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Antimicrobial Activity of Helicteres isora Root

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The aqueous ethanol extract of *Helicteres isora* root were partitioned using various solvents like petroleum ether, chloroform, ethyl acetate and butanol. Of the 10 tested microbial strains, all fractions exhibited antimicrobial activity against 9 microbial strains at concentrations of 10, 5, 2.5 mg/ml. Among tested organisms, *Micrococcus luteus, Aspergillus niger* and *Candida albicans* were most sensitive and *Salmonella typhimurium* was most resistant. Butanol extract was found to possess most potent antimicrobial activity.

Helicteres isora Linn. (Sterculiaceae), commonly known as east Indian screw tree is a large shrub or small tree occurs often gregariously, throughout India and in dry deciduous forests up to 1500 m on the hill slopes¹. In traditional medicine the root juice is claimed to be useful in cough, asthma, stomach affections, intestinal infections, diabetes and a cure for scabies when applied topically. Fruits are demulcent, mildly astringent and useful in griping and flatulence^{2,3}. The decoction of the root used to be mixed with turmeric powder and applied externally to treat cuts and wounds by the ethnic people of Rayalseema region of Andhra Pradesh, India⁴. The presence of cucurbitacin B and isocucurbitacin B were reported in roots⁵. Aqueous ethanol and butanol extracts of H. isora root has been reported to possess significant antihyperglycemic activity in both alloxan-6 and glucose-7 induced hyperglycemic rats at a dose of 250 mg/kg. The literature further revealed that, ethanol extract of root caused significant reduction in plasma glucose, triglycerides and insulin levels at 300 mg/kg dose after nine days of administration to insulin resistant and diabetic db/db mice8. The potent inhibitory activity of aqueous extract of H. isora fruits was reported against avian myeloblastosis virus9 and human immunodeficiency virus¹⁰.

To the best of our knowledge no report is available on the antimicrobial activity of *H. isora* roots. As there is no reference in literature regarding the antimicrobial aspects, it was considered worthwhile to investigate the antibacterial and antifungal properties of the roots of *H. isora* by its partitioning with various organic solvents and screening the resultant extracts for the antimicrobial activity.

*For correspondence E-mail: venkateshsama@hotmail.com *H. isora* roots were collected in the month of September 1999 from the Srisailam forest, A.P, India. Identification of the material was carried out at Kama Reddy Degree College, Kama Reddy, A.P, India. A voucher specimen (HI/Rt/99) is being maintained in the Phytochemistry and Pharmacognosy department of G. Pulla Reddy College of Pharmacy, Hyderabad, A.P, India.

The roots of *H. isora* were washed, air-dried and ground into a fine powder. The dried root powder (5 kg) was extracted with 80% aqueous ethanol by a maceration process for 3 days. The percent yield of crude aqueous ethanol extract was 2.26 (113 g). To the concentrated aqueous ethanol extract (113 g), 500 ml of water were added and fractionated with petroleum ether (4×500 ml), chloroform (4×500 ml), ethyl acetate (4×500 ml), and n-butyl alcohol (4×500 ml) in the increasing order of polarity of solvents. The resultant extracts were concentrated to dryness by rotary flash evaporator. All extracts were subjected to phytochemical screening^{11,12} and the results are tabulated in Table 1. For antimicrobial activity 10, 5, 2.5 mg/ml concentrations were made from the each crude extract. Dimethylsulphoxide was used as a solvent. Streptomycin and fluconazole at 500 µg/ml were used as standards for antibacterial and antifungal activities, respectively.

The *in vitro* antimicrobial activity of root extracts of *H. isora* was studied by Agar cup plate technique^{13,14}. The antibacterial studies were carried out against *Bacillus subtilis* NCIM 2063; *Micrococcus luteus* NCIM 2103; *Staphylococcus aureus* NCIM 2079; *Escherichia coli* NCIM 2068; *Proteus vulgaris* NCIM 2027; *Pseudomonas aeruginosa* NCIM 2200; *Salmonella typhimurium* NCIM 2501 using nutrient agar medium. Antifungal studies were carried out

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING AND PERCENTAGE YIELD OF ROOT EXTRACTS OF HELICTERES ISORA

Constituents	Aq. ethanol ext	Pet ether ext	Chloroform ext	Ethyl acetate ext	Butanol ext	Left over aq. ext
Alkaloids	_	_	_	_	_	_
Phenolic compounds and tann	nins +	_	_	+	+	+
Fixed oil and fats	_	_	_	_	_	_
Proteins and amino acids	+	_	_	+	+	_
Carbohydrates and glycosides	; +	_	+	+	+	+
Saponins	_	_	_	_	_	_
Steroids	+	+	+	+	+	_
Gums and mucilage	_	_	_	_	_	_
Coumarins	_	_	_	_	_	_
Flavonoids	+	_	_	+	+	_
% Yield (w/w)	2.26	0.04	0.48	0.25	0.90	0.55

+ indicates presence and - indicates absence

TABLE 2: ANTIMICROBIAL ACTIVITY OF HELICTERES ISORA ROOT EXTRACTS

Organisms Zone of inhibition (mm)																				
	Aq. ethanol ext		Pet. ether ext		Chloro-form ext		Ethyl acetate ext		Butanol ext		Left over aq. ext		Str	Flu						
	а	b	с	а	b	с	a	b	с	a	b	с	a	b	с	a	b	с		
B. subtilis	5	4	2	11	11	7	7	5	5	9	7	5	3	2	-	-	-	-	33	NT
M. luteus	19	14	14	7	6	5	10	10	9	5	4	4	32	27	22	9	8	6	22	NT
S. aureus	5	4	4	8	7	7	9	9	7	9	7	8	24	22	21	-	-	-	26	NT
P. vulgaris	3	2	-	4	4	3	2	2	-	4	4	2	-	-	-	-	-	-	14	NT
P. aeruginosa	4	3	2	7	6	5	9	8	7	10	7	7	6	6	4	-	-	-	16	NT
S. typhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	NT
E. coli	4	-	-	7	5	4	9	6	6	6	3	2	10	7	4	-	-	-	14	NT
A. niger	5	5	3	18	17	15	25	24	22	24	22	16	10	7	4	-	-	-	NT	15
C. albicans	8	7	4	14	13	9	13	10	9	23	14	11	12	11	9	-	-	-	NT	12
S. cerevisiae	17	12	11	17	14	12	18	16	11	13	12	10	20	17	13	-	-	-	NT	22

Bore diameter (8 mm) is subtracted from readings. a, b, c indicates 10, 5, 2.5 mg/ml concentrations, respectively; NT denotes not tested. Str and Flu indicates streptomycin and flucanozole, respectively at 500 µg/ml

against Aspergillus niger NCIM 620; Candida albicans NCIM 3471; Saccharomyces cerevisiae NCIM 3090 using MGYP medium. All microbial strains were procured from National Collection of Industrial Microorganisms, NCL, Pune, India (Ref. No: Bio/NCIM/2005-506). Bacterial concentration of 1×10^8 CFU/ml was used for antibacterial activity and fungal suspension of 1×10^6 CFU/ml for antifungal activity. In each plate wells of 8 mm diameter were made using a sterile borer. The wells were used in duplicate for each concentration. Solvent control (only DMSO) was also maintained throughout the experiments.

The aqueous ethanol extract of *H. isora* and its fractionated extracts viz., petroleum ether, chloroform, ethyl acetate, butanol and left over aqueous extracts were tested against 3 gram positive and 4 gram negative bacteria and 3 fungal strains. The results are reported in Table 2. All extracts at 10, 5, 2.5 mg/ml concentration exhibited appreciable antimicrobial activity against tested microbial strains, except left over aqueous extract. *M. luteus, A. niger* and *C. albicans* were the most sensitive and, had widest zone of inhibition, where as *S. typhimurium* was resistant

to all extracts of *H. isora*. The tested extracts also showed significant activity against fungal strains and are comparable with standards. Butanol extracts has more intensive antibacterial and antifungal activity than other extracts and the activity is comparable with standard drugs streptomycin and fluconazole. From the preliminary phytochemical screening it is revealed that *H. isora* root extracts showed positive results towards tannins, steroids and flavonoids. So the antimicrobial activity is due to any of these components or all the components. The susceptibility of various microbial agents to these extracts as observed in this preliminary study may suggest some information in developing natural antimicrobial herbal agents which need further evaluation.

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