

Helicobactericidal Activity of *Cissus quadrangularis* L. Variant I

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Abstract – *Cissus quadrangularis* L. variant I (Family: Vitaceae), the common variant with square stem is widely used for peptic ulcer disorders (PUD) in traditional medicine. Aerial parts were collected during flowering and vegetative seasons and analysed. Aqueous (hot and cold) and solvent extracts (acetone, chloroform and ethanol) were screened for their anti-*Helicobacter pylori* (Hp) activities. Among them chloroform extract was observed to recover bioactive principles markedly with low minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC). MIC was 30 µg in both samples and MLC was 35 µg for vegetative and 30 µg for flowering seasons, respectively. Extracts from samples collected during flowering season were better than those of vegetative season.

Keywords – *Cissus quadrangularis*, peptic ulcer disorders, ulcer, helicobactericidal, Vitaceae, *Helicobacter pylori*

Introduction

Helicobacter pylori (Hp) is an important etiological factor in chronic gastritis and peptic ulcer diseases (Graham *et al.*, 1989). Drugs for Hp eradication are available but costly and have documented side effects (Rauws and Tytgat, 1990). Little work has been carried out on the helicobactericidal activity of medicinal plants. So, in this view, a plant used for ulcer treatment traditionally was selected and screened for its helicobactericidal activity. *Cissus quadrangularis* is one such plant, which is considered to be a plant for gastric ulcer (Anoop Austin and Jegadeesan, 2002). Three morpho-variants of this plant viz., square-stemmed, round-stemmed and flat-stemmed are available and differentiated as variant I, II and III repetitively (Kannan and Jegadeesan, 1999). The most commonly available is variant I with square-stem. So, it was studied for its helicobactericidal activity *in vitro*. The plant was collected during vegetative and flowering seasons in order to ascertain the consistency in their efficacy during various seasons.

Materials and Methods

C. quadrangularis L. variant I (CQ); family Vitaceae,

selected for this study was identified according to standard methods (Gamble, 1967). A voucher specimen is deposited in the department herbarium for future reference (TUH No. 189). CQ was collected from Vallanad Hills from Tirunelveli District, Tamilnadu, India. Two seasonal samples were collected i.e., flowering at the month of November 2000 and vegetative at the month of May 2000. The aerial parts collected samples 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 ethanol) are prepared (Anonymous, 1966) by soxlet apparatus and subjected for screening against Hp. Hot aqueous extract yield 36.6%, cold extract yield 11.8%, Benzene yield 1.87%, acetone yield 0.37%, chloroform yield 2.07% and methanol yield 3.86%. Methanolic and aqueous extracts were brown in colour and oily in nature and others were green in colour and sticky in nature. Alkaloids, tannins, gums and mucilage and saponins were present in methanolic and aqueous extracts. Alkaloids were present in chloroform extract (Anoop Austin *et al.*, 2004a). The human isolate of Hp was collected from the gastric antral biopsy specimens at the site of active lesions with the help of sterile (2% glutaraldehyde) endoscopic forceps. It was transported to the laboratory in Brain Heart Infusion (BHI) soft agar tubes (Anonymous, 1998). The biopsy

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specimen was transferred to modified BHI agar plates as per our earlier studies (Anoop Austin *et al.*, 2003) 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 isolated 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 (Anonymous, 1998) and various extracts of the medicinal plant were incorporated aseptically in them at a concentration of 5 µg/disc. Simultaneously, broth culture of human Hp isolates was seeded on air-dried sterile Muller-Hinton agar plates (Anonymous, 1998) using a sterile cotton swab. The crude plant extract impregnated filter discs (5 µg/disc) were placed on the Hp inoculated plates with the help of flame-sterilised 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) of the crude plant drug preparations 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, chloramphenicol and tetracycline at 25 µg concentration; streptomycin, gentamycin, ampicillin 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 Hp. The zone of inhibition of these antibiotics are given in Table 2.

Results

Extracts of CQ exhibited slightly different anti-Hp activity profile (Table 1). Marginal anti-Hp activity was observed even in cold and hot aqueous extracts. Among the samples, plant materials drawn during flowering seasons seem to pose slightly higher quality of anti-Hp principles. Chloroform extract having bioactive principles effective against Hp was further subjected for MIC and MLC against Hp. The MIC at 30 µg/ml for both seasonal samples, whereas MLC was 35 and 30 µg/ml for vegetative and flowering periods. Table 2 categorically demonstrates the emergence of multiple drug resistance in Hp against a wide class of bacterials, which includes penicillins, aminoglycosides with relatively low sensitivity to cephalosporins,

Table 1. Helicobactericidal activity of *Cissus quadrangularis* L. variant I

Extracts	Zone of inhibition (mm)	
	Vegetative season	Flowering season
Control	Nil*	Nil*
Cold aqueous extract	< 1	< 1
Hot aqueous extract	Nil*	< 1
Acetone extract	3	1
Chloroform extract	15	16
Methanol extract	< 1	< 1

*Nil - No zone of inhibition Results are mean of 4 values

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

Antibiotics tested	Concentration (µg/disc)	Zone of inhibition (mm)	R/S pattern
Amikacin	30	18	S ^a
Kanamycin	30	0	R ^b
Vancomycin	30	0	R
Methicillin	30	0	R
Ceftazidime	30	14	R
Netillin	30	14	R
Tobramycin	30	23	S
Chloramphenicol	30	0	R
Tetracycline	30	12	R
Streptomycin	10	12	I ^c
Gentamycin	10	16	S
Ampicillin	10	0	R
Amoxycillin	10	1	R
Norfloxacin	10	31	S
Cotrimoxazole	25	13	I
Nitrofurantoin	300	0	R
Ciprofloxacin	5	38	S

^aS - Sensitive, ^bR - Resistant, ^cI - Intermediate

such as ceftazidime.

Discussion

Microbiological etiology of peptic ulcer was vague for very long time. Only in the recent past the etiological correlation of Hp (earlier known as *Campylobacter pylori*) was proved which has brought about a change in PUD management (Graham *et al.*, 1989). In view of the inherent cost, patient compliance, side effect profiles and the risk of drug resistant mutants (Rauws and Tytgat, 1990), a search for therapeutic alternative for *H. pylori* eradication is on. In this background, a medicinal plant used traditionally to treat PUD was screened for anti-HP

activity. CQ is a medicinal plant used therapeutically for PUD traditionally, which is also possessing anti-HP activities. Chloroform extract was observed to have potent anti-Hp activity with relatively low MIC and MLC. Antimicrobial activity of various extracts of CQ has been evaluated and reported with various extractions (Dhawan *et al.*, 1977; Abdel-Aziz *et al.*, 1990; Sivaswamy *et al.*, 1991). Anoop Austin and Jegadeesan (2004) has reported that antiulcer activity is due to its selectively inhibition of prostaglandin and on the other hand, the safety is also been established (Anoop Austin and Jegadeesan, 2002a).

Results observed in CQ are effective when compared with other variant, viz., *C. quadrangularis* variant II (Anoop Austin and Jegadeesan, 2002b, 2002c) which was having remarkably a high range of MIC and MLC (Anoop Austin *et al.*, 2004b). Flowering samples of variant II showed both MIC and MLC at 40 µg, whereas for that during vegetative sample MIC was 40 µg and MLC 45 µg. The additional activity of CQ as an antiulcer agent apart from its helico-bactericidal activity enhances the therapeutic effect of the drug in treating PUD. Nowadays multi drug regimens are required for eradication of Hp. Hence this plant is a promising drug, which has to be further evaluated. The results also show the bactericidal activity of CQ against *H. pylori* (Hp) is at par to many well-known antibiotics except Tobramycin, Norfloxacin and Ciprofloxacin. Characterization and isolation of the compound will throw more information on this plant in the future.

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