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Antimicrobial Activity of *Hemidesmus indicus* var. *indicus* R.Br. Against Human Isolates of *Helicobacter pylori*

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Abstract – *Hemidesmus indicus* var. *indicus* belonging to the family Asclepiadaceae was screened for its activity against *Helicobacter pylori* (Hp) human isolates. Flowering and vegetative period samples were analysed. Aqueous (hot and cold) and solvent extracts (acetone, chloroform and methanol) were screened. Among them chloroform extract was observed to recover bioactive principles with low MIC and MLC. MIC was 75 μg in both seasons and MLC was 100 μg for vegetative and 75 μg for flowering periods respectively. Extracts from samples collected during flowering period was better than that of vegetative period.

Keywords – Hp, Helicobactericidal, Hemidesmus, Asclepiadaceae, PUD, ulcer.

Introduction

Pharmacological suppression of gastric acid has been a most common approach on healing ulcer (Bardhan, 1989; Carlsson, 1989). Ulcers initially treated with antisecretory drugs, viz., H_2 receptor antagonist and proton pump inhibitors have a tendency of relapse after withdrawal of treatment. The discovery of *Helicobacter pylori* (Hp) (Coghlan, *et al.*, 1987; Drumm, *et al.*, 1990) lead to a better understanding of gastric physiology, etiology of peptic ulcer disease (PUD) and its treatment. Various therapeutic regimens for eradication of Hp were advocated but lacked consistency and did not offer advantageous in terms of side effects. So, in order to find a new anti *H. pylori* principle, a plant used for ulcer treatment was tried for its helicobactericidal effect. *H. indicus* var. *indicus* (HI) is one such plant used for treating ulcers (Anoop[a] *et al.*, 2002; Anoop[b] and Jegadeesan, 2002; Anoop[a] and Jegadeesan, 2003) and was tried for its helicobactericidal effect. The plant was collected during vegetative and flowering periods in order to ascertain the consistency in their efficacy during various seasons.

Materials and Methods

HI was collected from Coutrallam, Tirunelveli district, Tamilnadu, India and identified at Botanical Survey of India, Coimbatore. A voucher specimen is being deposited in the department herbarium for future reference (TU No. 186). Two seasonal samples were collected ie., flowering (October-January) and vegetative season (May-June) (Gamble, 1967). They were shade dried and pulverized in a stone mortar and filtered in a sieve (40 mm) and stored in an airtight container. This was used as a basic sample in this study. Aqueous extracts (hot and cold) and solvent extracts (acetone, chloroform and methanol) are prepared according to standard methods (Anonymous, 1980) by soxlet apparatus and subjected for screening against Hp study. Hot aqueous extract yield 32,53%, cold extract yield 29.50%, Benzene yield 16.26%, acetone yield 14.87% and methanol extract yield: 5.00%, methanolic extract and aqueous extracts were was dark brown colour and others were brown in colour and sticky in nature. Alkaloids, tannins, phytosterols and saponins were positive for aqueous and methanolic extracts (Anoop[a] *et al*, 2002).

The human isolated of Hp was collected from the gastric antral biopsy specimens at the site of active lesions with the help of sterile (2% gluteraldehyde) endoscopic forceps. It was transported to the laboratory in Brain Heart Infusion (Anonymous, 1998) soft agar tubes. The biopsy specimen was transferred to modified BHI agar plates (Calf brain infusion-200 g/l; Beef heart infusion-250 g/l; Proteose peptone-10 g/l; Dextrose-2 g/l; NaCl-5 g/l; Disodium phosphate-2.5 g/l; Agar-15 g/l; Triphenyl tetrazolium chloride-40 mg/l; Cefatoxime-10 mg/ml; Defibrinated sheep blood-50 ml; Final

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pH-7.4±0.2) and incubated at 37°C under microaerophilic conditions for 72-96 h. The bacterial outgrowth from the biopsy specimen was characterised on the basis of culture, microscopic characteristics, biochemical and physiological properties. Hp human isolates were stored in modified BHI agar slants at 4°C and used for this study.

Standard, pre-sterilised filter discs were obtained from Hi-media, Mumbai and various extracts of the medicinal plant were incorporated aseptically in them at a concentration of 5 µl/disc. Simultaneously, broth culture of human Hp isolates was seeded on air-dried sterile Muller-Hinton agar plates (Bauer, *et al.*, 1966) using a sterile cotton swab.

The crude plant extract impregnated filter discs (5 µl/disc) were placed on the Hp inoculated plates with the help of flame-sterilized forceps and pressed gently. These plates were incubated at 37°C under micro-aerophilic condition for 48-72 h and the zone of inhibition was recorded (Bauer, *et al.*, 1966).

Minimal inhibitory concentration (MIC) and Minimal lethal concentration (MLC) were determined as per standard methods (Presscot, *et al.*, 1996). For comparison, sterile filter discs with known concentration of antibiotics were obtained (Anonymous, 1998), namely Amikacin, kanamycin, vancomycin, methicillin, ceftazidime, netillin, tobramycin, chloromphenicol and tetracycline at 30 µg concentration; streptomycin, gentamycin, ampicillin, amoxycillin and norfloxacin at 10 µg concentration; cotrimoxazole at 25 µg concentration, nitrofurantoin at 300 µg concentration and ciprofloxacin at 5 µg concentration were used as standard antibacterials against *H. pylori*. The zone of inhibition for these antibiotics is given in Table 2.

Results

HI was found to a promising drug with antiHp activity. Among them, acetone and chloroform extracts were found to exhibit notable antiHp activities (Table 1). No significant difference was observed in antiHp activity among seasonal samples. From this table it is evident that chloroform extract is having a bioactive principle effective against the organism.

Table I. Helicobactericidal activity of *Hemidesmus indicus* var. *indicus* root extracts

Extracts	Zone of inhibition (mm)	
	Vegetative period	Flowering period
Cold aqueous extract	–	–
Hot aqueous extract	–	–
Acetone extract	1	2
Chloroform extract	8	11
Ethanol extract	–	–

– No zone of inhibition

So chloroform extract was further subjected for MIC and MLC against Hp. The MIC was 75 µg in both seasonal samples, but for MLC it was 100 µg for that collected during vegetative period and 75 µg for that collected during flowering period. Table II categorically demonstrates the emergence of multiple drug resistance in Hp, which also exhibited resistance to a wide class of bacterials, which includes pencillins, aminoglycosides with relatively low sensitivity to cephalsporins, such as ceftazimide.

Discussion

Hp (earlier known as *Camphylobacter pylori*) is spiral bacilli implicated recently as a causative agent in various PUD. This has brought a diabolic change in PUD management. In view of the inherent cost, side effect profiles and the risk of drug resistance, a search for therapeutic alternative for antibacterials in Hp eradication is on. *H. indicus* a medicinal plant, which is traditionally used to treat PUD, was screened for antiHp activity.

HI is one of the widely used medicinal plant, owing to its abundant occurrence and traditional usage. Ethanolic extracts has been evaluated for its antimicrobial and toxicity experiments (Dhar, *et al.*, 1968; Anoop[b] and Jegadeesan, 2003). Antibacterial activity of various extracts of *H. indicus* was evaluated and reported with differential observations (Naovi, *et al.*, 1991; Namba, *et al.*, 1985). Anoop[c] and Jegadeesan (2002) have reported the cytoprotective effect

Table 2. Antibiotic susceptibility pattern of human isolates of *Helicobacter pylori*

Antibiotics tested	Symbol	Concentration (µg/disc)	Zone of inhibition (mm)	R/S pattern
Amikacin	Ak	30	18	S
Kanamycin	K	30	–	R
Vancomycin	Va	30	–	R
Methicillin	M	30	–	R
Ceftazidime	Ca	30	14	R
Netillin	Nt	30	14	R
Tobramycin	Tb	30	23	S
Chloromphenicol	C	30	–	R
Tetracycline	T	30	12	R
Streptomycin	S	10	12	I
Gentamycin	G	10	16	S
Ampicillin	A	10	–	R
Amoxycillin	Am	10	1	R
Norfloxacin	Nx	10	31	S
Cotrimoxazole	Co	25	13	I
Nitrofurantoin	Nf	300	–	R
Ciprofloxacin	Cf	5	38	S

– : No zone.
R : Resistant.
S : Sensitive.
I : Intermediate.

of (50%) ethanolic extract in PUD. Chloroform extract was found to exhibit significant antiHp activity which is comparable with that of conventional therapeutic, which is used in the eradication of Hp. The triterpenoid or saponins fraction might be the active principle responsible for the action which is also the responsible for its antiulcer activity (Anoop[b] and Jegadeesan, 2003).

Since a multi-drug regimen is required in the eradication of Hp nowadays, this plant is a promising drug and it has to be further evaluated. Structural and compound isolation of this drug are being carried out in our laboratory to have a better insight on this drug. Varieties are also occurring in this plant namely var. *pubescens* (Anoop[c] and Jegadeesan, 2003), which is also being screened for its probable common active constituents in our laboratory.

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