SCREENING OF ANTIBACTERIAL ACTIVITY IN MEDICINAL CLIMBER *HEMIDESMUS INDICUS* L.B.BR

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Abstract : *Hemidesmus indicus* the milky weed is a pharmacologically important plant. It is widely used in Indian and also an official drug in Indian pharmacopoeia and British pharmacopoeia. The aim of this study was to investigate the antibacterial activity of *Hemidesmus indicus* against *Entero bacter, E.coil, Staphylococcus aureus, Klebsiella pneumoniae, and proteus vulgaris* by disc diffusion method. Results showed that the maximum zone of inhibition was observed in *Staphylococcus aureus* in ethanol extract and the minimum zone of inhibition was observed in *Entero bacter* in ethanol extract.

Key Words: *Hemidesmus indicus*, climber, antibacterial activity, pharmacopoeia.

INTRODUCTION

According to the world health organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants therefore, such plants should be investigated to better understand their properties, safety and efficiency (Gislene, 2000). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind (Jigna and Sumitra, 2007). Medicinal plants are the great and have economic value. The extraction and characterization of bioactive compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value. Treatment using medicines of natural origin is gaining momentum nowadays on account of increasing concern about potentially harmful synthetic additives (Resche, 1998).

Hemidesmus indicus (L.) R.Br. Commonly known as Indian sarsaparilla. It belongs to the family Asclepiadaceae. The roots and woody portion has been used traditionally for curing various ailments like stomach pain, fever, veneral diseases, rheumatism and also act as blood purifier. It serves as an alternative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases and urinary infections (Das, 2010). Plants have been a valuable source of natural products for maintaining human health especially; in the last decades, with more intensive studies for natural therapies. The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world. Therefore, it is essential to find new compounds that have antimicrobial properties and it is worthwhile to screen plant species which have the above properties to synthesize new drugs (Nascimento, 2000).

MATERIALS AND METHODS

Sample collection and solvent extraction

The material selected for the present study was *Hemidesmus indicus*. The plant was collected from Nagercoil, Kanyakumari District, TamilNadu, India. Plant was dried under shade condition, cut into small pieces, powered and stored in sterile containers for further use. A soxhlet apparatus was used for extraction, with ethanol, n-butanol and Aqueous solvents.

Bacterial strains

Five human pathogenic pathogens were used namely *Entero bacter, E.coil, Staphylococcus aureus, Klebsiella pneumoniae,* and *proteus vulgaris* obtained from MTCC Chandigar. Stock culture were maintained in nutrient agar medium at 40^0c, and then subcultured in nutrient broth at 37^0c prior to each microbial test.

Extraction of plant material (Maceration)

The shade dried 100gm coarse powered of selected climber *Hemidesmus indicus* was immersed in 200ml of different solvents (ethanol, n-butanol and Aqueous) contained in 500 ml sterile conical flasks and covered with cotton wool separately. It was placed aside with intermittent shaking for one week. They were first filtered with double layered muslin cloth and then through Whatman No. 1 filter paper. The filterate was subjected to evaporation by treating at 40°C in an oven to obtain a dried extract. The dried extract was stored at 40°C until used for further study (Atata *et al*., 2003; Jeyaseelan *et al.*, 2012).

Antibacterial activity by agar well diffusion assay method

The antibacterial activity of crude solvents (Ethanol, N-butanol and Aqueous) plant extracts of *Hemidesmus indicus* against gram-positive as well as gram-negative bacterial strains were evaluated by agar well diffusion assay (AWDA) method (Parekh and Chanda, 2007; Kumar and Gitika, 2014). The diameters of the inhibition zones were measured in millimeters (mm). For this, a well (6mm diameter) was made with the help of a borer in cooled nutrient agar plate, overlaid with soft agar (5ml), seeded (with a target strain (-106 cfu/ml). Aliquots of the test compound were introduced into the well and the plates were incubated for overnight at 370C. For each bacterial strain, the dissolving solvent 10% DMSO and Amikachin (50µg/ml) were used as negative and positive controls respectively. To test the antibacterial activity of all extracts were dissolved in 10% DMSO solvent to make a final concentration 200 mg/ml.

Determination of Minimum Inhibition Concentration (MIC)

The MIC is the concentration giving the least inhibitory activity and below which there is no further inhibition were determined by using the Broth dilution method (Adesokan *et al.,* 2007). Briefly, 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100 mg/ml. 1.0 ml of this dilution was transferred to another test tube till the 7^{th} test tube was reached. The 8^{th} test tube did not contain any extract, but a solution of pure solvent and served as negative control. Then 1 ml of 18 hr grown cultures of each bacterial strain, adjusted at ~1×10^6 cfu/ml was put into each tube and thoroughly mixed by vortex mixer. The tubes were incubated at 37^0C for 18hr and observed the growth in the form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection considered the MIC value.

Determination of Minimum Bacterial Concentration (MBC)

The MBC values were determined by removing 100 µl of bacterial suspension from the MIC positive tube as well as one above and one below the same tube, spread on nutrient agar plates and incubated at 37^0C for 18hr, After incubation, the plates were examined for colony growth and MBC's were recorded (Rahman *et al.,* 2008; Nand *et al*., 2012).

RESULTS AND DISCUSSION

The results of antibacterial activity in medicinal climber against human pathogens were presented in Table1 and plate1. The present investigation showed that the tested plant extract possess potential antibacterial activity against *E. coli, Klebsiella pneumonia, Proteus vulgaris, Staphylococcus aureus,* and *Entero bacter*. The ethanol extract of *Hemidesmus indicus* showed the antibacterial activity against five pathogens with the inhibition zones of *Staphylococcus aureus* 28.66±0.94 mm, *Klebsiella pneumoniae* 24.00±0.81mm, *E. coli* 19.33±0.94 mm, *Proteus vulgaris* 17.33±0.47 mm, and *Entero bacter* 11.66±0.47 mm. The n-butanol extract of *Hemidesmus indicus* showed the inhibition zone of *Klebsiella pneumoniae* 13.66±0.47 mm, *Staphylococcus aureus*16.66±0.47 mm, *Entero bacter*15.33±0.52 mm, *E. coli*, and *Proteus vulgaris* have no activity. The maximum zone of inhibition was observed in ethanol extract against *Staphylococcus aureus* and the minimum zone of inhibition was observed in *Entero bacter* in ethanol extract. Aqueous extract of *Hemidesmus indicus* does not showed any activity.

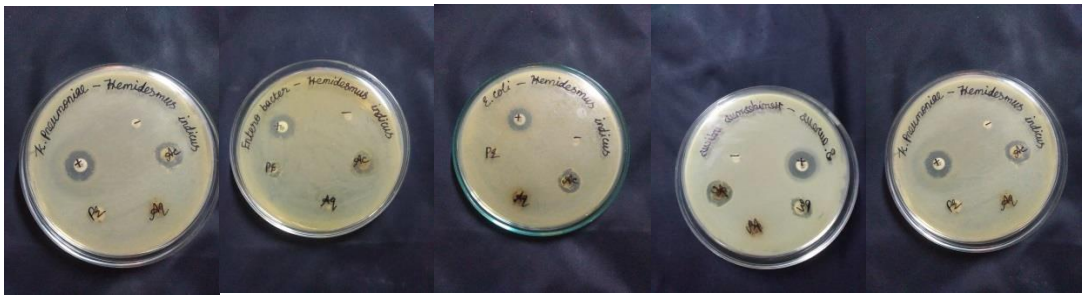
Mohamed *et al.* (2012) reported that the ethanol and acetone extract gave the highest inhibitory activity against all the tested five different bacterial strains. Ratha *et al*., 2012; Nagat *et al*., 2016 suggested that the ethanol extract of *Hemidesmus indicus* (L.) exhibited maximum zone of inhibition against *Vibrio cholera*. The methanol extract of *Hemidesmus indicus* (L.) showed maximum zone of inhibition against *Klebseilla pnemoniae*. The aqueous extract of *Hemidesmus indicus* (L.) does not show any activity against selected pathogenic bacteria. Similar results were observed the present study.

Table1: Inhibition Zone (mm) in *Hemidesmus indicus* using solvent extracts.

Sl.No.	Pathogens	Solvents		
		Ethanol	n-butanol	Aqueous
1	*E. coli*	20.33±0.94	-	-
2	*K. pneumoniae*	24.00±0.81	13.66±0.47	-
3	*P. vulgaris*	17.33±0.47	-	-
4	*S. aureus*	28.66±0.94	16.66±0.47	-
5	*E. bacter*	11.66±0.47	15.33±0.52	-

- (-) No activity
- Each value is a mean of three data
- Values were taken after subtracting the standard disc value 6mm.

Plate1:Inhibition Zone in *Hemidesmus indicus* using solvent extracts.



MIC and MBC values of *Hemidesmus indicus*

MIC and MBC values (mg/ml) of *Hemidesmus indicus* in ethanol and n-butanol extracts against bacterial pathogens were presented in table 2.

The MIC value of *Hemidesmus indicus* showed highest inhibition activity 41±0.57 mg/ml in ethanol extract against the pathogen *K. pneumonia* and lowest inhibition activity 19±0.12 mg/ml against the bacteria *E. bacter*. The MBC value of *Hemidesmus indicus* in ethanol extract showed highest colony forming unit 58±0.19 mg/ml in the pathogen *K. pneumonia* and lowest colony forming unit 22±o.46 mg/ml against the bacteria *E. coli*.

The MIC value of n-butanol in *Hemidesmus indicus* showed highest inhibition activity 30±0.46 mg/ml against the bacteria *K. pneumonia* and the lowest MIC 18±0.26 mg/ml against *E. coli*. The MBC value of *Hemidesmus indicus* showed highest colony forming unit 48±0.61 mg/ml against *E. coli* and lowest colony forming unit 36±0.01 mg/ml against *E. bacter*. The ethanol extract showed highest inhibition activity and colony forming activity (Rabe and Vanstaden, 1997; Goncalves *et al.*, 2008). The present result correlated with these findings.

Table 2: MIC and MBC values (mg/ml) of *Hemidesmus indicus* using different solvents

Pathogen	Ethanol		n-butanol	
	MIC	MBC	MIC	MBC
E. coli	23±0.16	22±o.46	18±0.26	48±0.61
K. pneumoniae	41±0.57	58±0.19	30±0.46	43±0.62
P. vulgaris	25±0.18	40±0.08	28±0.04	39±0.37
S. aureus	36±0.08	52±0.77	26±0.03	44±0.03
E. bacter	19±0.12	33±0.08	20±0.05	36±0.01

➢ Each value is a mean of 3 data

CONCLUSION

The results observed in the present investigation showed that *Hemidesmus indicus* possess antibacterial activity. It can be a source of newer useful drugs and of greater pharmacological importance. Thus, further research needs to be carried out for effective utilization and conservation of *Hemidesmus indicus*.

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