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Antimicrobial Activity of *Heliotropium Curassavicum* a Mangrove Plant.

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ABSTRACT

Extraction of *Heliotropium curassavicum*. Aerial parts resulted . The crude extract was evaluated for their antibacterial and antifungal activity. The results indicate that Ethyl acetate and methanolic extracts, exhibited significant antimicrobial activity.

Keywords: *Heliotropium curassavicum*, Extract, Antimicrobial activity.

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INTRODUCTION

Heliotropium curassavicum Var.. argentinum is widely employed in gout, rheumatism, neuralgias, arteriosclerotic disorders, muscular algias, phlebitis, varix and other illnesses [1]. Two basic proteins were identified from this Mangrove and studies indicate that they possess potential antimicrobial activity [2] and cardiovascular system activity [3]. In Saudi Arabia, *Heliotropium curassavicum* L. and *Heliotropium bacciferum* (Boraginaceae) have become two of the most common polycarpic weeds infesting many Wadis and newly reclaimed fields at many areas of Taif regions [4,5]. Because of their vigorous growth and natural ability to colonize the disturbed salt affected sand flats, the species spreads rapidly invading the newly reclaimed lands and the surrounding fields as a troublesome weed [6,7]. The success of different *Heliotropium* species as weeds can be attributed to a large extent to their ability to produce adventitious root buds which allow for the plant's perennation and spread. The aim of the current work is to study the antimicrobiological studies of the species belongs to the genus *Heliotropium* (Boraginaceae); namely, *Heliotropium curassavicum* L.

EXPERIMENTAL

Materials and Methods

The stem of *Heliotropium curassavicum*, was collected from Gullalamoda (16°35'N latitude and 84°15'E longitude) Nagayalanaka estuary, Krishna District, Andhra Pradesh of India in Dec. 2012. Shade dried and finely powdered stems (3 Kgs) of *Heliotropium curassavicum* were extracted with Ethyl acetate and methanol successively.

Study of Microbial activity

Literature survey reveals that this mangrove plant crude extract exhibits Antimicrobial activity. Hence in this present investigation it is proposed to study the activity of the extract as well as the crystalline constituents. The results are presented in Table II and I. The Antimicrobial activity of *Heliotropium curassavicum*: (i) Methanolic extract & (ii) Ethyl acetate extract, were investigated by the cup diffusion method of Murry et al. [8], on nutrient agar medium for bacteria and potato dextrose agar for Fungi. The sterile medium (20ml) in Petri dishes were uniformly smeared with culture of tested organisms. Wells of 6mm diameter were made in each Petri dish, to which 50µl of the different concentrations of plant extracts; concentration 1µg/µl was added. The study also include with Beacon Multi Disc antibiotics and Bavistin fungicide standard drug for comparison. For each treatment, triplicates were examined and recorded.

The plates were incubated at 37° C for 18 hrs for Bacteria or at 25° C for 48 hrs for fungi. The diameter of the resulting zone of inhibition was determined with Hi Antibiotic Zone Scale. The data were presented in Table –I and II.

Ten pathogenic bacteria and ten phytopathogenic fungi were obtained from the microbial type culture collection (MTCC) Chandigarh. and ATCC from New Delhi as detailed below.

Micro Organisms: - Bacteria**G +Ve**

<i>Bacillus Subtilis</i>	MTCC - 2274
<i>Bacillus pumilis</i>	MTCC - 2296
<i>Enterococcus faecalis</i>	MTCC - 0439
<i>Micrococcus luteus</i>	MTCC - 1538
<i>Streptococcus faecalis</i>	MTCC - 0459

G – Ve

<i>Erwinia Carotovora</i>	MTCC - 1428
<i>Escherichia coli</i>	ATCC - 9637
<i>Klebsiella pneumoniae</i>	MTCC - 2405
<i>Pseudomonas marginalis</i>	MTCC - 2758
<i>Proteus vulgaris</i>	MTCC – 0426

Micro Organisms :- Fungi

<i>Acremonium strictum</i>	MTCC -3072
<i>Aspergillus flavus</i>	MTCC -1884
<i>Aspergillus niger</i>	MTCC -1881
<i>Cladosporium herbarum</i>	MTCC -2143
<i>Fusarium oxysporum</i>	MTCC -1755
<i>Lasiodiplodia theobromae</i>	MTCC -3068
<i>Penicillium expansum</i>	MTCC - 2006
<i>Penicillium chrysogenum</i>	MTCC -1996
<i>Physoderma maydis</i>	MTCC -2802
<i>Ustilago maydis</i>	MTCC -1474

RESULTS AND DISCUSSION

Phytochemical studies on this species has resulted the identification of different extracts. The results obtained in this microbial study indicate a prominent antimicrobial activity among microbes tested against *Heliotropium curassavicum* (Ethyl acetate and Methanolic extracts). Activity was more pronounced against human pathogenic bacteria. Out of the ten bacteria screened, seven showed significant antibacterial Activity as evidenced by zone of inhibition .and the data is recorded in Table –I.

Investigations on fungal species, viz) *Aspergillus flavus* and *Lasiodiplodia theobromae* , which are important post-harvest seed and fruit rot disease causing fungi ; the zone of inhibition against the plant extract and the compounds are significant. The data is presented in Table-II.

In case of some fungi viz) *Fusarium oxysporum*, *Acremonium strictum* and *Ustilago maydis* the Ethyl acetate and Methanolic extracts possess antimicrobial activity against these organisms. The results showed that the Ethyl acetate and methanolic extracts of this mangrove species exhibits the synergistic effect of the compounds.

The increasing prevalence of multidrug resistant strains of untreatable microbial infections adds urgency to the search for new infection fighting strategies. This in vitro screening of plant extract and compound, for antimicrobial bioassay, infers their potential use in the management of diseases caused by microbes.

Table I: Antibacterial activity of *Heliotropium curassavicum* : Methanolic & Ethyl acetate extracts

Micro organisms	Minimum inhibitory concentration (100mg/ml) ^a of extracts Zone of Inhibition (mm)		Beacon multidiscs for Standard Antibiotics						
	EtOAc extract	MeOH extract	CL5	CZ30	XO30	SP5	J10	AK30	OF5
			5µg/disc	30µg/disc	30µg/disc	5µg/disc	10µg/disc	30µg/disc	5µg/disc
<i>Bacillus Pumilis</i> (MTCC - 2296) +Ve	21	21	25	20	20	27	15	25	27
<i>Enterococcus faecalis</i> (MTCC - 0439) +Ve	NA	NA	15	NA	NA	NA	NA	NA	12
<i>Micrococcus luteus</i> (MTCC - 1538) +Ve	23	19	24	15	NA	22	NA	20	23
<i>Streptococcus faecalis</i> (MTCC - 0459) +Ve	15	NA	22	NA	26	16	NA	16	18
<i>Escherichia coli</i> (ATCC - 1937) -Ve	22	25	17	12	10	17	NA	17	23
<i>Klebsiella pneumonia</i> (MTCC - 2405) -Ve	14	11	20	12	NA	20	8	18	20
<i>Proteus vulgaris</i> (MTCC - 0426) -Ve	23	25	20	8	10	20	NA	10	20

Values including diameter of the well (06.0mm), are the mean of three replicates. a = 50µl of solution (50mg/ml) was applied to each well, NA = Not Active

Used antibiotics names: - CL=Ciprofloxacin, CZ=Cettazidine, XO=Ceftriaxone, SP= Sparfloxacin, J=Gentamycin, AK=Amikacin, OF= Ofloxacin

Table II: Antifungal activity of *Heliotropium curassavicum* : Methanolic & Ethyl acetate extracts

Micro Organisms	Minimum inhibitory concentration of extracts(100mg/ml) ^a Zone of Inhibition(mm)		Standard Fungicide Minimum inhibitory concentration of Bavistin Zone of mg/ml inhibition	
	EtOAc extract	MeOH extract		
<i>Ascremonium strictum</i> (MTCC - 3072)	15	12	0.01	14
<i>Aspergillus flavus</i> (MTCC - 1884)	14	16	0.01	19
<i>Aspergillus niger</i> (MTCC - 1881)	10	12	10	19
<i>Cladosporium herbarum</i> (MTCC - 2143)	18	15	0.01	16
<i>Fusarium oxysporum</i> (MTCC - 1755)	17	16	100	23
<i>Lasiodiplodia theobromae</i> (MTCC - 3068)	18	17	6	13
<i>Penicillium expansum</i> (MTCC - 2006)	19	16	0.001	15
<i>Penicillium chrysogenum</i> (MTCC - 1996)	18	20	0.001	15
<i>Physoderma maydis</i> (MTCC -2802)	17	17	0.1	12
<i>Ustilago maydis</i> (MTCC - 1474)	30	20	0.1	14

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