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Determination of Antibacterial Activity using the Aqueous Leaf Extract of *Eichhornia crassipes* against Few Common Bacterial Pathogens on Fresh Water Fish *Oreochromis mossambicus*

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A B S T R A C T

The aim of the study was to investigate the antibacterial activity of aqueous leaf extract of *E. crassipes* against fish pathogenic organisms like *P.aeruginosa, A.hydrophila, Vibrio sp., E. coli and S.aureus. O.mosambicus* was collected from Kolathur area, Chennai. Fishes were packed aseptically and transported to lab. Organisms were isolated and identified from fishes and used for further studies. The aqueous leaf extract of *E. crassipes* was extracted by using Soxhlet apparatus and concentrated by Vacuum evaporator to remove the moisture. The phytochemical screening was done to show that the aqueous leaf extract of *E. crassipes* contain alkaloids, sterols, flavonoids, carbohydrates, and terpenoids, anthoquinones and proteins. The occurrence of these biologically active compounds of the plants may justify their wide usage in traditional medicine and effective against various disease causing pathogens. TLC, HPTLC and GC-MS were performed to study the qualitative and quantitative analysis of various compounds present in the plant extract. Due to the presence of these phytochemical compounds, the leaves can be used as a natural source to treat against the selected fish pathogenic organisms. MIC and the antibacterial activity of the aqueous plant extract against the selected pathogens were done by well diffusion method. The diameters of growth inhibition zone formed were measured in mm along with tetracycline as a reference standard.

Introduction

Eichhornia crassipes (Mart) *Solms* commonly known as Water hyacinth is a warm water aquatic plant belonging to the family Pontideraceae. It is listed as one of the most productive plant on earth and is considered the world worst aquatic weed (Grodowitz, 1998). Water hyacinth, *Eichhornia crassipes* also known as 'blue devil', grows rapidly as a dense green mat over stagnant water bodies such as lakes, streams, ponds, waterways, ditches and backwaters. It has been considered as an uncontrolled nuisance many a time. The capacity of water hyacinth to invade and overtake aquatic

habitats is astounding. It can quickly dominate natural areas and can dramatically alter the species composition, structure and function of native plant and animal communities. It alters the ecosystem of the water body, causing dissolved oxygen fluctuations and raising the water temperature (Vilaseca *et al.,* 2010; Awomeso *et al.,* 2010).

Aquatic plants have economic and environmental uses, depending on the natural characteristics. Some are consumed in human diet, while other species have medicinal values and still other species are good resource of minerals and vitamins.

Since aquatic weeds are known to differ widely in their chemical composition depending upon species, season and location, an insight into their chemical composition is essential if utilization prospects are to be considered (Lata and Dubey, 2010). Water hyacinth can also cause many problems for the fisherman such as decreased fish population, difficult access to the fishing sites and loss of fishing equipment, resulting in reduction in catch and subsequent loss of livelihood (Anushree Malik, 2007).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Herbal remedies and alternative medicines are used throughout the world and in the past, herbs often represented the original sources of most drugs (Sa'ad *et al.,* 2005).

Most important of these bioactive constituents of *Eicchornia crassipes* leaves of the plants are alkaloid, tannins, sterols, anthroquinones, flavonoids, phenolic compounds and proteins (Kayathri *et al.,* 2014).

Plants contains flavonoids, alkaloids, Tannins, phenols etc., which have biological significance in terms of medicine development and extracts of aqueous, methanol and ethanol are good source of antiviral, antitumor and antibacterial agents (Cowan, 1999; Harborne, 1998).

Here in this paper we are focusing on the minimum inhibitory concentration, antimicrobial activity and analysis of the phytochemical compounds from the aqueous extract of *Eicchornia crassipes* by using GC-MS.

Materials and Methods

Collection of fish

Same size group of healthy *Oreochromis mossambicus* were purchased from Kolathur, Chennai, India. Fish were aerated properly and transported to the lab. Bacteria were isolated from Gills, Muscles, Liver and Kidney of *O.mosambicus*. *E.coli, S.aureus, P.aeruginosa, Vibrio sps and A.hydrophila* was isolated from *O.mosambicus*.

Collection and extraction of plant material

The fresh aquatic leaves of *Eichhornia crassipes* were collected from Pallavaram Lake, Tamil Nadu, and India.

The leaves were washed thoroughly in running tap water and finally washed with sterile distilled water. The leaves were shade dried and ground into coarse powder. The Powdered materials were stored in air tight polythene bags until use.

Twenty five grams of powdered sample was extracted with 250 ml distilled water for 24 hrs using Soxhlet apparatus and filtered through what mann filter paper. The filtrate was then subjected to Vacuum evaporator for getting the crude extract.

Thin layer chromatography

TLC technique was used for separation of phytochemical compounds twenty microlitres of plant extract were spotted on glass plate (2 × 10 cm) covered by silicagel and using Petroleum ether: Formic acid: water with the ratio of 8:1:1 and the separation time was 20 minutes. The TLC was performed using the standard phytochemical methods (Cowan, 1999).

HPTLC

The HPTLC system (Camag, Muttenz, Switzerland)

TLC scanner connected with a PC running WinCATS software under MS Windows NT

Linomat V Sample applicator

GC-MS

The TLC and GC-MS were performed using the standard phytochemical procedure (Cowan, 1999).

Minimum inhibitory concentration

The minimum inhibitory concentration was done for the selected bacterial strains against the crude aqueous extract of *E.crassipes*.

Agar-Well diffusion method

The agar well diffusion method was performed for determining the antibacterial activity of crude extract of *E.crassipes* against all the selected bacterial isolates of *P.aeruginosa, A.hydrophila, Vibrio* sp., *E. coli and S.aureus.* The tetracycline was used as control. Antibacterial activity was obtained by determining the zone of inhibition around the well.

Results and Discussion

Minimum inhibitory concentration

The minimum inhibitory concentration for *P.aeruginosa* and *E.coli* were 1.25mg/ml. *A.hydrophila* showed 0.625mg/ml, *Vibrio sps* showed 0.312mg/ml and *S.aureus* showed 0.156mg/ml. The MIC results indicate that the plant crude extract was very active against the species for its particular concentration. The plant crude extract was very effective even in the lower concentration for *Vibrio sps* and for *A.hydrophila*. The minimum inhibitory concentration for *P.aeruginosa* and *E.coli* requires little high when compared to other organisms.

Antibacterial activity by Agar-well diffusion method

The clear zone of inhibition was noted around the well due to diffusion of drug and the inhibition of bacterial growth. The antibacterial studies show the effective and greatest potential of the aqueous leaf extracts of water hyacinth. The results of phytochemical screening indicate that the plant extracts contains alkaloid and flavonoids. The antimicrobial activity of the extracts might be attributed to the presence of the foresaid secondary metabolites in the extracts.

Thin layer chromatography

The TLC was performed for the separation of phytochemical compounds from the aqueous leaf extract. More number of colored bands indicates the presence of phytocompounds which are responsible for the plant defense mechanism against the pathogenic microorganisms.

High performance thin layer chromatography

In this study the HPTLC fingerprinting of aqueous leaf extract of *E. crassipes* with different concentrations **(5** µl, 10 µl, 15 µl and 20 µl) revealed 4 spots with Rf values 0.07, 0.15, 0.35, 0.69 and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

The GC-MS study reveals that there are many different compounds were identified from the aqueous leaf extract of *E.crassipes*.

Table.1 Minimum Inhibitory Concentration

S. No.	Organisms	Concentration Of Crude Extract							
		5	2.5	1.25	0.625	0.312	0.156	0.078	0.037
1.	*P.aeruginosa*	_	_	_	+	+	+	+	+
2.	*A.hydrophila*	_	_	_	_	+	+	+	+
3.	*Vibrio sps*	_	_	_	_	_	+	+	+
4.	*E.coli*	_	_	_	+	+	+	+	+
5.	*S.aureus*	_	_	_	_	_	_	+	+
6.	Negative control	+							
7.	Positive control	+							

Table.2 Agar-Well Difussion Method

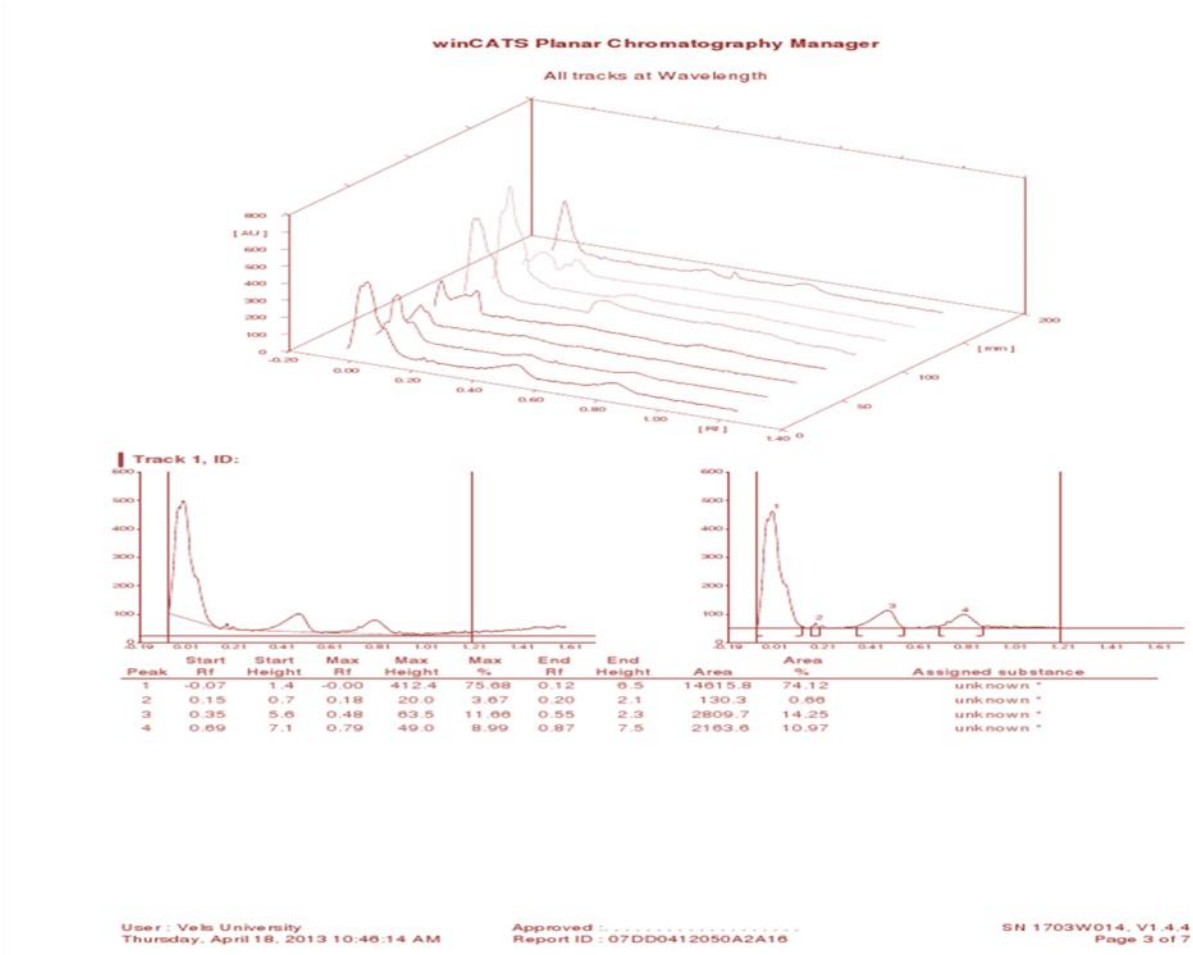
S. No.	Organisms	Zone of Inhibition		
		Aqueous Extract	Tetracycline	Distilled water
1.	*P.aeruginosa*	6	11	_
2.	*A.hydrophila*	8	11	_
3.	*Vibrio sps*	10	11	_
4.	*E.coli*	6	11	_
5.	*S.aureus*	11	11	_

Table.3 Components identified in the leaf extract of *E.crassipes* sample [GC MS study]

S. No.	Name of the compound	Retention Time	% Peak area
1.	Butane, 1,1-diethoxy-	3.02	6.60
2.	4-Tridecene, (Z)	6.48	0.2260
3.	4H-Pyran-4-one, 2,3-dihydro3,5-dihydroxy6-methyl-	6.94	0.65
4.	Methyl Salicylate -	8.75	0.80
5.	1-Octene, 6-methyl-	8.98	0.2038
6.	3-Tetradecene, (Z)-	13.09	0.4070
7.	Undecane, 4,7-dimethyl-	13.93	0.2742
8.	Dodecane, 2,6,10-trimethyl	15.75	0.1505
9.	Phenol, 2,4-bis(1,1-dimethylethyl)-	16.16	0.5696
10.	9-Octadecene, (E)-	16.90	0.4355
11.	Dodecanoic acid	17.32	1.0117
12.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	20.59	2.7839
13.	9-Octadecenoic acid (Z)-, methyl ester	25.83	0.8090
14.	Phytol	26.18	0.6163

Fig.1 Thin Layer Chromatography

Fig.2 High Performance Thin Layer Chromatography



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	1.4	-0.00	412.4	75.68	0.12	6.5	14615.8	74.12	unknown *
2	0.15	0.7	0.18	20.0	3.67	0.20	2.1	130.3	0.66	unknown *
3	0.35	5.6	0.48	63.5	11.66	0.55	2.3	2809.7	14.25	unknown *
4	0.69	7.1	0.79	49.0	8.99	0.87	7.5	2163.6	10.97	unknown *

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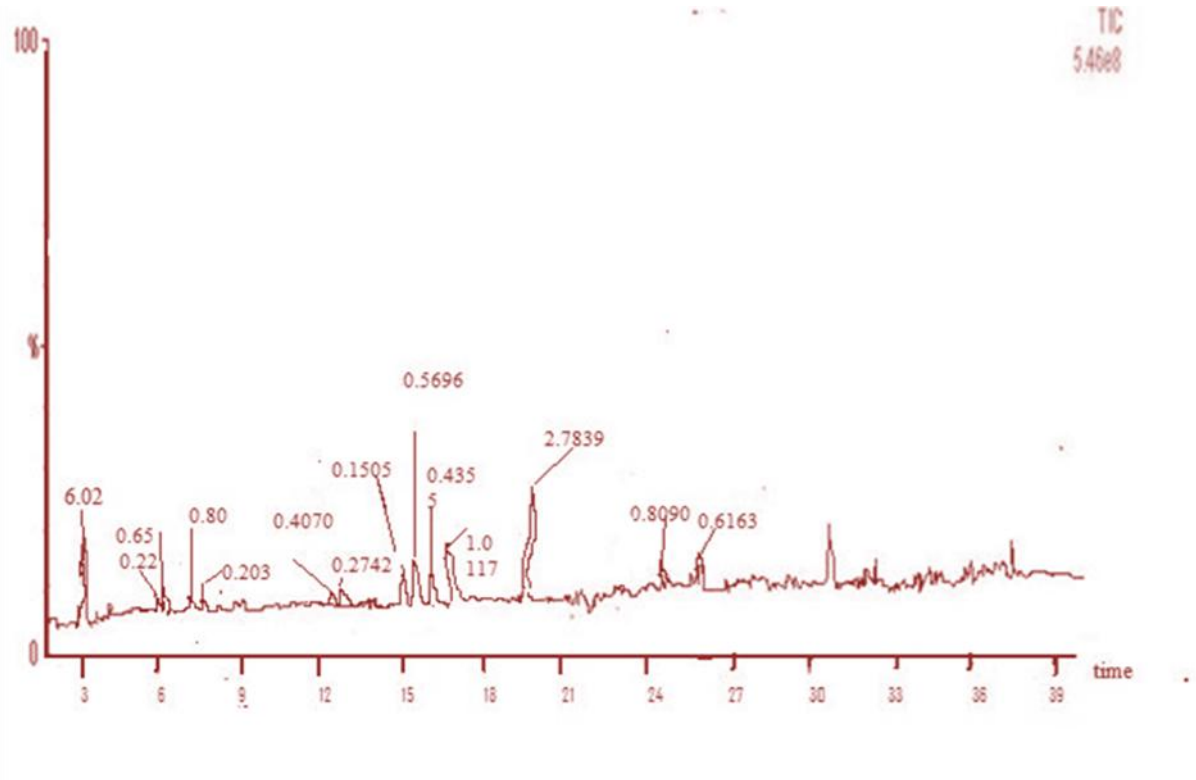
Fig.3 GC-MS Chromatogram of aqueous leaf extract of *E. crassipes*



Minimum inhibitory concentration and the antibacterial potency of aqueous plant extract showed higher activity in *S.aureus* and *vibrio* species, medium activity showed in *A.hydrophila* and less activity was showed in *P.aeruginosa* and *E.coli.* The phytochemical screening of the aqueous leaf extract for TLC, HPTLC and GC-MS indicates that the presence of secondary metabolites present in the leaf extract was attributed to its antibacterial activity.

The results obtained in the present study shows the presence of phytochemicals which take part in defense mechanism of the plant and these phytochemical compounds may enhance the antibacterial activity of certain microorganisms. Therefore, we have to exploit the potent possibilities of this plant which possess high therapeutic value and thus, we can use this plant mediated defense mechanism in the aquaculture improvement by enhancing the innate immunity of the fish as well as we can prevent it from infectious diseases.

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