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IN-VITRO SCREENING OF *CISSUS QUADRANGULARIS* L. VARIANT II AGAINST *HELICOBACTER PYLORI*

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ABSTRACT : *Cissus quadrangularis L. variant II belonging to the family Vitaceae was screened for its activity Hellcobacter pylori (Hp) human isolates. Flowering and vegetative period samples were analyzed. Aqueous (hot and cold) and solvent extracts (acetone, chloroform and methanol) were screened. Among them chloroform was observed to recover bioactive principles with low MIC and MLC. MIC and MLC was 40 µg/ml for flowering period. Whereas for vegetative period MIC was 40 µg/ml and MLC was 40 µg/ml respectively. Extracts from samples collected during flowering period were better than that of vegetative period. The results confirm the traditional use of the plant in PUD.*

KEYWORDS: *Cissus quadrangularis, PUD, ulcer, Helicobacter pylori, Vitaceae.*

INTRODUCTION

Helicobacter pylori (Hp) is an important etiological factor in chronic gastritis and peptic ulcer diseases[1]. Drugs of Hp eradication are available but are costly and have documented side effects[2]. Plants are not reported for their helicobacericidal activity yet. So, in this view, a plant used for treating ulcer traditionally viz., *Cissus quadrangularis*[3,4] was screened for its helicobatericidal activity. Three morphovariants of this plant viz., square-stemmed, round-stemmed and flat-stemmed are available and differentiated as variant I, II and III respectively. Variant I is a common plant widely distributed in hotter parts of India widely used and studied for gastric problems[5, 6]. The round-stemmed variety (variant II) is not much studied[7], which is also considered to be a plant for gastric ulcer. The antiulcer potential of this plant has been carried out pharmacologically[8]. Hence, microbiological screening for its helicobactericidal activity was carried out.

MATERIALS AND METHODS

C.quadrangularis L. variant II (Vitaceae) was identified according to standard methods[9]. A voucher specimen has been deposited in the department for future reference (TU 190). *C.quadrangularis* variant II was collected form the herbal garden of Tamil University, Thanjavur, Tamilnadu, India. Two seasonal samples were collected i.e., flowering in the month of April (2000) and vegetative during October (2000) with a difference of six

months between the periods of sample collection[9]. Plant samples were shade dried, pulverized, filtered (40 mm mesh) and stored. Aqueous extracts (hot and cold) and solvent extracts (Acetone, Chloroform and Ethanol) were prepared according to standard methods[10].

Human isolates of *H.pylori* were collected from gastic 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[11] (BHI) soft agar tubes. The biopsy specimen was transferred to modified BHI agar (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; Disadium 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 pH – 7.4 ± 0.2) plates and incubated at 37^0C under microaerophilic conditions for 72 – 96 h. Bacterial outgrowths from the biopsy specimen were characterized on the basis of culture, microscopic characteristics, biochemical and physiological properties. *H.pylori* was stored in modified BHI agar slants at 4^0C and used for this study.

Extracts were incorporated aseptically in Standard, pre-sterilized filter discs[12] at a concentration of 5µl / disc. Simultaneously, broth culture of human *H.pylori* isolates was seeded on air-dried sterile Muller-Hinton agar plates[12] using a sterile cotton swab. Filter discs were placed on the *H.pylori* inoculated plates with the help of flame sterilized forceps and pressed gently. Plates were incubated at 37^0C under micro-aerophilic condition for 48-72 h and zone of inhibition was recorded[13].

Minimal inhibitory concentration (MIC) and determined as per standard methods[11]. For comparison, sterile filter discs with known concentration of antibiotics were obtained[10] from Hi-media and used as standard antibacterial against Hp.

RESULTS

The zone of inhibition is given in table 1. Marginal inhibitory activity was observed in cold and hot aqueous extracts. Among the samples, plant materials drawn during flowering season is found to possess slightly higher quality of inhibitory principles. On the basis of MIC and MLC of variant II is observed to be potent against *H.pylori.* Chloroform extract of samples collected during flowering period showed both MIC and MLC at 40µg/ml, whereas for that during vegetative period the MIC was 40µg/ml and MLC was 45µg/ml.

Table II categorically demonstrates the emergence of multiple drug resistance in *H.pylori*. In this the human *H.pylori* isolate has exhibited resistance to a wide class of bacteria's, which includes pencillins, aminoglycosides with relatively low sensitivity to cephalsporins, such as ceftazimide.

DISCUSSION

Microbiological etiology of peptic ulcer was vague for a very long time. Only in recent past the etiological correlation of Hp (earlier known as *Campylobacter pyiori)*[14] was proved which has brought about a change in PUD management[15]. In view of its inherent cost, patient compliance, side effect profiles and risk of drug resistant mutants, a search for therapeutic alternative for Hp eradication is on. In this background, medicinal plant, which is traditionally used to treat PUD was screened against Hp.

Chloroform extracts were observed to be potent against Hp with relatively low MIC and MLC. The terpenoidal fraction might posses the helicobactericidal activity. Even though this is a promising candidate for eradication of Hp, its lower occurrence is indeed an inhibitory factor for its medicinal use in PUD[4,16]. Pharmacological studies were carried out previously on the cytoprotective activity17 which was found to increase potassium ion concentrations. This plays an important role in protecting the gastric and duodenal mucosa. This result also support our finding wherein the increase in bicarbonates helps in building up of mucosal barrier against Hp. Further characterization, structural elucidation and *in-situ* clinical evaluation on human volunteers will open a new view for the traditional usage, which would implement the therapeutic strategies for the eradication of the harboring bacteria of ulcerated site in the gastric tract.

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Table I : Helicobactericidal activity of *Cissus quadrangularis L.* variant II

Extracts	Zone of inhibition (mm)	
	Vegetative period	Flowering period
Control	-	-
Cold extract	Trace	Trace
Hot extract	-	Trace
Acetone extract	1	3
Chloroform extract	15	16
Ethanol extract	Trace	Trace

Zone of inhibition is given in m.m.
- - No zone of inhibition
Results are mean of 4 values

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

Antibiotics tested	Concentration (ug/disc)	Zone of inhibition (mm)	R/S pattern
Ciprofloxacin	5	38	S
Amoxycillin	10	1	R
Ampicillin	10	-	R
Gentamycin	10	16	S
Norfloxacin	10	31	S
Streptomycin	10	12	I
Cotrimoxazole	25	13	I
Amikacin	30	18	S
Ceftazidime	30	14	R
Chloromphenicol	30	-	R
Kanamycin	30	-	R
Methicillin	30	-	R
Netillin	30	14	R
Tetracycline	30	12	R
Tobramycin	30	23	S
Vancomycin	30	-	R
Nitrofurantoin	300	-	R