indicates the medical importance of this untapped resource. The ethanol extract of the sample was separated as different eluents using column chromatography and preliminary initiation of the compound elucidation was performed using Thin Layer Chromatography. The revealing agents and the standardized solvent displayed the separation of tannins and flavonoids in the extracts. The ethanol extracts when subjected to GC-MS analysis which revealed the presence of volatile organic compounds, mainly, sterol based bioactives like, aldehydes, ethers and esters. Stigmasterol, a precursor

for the synthesis of semisynthetic progesterone and an inhibitory agent against ovarian, prostate, breast, and colon cancers was mainly present in the ethanolic shoot extracts. Another common fatty acid, n-hexadecanoic acid, was identified to have immense pharmacological properties and was identified in both the shoot and root extracts. Hence, *Eicchornia crassipes* can be commercially used as an effective source of bioactive compounds in pharmaceutical industries.

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References

1. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M *et al*. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical Biology & Drug Design. 2012; 80(3):434-439.
2. Chauhan A, Goyal MK, Chauhan P. GC-MS technique and its analytical applications in science and technology. J Anal. Bioanal. Tech. 2014; 5(6):222.
3. Gowthama Prabu U, Baskar R, Kumaresan K, Poorani, G. Investigations into the phytochemical profile in the seeds of Elaeocarpus variabilis fruits: A potential untapped source endemic to Western Ghats. Journal of Pharmacognosy and Phytochemistry. 2020; 9(4):566-571.
4. Jayanthi P, Lalitha P, Shubashini KS. Phytochemical investigation of the extracts of Eichhornia crassipes and its solvent fractionates. Journal of Pharmacy Research. 2011; 4(5):1405-1406.
5. Kumar S, Kumar R, Dwivedi A, Pandey AK. *In vitro* antioxidant, antibacterial, and cytotoxic activity and *in vivo* effect of Syngonium podophyllum and Eichhornia crassipes leaf extracts on isoniazid induced oxidative stress and hepatic markers. BioMed Research International, 2014.
6. Lata N, Dubey V. Preliminary phytochemical screening of Eichhornia crassipes: the world's worst aquatic weed. Journal of Pharmacy Research. 2010; 3(6):1240-1242.
7. Lila MA. Anthocyanins and human health: an *in vitro* investigative approach. Journal of Biomedicine and Biotechnology. 2004; (5):306.
8. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia. 2009; 80(2):123-126.
9. Perchellet EM, Magill MJ, Huang X, Dalke DM, Hua DH, Perchellet JP. 1, 4-Anthraquinone: an anticancer drug that blocks nucleoside transport, inhibits macromolecule synthesis, induces DNA fragmentation, and decreases the growth and viability of L1210 leukemic cells in the same nanomolar range as daunorubicin *in vitro*. Anti-Cancer Drugs. 2000; 11(5):339–352.
10. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2011; 8(1).
11. Silva RP, de Melo MMR, Silvestre AJD, Silva CM. Polar and lipophilic extracts characterization of roots, stalks, leaves and flowers of water hyacinth (Eichhornia crassipes), and insights for its future valorization. Industrial Crops and Products. 2015; 76:1033-1038.
12. Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor WC. Cytotoxic diterpenoid constituents from Andrographis paniculata Nees leaves. J Sci Soc Thailand. 1992; 18(4):187-194.
13. Sonam M, Singh RP, Pooja S. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(4):523-527.
14. Surendraraj A, Farvin KHS, Anandan R. Antioxidant potential of water hyacinth (Eichornia crassipes): *In vitro* antioxidant activity and phenolic composition. Journal of Aquatic Food Product Technology. 2013; 22(1):11-26.
15. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale Pharmaceutica Sciencia. 2011; 1(1):98-106.
16. Zhang Z, Pang X, Xuewu D, Ji Z, Jiang Y. Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. Food Chemistry. 2005; 90(1-2):47-52.