ANTIBACTERIAL ACTIVITY OF THE ROOT EXTRACTS OF *HEMIDESMUS INDICUS*

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Summary

Hemidesmus indicus is a pharmacologically important plant. The *in vitro* experiment by the disc diffusion method established that *Hemidesmus indicus* root extracts posses a bioactive compound, which inhibits the growth of the microorganisms. The root extracts of *Hemidesmus indicus* in different ratios of Petroleum ether and Ethyl acetate, tests have shown significant zone of inhibition to all these microorganisms viz., *Escherichia coli* (75 mm), *Bacillus subtilis* (56 mm) *and Pseudomonas aeruginosa* (54 mm).

Key words: Antibacterial activity, *Hemidesmus indicus*, Medicinal plants.

Introduction

Hemidesmus indicus R. Br (Periplocaceae) commonly known as 'Indian Sarsaparilla' is an aromatic medicinal twining shrub distributed in moist localities of India and Srilanka. *Hemidesmus indicus* is a pharmacologically important plant belonging to the Asclepiadaceae family or the milk weed or calotropis family includes 320 genera and 1,700 species of world wide distribution but most abundant in the subtropics and tropics. Herbal medicines generally have fewer side effects than synthetic compounds, and their effectiveness can be improved by modern pharmacological methods [1]. *H. indicus* extract is also found to inhibit lipid peroxidation and scavenge hydroxide radicals *in vitro* [2]. The glycosides of *H. indicus* root inhibited *S. typhimurium* induced pathogenesis nonspecifically, by reducing bacterial surface hydrophobicity and perhaps also by mimicking host cell receptors, there by blocking its attachment to host cell and further pathological effects [3]. The *H. indicus* root powder or its water extract can be incorporated in oral rehydrating salt solution (ORS) for increasing its anti-diarrhoeal efficacy [4]. It was observed that the cell culture extract of *H. indicus* had prevented hyper cholesterolemia in rats [5]. *In vitro* cultures of this species might offer an alternative method for production of these important pharmaceuticals, which would reduce the collection pressure on this plant [6]. It is also used to treat nutritional disorders, syphilis, fever, foul odour from the body, bronchitis, piles, rat bite poisoning, epileptic fits in children and 'tridosha' disease of the blood, leukorrea, kapha and vata [7]. *H. indicus* root extract was found to protect microsomal membranes as evident from reduction in lipid peroxidation values. As there is a growing worldwide demand for alternative medicine. The present study was under taken to evaluate the antibacterial activity of root extract of *H. indicus in vitro* against *E. coli*, *B. subtilis* and *P. aeruginosa*

Methods

Collection and processing of the roots of *Hemidesmus indicus*

The fresh plant roots of *H. indicus* were collected from Western Ghat of Karnataka. The material has been processed for extraction. Roots were washed in tap water for 2 to 3 times and then rinsed in distilled water. The clean roots were shade dried for about 15 to 20 days. Further, the roots were finely powdered with the help of blender and stored for further utilization.

Extraction and separation of crude sample

The dried roots were powdered and extracted successively by soaking 100g of plant material in Petroleum ether and Ethyl acetate for 24 hours. After 24 hours the extract was separated by filtration through filter paper. The Petroleum ether extract and Ethyl acetate extract were then concentrated using Rota vapour equipment, under compressed pressure and constant temperature (60°C & 76°C) respectively. The extracted crude drug was subjected to column chromatography using different ratios of Petroleum ether and Ethyl acetate. A column was prepared using silica gel 60-120 mesh. About 2 g of crude extract collected from Rota vapour was loaded to the column. Then different ratios of solvents are prepared. The elutants were collected in a separate tube of 20 ml capacity, about 5 elutants of each percent were collected.

Microbiological assay

Microbiological evaluation technique is employed for screening of antibacterial activities of bioconstituents. The method adopted for this was Agar diffusion assay of [8,9]. Antibiotic namely Red Clox (Amoxicillin and Cloxacillin) was used as a positive control and solvents Chloroform, Petroleum ether, Ethyl acetate, Methanol were used as a control. The pure bacterial cultures were collected from Department of Microbiology, Kuvempu University, Shimoga. Non-chemically defined nutrient agar medium was used for antimicrobial assay. The pure cultures of bacteria on agar medium subcultured in nutrient broth of pH 7.2. The broth was inoculated on Petriplate by spread plate method. The plates are kept in such a manner so as to ensure that no significant growth of any organisms occur before the plate is inoculated with desired inoculum.

The filter paper discs (Whatman No.1) of diameter 6mm were soaked in different ratios of elutants and placed on the inoculated agar plates with gentle pressure. The plates were incubated at 37ºC for 24 hours. The zone of inhibition was recorded, the resultant clear zones around the discs were measured in mm. Clear zones of growth inhibition indicated the anti microbial activities of plant extracts.

Results

The results of the experiments are summarized in Table 1 and 2. The active fractions of extractions of *H. indicus* which are found to possess alkaloid have been tested for their antimicrobial assay. The antibacterial activity of the root extracts of *H. indicus* was assessed by disc diffusion method and the elutant extract exhibited the zone of inhibition on three microorganisms viz., *Escherichia coli*, *Bacillus subtilis*, *and Pseudomonas aeruginosa*. The extracts of *H. indicus* exhibited the maximum zone of inhibition along with 6 mm diameter disc are (60, 49, 40), (64, 51, 36), (68, 53, 51), (70, 53, 52) and (75, 56, 54) in (mm) at 80:20 ratio (Table.1). In the control experiments of antibiotics and solvents, insignificant zone of inhibition was noticed (Table.2). Keeping in view the experimental parameters it is concluded that the elutant-5 recorded more zone of inhibition followed by E_4, E_3,, E_2, and E_1 and *Hemidesmus indicus* root extract recorded highly significant zone of inhibition on *E. coli* (75mm), *B. subtilis* (56mm), and *P. aeruginosa* (54mm) respectively. It is also clear that, *H. indicus* possesses bioactive compounds which inhibit the growth of *E. coli*, *B. subtilis*, *P. aeruginosa* microorganisms under laboratory conditions compared to reference antibiotics.

Table: 1. Antibacterial activity of *H. indicus* on three bacterial species: Zone of inhibition in (mm).

Ratios:- Petroleum ether and Ethyl acetate						
Elutants	**Escherichia coli**					
	100:00	80:20	60:40	40:60	20:80	00:100
01	-	60	56	54	54	50
02	-	64	62	56	50	53
03	-	68	64	59	54	55
04	-	70	65	61	57	55
05	-	75	67	63	60	57
Bacillus subtilis						
Elutants	100:00	80:20	60:40	40:60	20:80	00:100
01	-	49	43	42	40	30
02	-	51	47	41	40	36
03	-	53	50	44	42	35
04	-	53	52	47	44	39
05	-	56	55	55	50	32
Pseudomonas aeruginosa						
Elutants	100:00	80:20	60:40	40:60	20:80	00:100
01	-	40	36	34	30	28
02	-	36	27	26	25	26
03	-	51	29	32	29	29
04	-	52	42	37	31	30
05	-	54	44	40	31	27

- = Absent. Diameter of disc: 6 mm

Table: 2. Bacterial activity for Normal Solvents & Antibiotics used as a control: Zone of inhibition in (mm).

Test organisms	Diameter of zone of inhibition in (mm) Normal Solvents				Concentration of antibiotic added in mg/ml				
	Chloroform	Petroleum ether	Ethyl acetate	Methanol	10mg/ml	8mg/ml	6mg/ml	4mg/ml	2mg/ml
Escherichia coli	2	-	-	-	16	15	13	12	10
Bacillus subtilis	2	-	4	2	14	12	11	10	9
P. aeruginosa	-	-	4	2	14	12	12	11	9

Discussion

As per the results of our investigation we have found that the root extract of the plant *H.indicus* shows good antimicrobial potential Against the three microorganisms viz*., Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa.* Significant zone of inhibition were produced by the different elutents extracted from column. These strong antimicrobial activities can be due to the bioactive compounds present in the roots The ethanolic extract of root is reported to contain triterpenes, Flavonoids, tannins, coumarins and glycosides[10]. The glycoside of *H.indicus* roots inhibited *S. typhimurium* induced pathogenesis non-specifically, by reducing bacterial surface hydrophobicity and perhaps also by mimicking host cell receptors, thereby blocking its attachment to host cell and further pathological effects [11]. The aqueous- ethanolic root extract is also reported to contain alkaloids, saponins, phenols and tannins [12]. One mode of action of plant tannins is to complex with dietary nutrients through hydrogen and hydrophobic effects, as well as by covalent bond formation [13,14].Thus, their mode of antimicrobial effect may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and mineral uptake [14,15,16]. Aqueous extract of roots of the plantexhibited bacteriostatic activity in mice infected with *Mycobacterium leprae*. P- methoxy salicylic aldehyde present in the extract was considered to be responsible for the activity [17]. Cooper (1912) [18] notably described the relationship between phenolics (phenol and meta-cresol) and bacterial proteins as being of importance in their mechanism of disinfection. In particular, it was considered that the protein structure inside the bacterial cell was affected.

Conclusion

Overall results indicate that the *Hemidesmus indicus* root extracts has the potential bioactive components which inhibit the growth of microorganisms significantly compared to reference antibiotics.

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