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Phytopharmacological evaluation of leaf extracts of *Hemidesmus indicus* (L)

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For centuries, indigenous medicinal plants have been used against bacterial-induced pathogenesis. *Hemidesmus indicus* is a pharmacologically important plant. The *in vitro* experiment by the agar well diffusion assay showed the presence of bioactive components in *H. indicus* leaf extract through zone of inhibition. In different ratios of petroleum ether and ethyl acetate test have shown significant zone of inhibition against gram positive and gram negative bacteria: *Bacillus subtilis* (54 mm), *Escherichia coli* (70 mm) and *Pseudomonas aeruginosa* (50 mm).

Key words : Antibacterial activity, *H. indicus*, Medicinal plants, Bioactive components.

INTRODUCTION

Finding healing powers in plants is an ancient idea. Since the advent of antibiotics in the 1950s the use of plant derivatives as antimicrobials has been virtually nonexistent. *Hemidesmus indicus*, commonly called Indian Sarsaparilla a climbing vine found throughout India which belongs to family Asclepiadaceae. *H. indicus* has been used as a folkloric medicine and found to be an ingredient in Ayurvedic and Unani preparations which are usually prescribed against inflammation, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, urinary disorders, loss of appetite, burning sensation, rheumatism and especially for epileptic fits in children (Lakshman *et al*., 2006). Considerable research on pharmacognosy, chemistry, pharmacology, and clinical therapeutics has been carried out on Ayurvedic medicinal plants of India (Patwardhan, 2005). Herbal medicines generally have fewer side effects than synthetic compounds, and their effectiveness can be improved by modern pharmacological methods (Wilasrusmee *et al*., 2002). Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. Worldwide spending on finding new anti infective agents is expected to increase 60% from the spending levels. New sources, especially plant sources, are also being investigated, secondly the public is becoming increasingly aware of the problems with over prescription and misuse of antibiotics. Moreover, plant based medicines being effective and cheaper are responsible for the fast growth of industries of herbal medicines (Rojas *et al*., 1992). In view of the alarming incidence of antibiotic resistance in bacteria and fungi there is a need to identify new antimicrobial formulations (Cowan, 1999).

H. indicus extract is also found to inhibit lipid peroxidation and scavenge hydroxyl radicals *in vitro* (Amirghofran *et al*., 2000). It was observed that the cell culture extract of *H. indicus* had prevented hypercholesterolemia in rats (Bopanna *et al*., 1997). *In vitro* culture of this species might offer an alternative method for production of these important pharmaceuticals which would reduce the collection pressure on this plant (Neeta *et al*., 2005). As there is a growing world wide demand for alternative medicine, in our continuing search for antimicrobial agents from plants, number of secondary metabolites have been characterized as active principles. *P. aeruginosa* and *E. coli* are opportunistic pathogens that cause variety of infections, usually in immune compromised host. The aim of the present study was to screen for the antibacterial property of leaf extracts of *H. indicus in vitro* against *E. coli, B. subtilis* and *P. aeruginosa*.

MATERIALS AND METHODS

Collection and processing of plant material :

The fresh plant leaves of *H. indicus* were collected from Western Ghats of Karnataka. The material has been processed for extraction. Leaves were washed in tap water for two to three times and then rinsed in distilled water. The clean leaves were shade dried for 15-20 days. Further, the leaves were finely powdered with the help of blender and stored for further utilization.

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Extraction and separation of crude sample:
The dried leaves were powdered and extracted successively by soaking 100g of plant material in petroleum ether and ethyl acetate for 24 hours. The extract was separated by filtration through filter paper. The petroleum ether extract and ethyl acetate extracts were then concentrated using Rota vapor equipment, under compressed pressure and constant temperature 60ºC and 76°C, respectively. The crude extract 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 2g of crude extract was collected from Rota vapour and the same was loaded to the column for separation. Then different ratios of solvents are prepared. The elutants were collected in a separate tube of 20 ml capacity, about 5 elutants of each per cent were collected.

Screening for antimicrobial activity:
Microbial evaluation technique was employed for screening the antibacterial activities of bioactive components. The method adopted for this was agar well diffusion assay. Antibiotics namely Amoxycillin and Cloxacillin was used as a positive control and solvents chloroform, petroleum ether, ethyl acetate, methanol were used as control. Non-chemically defined nutrient agar medium was used for antibacterial activity. The bacteria on the agar slant were subcultured in nutrient broth at pH 7.2 and an aliquot of the broth was transferred to the Petriplate and spread on the nutrient agar media.

The filter paper discs (Whatman No.1) of 6mm diameter were moistened thoroughly 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 resultant clear zone around the discs were measured in mm. Clear zones of growth inhibition indicated the antimicrobial activities of plant extracts.

RESULTS AND DISCUSSION

Phytochemicals usually exhibit less antimicrobial activity than agents derived from microbial sources. This is likely due to the multichemical approach of plants defense, which utilizes various compounds with moderate antimicrobial activity. Thus, Phytochemicals have often been overlooked as agents for antimicrobial therapy. Many microorganisms which can disturb the human health, exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the discovery of new effective drugs from natural resources, including medicinal plants. In the present work, the antimicrobial activity of the leaf extracts of *H. indicus* was evaluated by agar well diffusion assay method. The results of the experiments are summarized in the Table 1 and 2. The active fractions of extractions of *H. indicus* which are found to possesses alkaloids have been tested for their antibacterial activity. The elutant extracts exhibited the significant 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 6mm diameter disc are (55, 47, 38), (60, 50, 32), (63, 51, 50), (66, 51, 49) and (70, 54, 50) in (mm) at 80:20 (Table 1).

In the control experiments of antibiotics and solvents, insignificant zone of inhibition was noticed (Table 2). Concerning the experimental parameters it is concluded that the leaf extract of *H. indicus* showed substantial effect and maximum zone of inhibition on *E. coli* (70 mm), *B. subtilis* (54 mm) and *P. aeruginosa* (50 mm), respectively. Elutant-5 (E_5) recorded more zone of inhibition followed by E_4, E_3, E_2 and E_1. It is also clear that leaf extracts of *H. indicus* possesses bioactive components which inhibit the growth of *E. coli, B. subtilis* and *P. aeruginosa* microorganisms under *in vitro* conditions compared to standard antibiotics.

Table 1 : Antibacterial activity of the leaf extracts of *H. indicus* on three bacterial species: zone of inhibition in (mm)

Elutant	Petroleum ether and Ethyl acetae																	
	Escherichia coli						*Bacillus subtilis*						*Pseudomonas aeruginosa*					
	100:00	80:20	60:40	40:60	20:80	00:100	100:00	80:20	60:40	40:60	20:80	00:100	100:00	80:20	60:40	40:60	20:80	00:100
01	-	++	++	++	++	+	-	+	+	+	+	+	-	+	+	+	+	+
02	-	++	+++	++	+	+	-	+	+	+	+	+	-	+	+	+	+	+
03	-	+++	+++	++	++	++	-	++	+	+	+	+	-	+	+	+	+	+
04	-	+++	+++	++	++	++	-	++	++	+	+	+	-	+	+	+	+	+
05	-	+++	+++	++	++	++	-	++	++	++	+	+	-	+	+	+	+	+

+=> 20-50; ++=> 50-60; +++=> 60-80; ++++=> 80-100
- = Absent Diameter of disc: 6 mm

Table 2 : Bacterial activity for normal solvents and 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	10	8	6	4	2
Escherichia coli	01	01	01	01	13	14	11	10	09
Bacillus subtilis	01	01	03	01	13	11	12	09	08
P. aeruginosa	01	-	03	01	12	11	10	10	07

- = Absent Diameter of disc: 6 mm

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