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GC-MS-MS analysis of Alien Invasive Aquatic weed, *Eichhornia crassipes* (Mart.) Solms.

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ABSTRACT

GC-MS analysis of methanolic extract of aerial parts of Eichhornia crassipes revealed the presence of 26 compounds. The identified compounds such as Goniothalamicin, Arborescidine were reported to have anticancer properties. Compounds such as 1, 8 Dipropoxyanthraquinone (91.33%) reported to have antioxidant property. Ambrosial, Camarolide and trace amount of Muricin were also recorded and reported as antimicrobial compounds. Therefore methanol extract of E.crassipes proves as a potential source of bioactive compounds of pharmacological importance.

Keywords: GC-MS analysis, phytochemicals and *Eichhornia crassipes*

INTRODUCTION

Plant secondary metabolites are unique resources for pharmaceuticals, food additives and fine chemicals. The different compounds can be classified as carotenoids, phenolics, alkaloids and nitrogen containing compounds based on their common structural features. Phenolics and flavonoids have raised particular interest because of their potential effects as antioxidant, anti inflammatory, cardioprotective and anticarcinogenic compounds. Plant extracts have great potential as biologically active compounds against pathogens, including microorganisms [1]. A large number of biologically active compounds have been extracted from *E. crassipes* since it is endowed with many potent phytochemicals like flavonoids, tannins, terpenoids, saponins, cardiac glycosides, quinones and many others [2]. 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. Hence commercial value of this aquatic weed needs to be investigated. Our preliminary studies on *E. crassipes* revealed that it is an alternate source of shikimic acid and a precursor for synthesis of oseltamivir commercially known as Tamiflu® in treating human influenza virus of swine origin and mild anticancer activity [3-4]. Hence, the present study is aimed at identification of bioactive compounds of the said properties through GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material

Leaves of *Eichhornia crassipes* (Mart.) solms, (Pontederiaceae), were collected from Kurichi Kulam (Kurichi lake), Coimbatore city, Tamil Nādu, India. It is situated between 10°57'57.6" latitude and 76°57'48.96" longitude. Kurichi Kulam in Coimbatore city is one of the major water bodies enhances the ground water level around this area.

Preparation of plant extracts

The collected plant materials were air dried and ground into uniform powder. Dry powder of plant sample was extracted with methanol using soxhlet apparatus for 6 hours. The extract was filtered over anhydrous sodium sulphate 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 n-hexane at the concentration of 100mg/ml (w/v) followed by filtration through Varian Bond Elute C_{18} solid phase extraction to remove impurities. 1µl of this solution was employed for GC-MS-MS analysis.

GC-MS-MS analysis

The GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS 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 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

Identification of phyto-compounds

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

RESULTS AND DISCUSSION

The GC/MS/MS analysis of methanol extract of leaf of *E. crassipes* gave us twenty six major compounds (Fig.1). All compounds identified by GC/MS/MS screening were assessed for their biological property using physico-chemical property calculations according to Tice Rules [5]. As per Tice rule compounds are more likely to have properties of antimicrobial, anticancerous antioxidants and anti-insect if molecular weight is within ≥ 150 and ≤ 500; theoretical logarithm of the noctanol/water partition coefficient (log P), is less than or equal to 5.0; hydrogen bond acceptor is within 1-8; hydrogen bond donar is less than or equal to 2 and the number of rotatable bond is less than or equal to 12 (Table.1). The compounds those are strictly following the Tice rules are considered as antimicrobial, anticancerous, and antioxidant potential compounds for new or novel drugs [6]. Almost all the compounds detected in GC-MS analysis follow Tice rule. The compound Goniothalamicin identified in the present study is reported to have anticancer property which inhibited the growth of human lung cancer cells [7]. In our earlier study on anticancer activity of methanol extract of aerial parts of *E. crassipes* on the human cervical cancer cell line (HeLa) was found to have mild cytotoxic activity of 17% cell death [3].

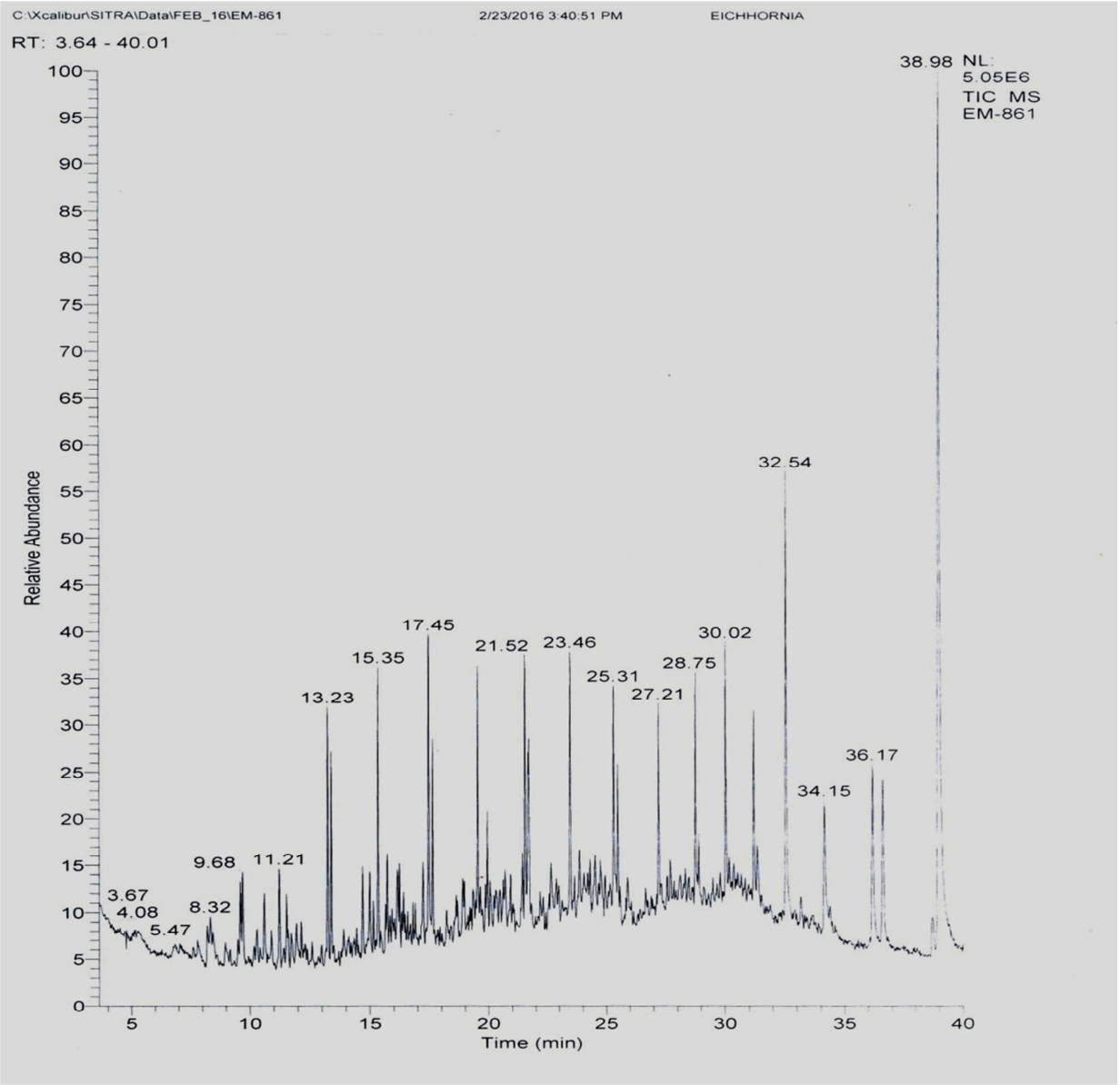

Fig. 1. GC-MS Chromatogram of methanol extract of *E. Crassipes*

Compounds namely 2-Allyl-3, 6-dimethoxybenzyl alcohol (6.98%) and 1, 8-Dipropoxyanthraquinone (91.33%) were reported to have antioxidant properties [8]. Nonacosane (10.88%) was reported for its nematicidal activity [9]. Camarolide (19.11%) was reported for its antibacterial activity [10]. Arborescidine B (55.43%) was reported for antiproliferative property [11]. E-10, 13, 13-Trimethyl-11-tetradecen-1-ol acetate (6.80%) was reported for its antidandruff property and application in hair oil [12]. Docosane (13.84%) was accounted for bioremediation [13]. Hence, isoaltion and bioassay studies with respect to aforementioned compounds will be an insight into develop novel drugs for application in pharmacology.

Table 1. GC-MS analysis of methanol extracts of *E. crassipes*

S.no	Retention time	Lead compound	Molecular formula	Molecular weight	%	HD	HA	RB	LogP	Biological properties
1	23.38	9,9-Ethylenedioxybicyclo[3.3.1]non-2-en-7-ol	C11H16O3	196	6.40	1	3	0	0.87	unknown
2	26.37	2-Allyl-3,6-dimethoxybenzyl alcohol	C12H16O3	208	6.98	1	3	5	1.97	Antioxidant activity
3	26.31	Ambrosiol	C15H22O4	266	3.90	2	4	0	1.24	Nematicidal and antimicrobial activity
4	26.62	1,8-Dipropoxyanthraquinone	C20H20O4	324	91.33	-	-	-	-	Antioxidant
5	27.39	Dictysine - Trideuterio Derivative	C21H30D3NO3	347	2.45	3	4	1	1.77	Anesthetic activity
6	27.88	1-(2-Hydroxy-3,4-dimethoxybenzyl)isoquinoline	C18H17NO3	295	11.32	1	4	4	2.85	unknown
7	29.35	1-[2'-(t-Butyldimethylsilyloxy)phenyl]-3-phenyl-2-propyn-1-one	C21H24O2Si	336	21.28	0	2	6	2.70	unknown
8	29.35	3-Buten-2-ol (CAS)	C4H8O	72	14.17	1	1	1	0.52	unknown
9	29.35	2-N-Hexadecyl-hexahydroindan	C25H48	348	2.41	0	0	15	12.83	Antioxidant activity
10	29.90	trans-3,4,5-Trimethoxy-á-methyl-á-nitrostyrene	C12H15NO5	253	8.01	0	6	5	2.46	unknown
11	30.22	2-(2-furyl)-phenol	C10H8OS	176	18.55	1	2	1	2.13	unknown
12	30.91	Camarolide	C30H44O3	452	19.11	0	3	0	6.98	Antibacterial activity
13	30.91	1-AZAFLUOREN-9-ONE	C12H7NO	181	1.88	0	2	0	2.28	
14	32.10	8-Oxabicyclo[3.2.1]octan-3-one	C7H10O2	126	5.43	0	2	0	-0.37	Allelopathic property
15	33.20	1H-Indene, 5-decyloctahydro- (CAS)	C19H36	264	6.64	0	0	9	9.64	unknown
16	33.60	3,6,6-Trimethylundecane-2,5,10-trione	C14H24O3	240	10.10	0	3	8	1.23	unknown
17	33.85	4,6-Dibromo-2-diethylamino-3,5-difluoropyridine	C9H10Br2F2N2	342	16.00	0	2	3	4.91	unknown
18	34.17	Docosane (CAS)	C22H46	310	13.84	0	0	19	12.44	Bioremediation
19	34.17	Nonacosane (CAS)	C29H60	408	10.88	0	0	26	16.17	Nematicidal activity
20	34.99	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	C19H36O2	296	6.80	0	2	13	7.16	Anti-dandruff
21	36.25	Arborescidine B	C16H17BrN2	316	55.43	0	2	0	4.24	Antiproliferative activity
22	38.61	Trimethyl 2-phenyl-5-(4'-cyanophenyl)-3-pyrroline 2,3,4-tricarboxylate	C23H20N2O6	420	90.31	-	-	-	-	Antibacterial
23	38.98	13-Docosenamide, (Z)-	C22H43NO	337	66.91	2	2	19	8.87	Antimicrobial
24	38.98	Erucylamide	C22H43NO	337	66.91	2	2	19	8.87	Fabric softner, pesticide
25	26.62	Goniothalamicin	C35H64O7	596	1.05	4	7	26	6.46	Anticancer property
26	26.62	Muricin B	C35H64O7	596	0.00	4	7	26	6.06	Antibacterial

CONCLUSION

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. The present study therefore has provided some biochemical basis for biological values of *E. crassipes.* As rich source of phytochemicals such as, alkaloids, phenols and flavonoids *E. crassipes* could be a potential source of useful compounds as mentioned. GC-MS analysis of methanol extract of aerial parts of *E. crassipes* revealed the presence of secondary metabolites of anticancerous, antimicrobial, antioxidant, antidandruff, antiproliferative activities and provides a potential source of industrial application.

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