International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 2 (6): 674-677, 2006
ISSN 1811-7775

In vitro Antibacterial Screening of *Anethum graveolens* L. Fruit, *Cichorium intybus* L. Leaf, *Plantago ovata* L. Seed Husk and *Polygonum viviparum* L. Root Extracts Against *Helibacter pylori*

[1]Rifat-uz-Zaman, [2]Muhammad Shoaib Akhtar and [1]Muhammad Shafiq Khan
[1]Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[2]Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan

Abstract: In the present study, extracts from four folkloric medicinal plants, previously reported potential anti-ulcer; *Anethum graveolens* L. fruit, (AG), *Cichorium intybus* L. leaf, (CI), *Plantago ovata* L. seed husk (PO) and *Polygonum viviparum*, L. root, (PV), were screened for anti-*Helicobacter pylori* (HP) activity. Anti-HP activity of the powdered drugs extracted by water, ethanol, ethyl acetate and acetone, obtaining yields of 2.34-13.43 g kg^{-1} (w/w), by using the turbidity method was determined. The aqueous extract exhibited the lowest minimum inhibitory concentrations (MICs) against *H. pylori* strain (obtained from ulcer patients), of which ranged from 0.6 to 10.5 mg mL^{-1}, followed, in ascending order, by the *Cichorium intybus* leaf (CI), *Polygonum viviparum* root (PV), *Anethum graveolens* fruit (AG) and *Plantago ovata* seed husk (PO). Antibacterial activity was determined CI, PV, PO, AG extracts, with the highest percentage of inhibition (1.2-62.6%) demonstrated for the water, followed, in descending order, by the ethanol, ethylacetate and acetone analogs. Anti-HP activity appeared to be in a dose-dependent manner. The extracts (aqueous and ethanol) of CI and PV due to potent and AG and PO due to the moderate potent anti-*H. pylori* activities might ultimately be proved, the preferred and curative anti-ulcer agent.

Key words: Anti-bacterial, anti-*H. pylori*, *Anethum graveolens*, *Cichorium intybus*, *Plantago ovata*, *Polygonum viviparum*

INTRODUCTION

There is overwhelming and convincing evidence that *Helicobacter pylori* (HP) is of major importance in the pathogenesis of gastric and duodenal ulcers. In addition, infection has been linked to the development of chronic active gastritis, gastric carcinoma, mucosa-associated lymphoid tissue (MALT) lymphoma and possibly non-ulcer dyspepsia (NUD). Eradication of HP is a challenge because of the unique niche it occupies in the gastric mucous layer of the stomach (Podolski, 1996; Joette *et al*., 1999; Czesnikiewicz-Guzik *et al*., 2004; Wang and Huang, 2005a). Approximately 50% of the world's population is believed to be infected with HP. In western medicine, a 3-fold therapeutic regimen, emphasizing the use of antibiotics, is typically used to suppress HP activity (Podolski, 1996; Joette *et al*., 1999; Czesnikiewicz-Guzik *et al*., 2004).

To date, there has been no consensus on the optimum treatment regimen for *H. pylori* (HP) infection (Joette *et al*., 1999). Antimicrobial(s)/antibiotic(s) such as Metronidazole, Tetracycline, Clarithromycin and Amoxicillin, have been used in appropriate dosage to eradicate/kill the bacteria (Manes *et al*., 1998; Malfertheiner *et al*., 2000; Hopkins *et al*., 2002). In spite of the current natural and/or synthetic antimicrobial/ antibiotic agents, the occurrence of side/adverse effects and resistance against HP-infection even after long-term therapy has been reported (Choudhary *et al*., 2001; Rifat-uz-Zaman *et al*., 2002). Therefore, the infection with HP has continued to be the big therapeutic challenge to the microbiologists/pharmacologists.

Earlier we reported the preliminary anti-ulcer efficacies of few highly reputed and quite frequently used medicinal plants in the traditional medicines; *Plantago ovata* seed husk (Rifat-uz-Zaman *et al*., 2002), *Anethum graveolens* fruit (Rifat-uz-Zaman *et al*., 2004), *Cichorium intybus* leaf (Rifat-uz-Zaman *et al*., 2006) and *Polygonum viviparum* root (Rifat-uz-Zaman *et al*., 2005). The present study was undertaken to search for the effective anti-ulcer tool(s) which can eradicate HP infection. Therefore, the extracts; aqueous, ethanol, ethylacetate and acetone, of above mentioned plant drugs were tested for the possible anti-HP activity. So, to serve the ailing humanity and to search the complete cure of the peptic ulcer disease by the use of the medicinal plants (natural herbal wealth) the present project was planned.

Corresponding Author: Rifat-uz-Zaman, Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

MATERIALS AND METHODS

Plant drugs: *Anethum graveolens* fruit (AG), *Cichorium intybus* leaf (CI), *Plantago ovata* seed husk (PO) and *Polygonum viviparum* root (PV) were purchased locally from herbal dealers in Bahawalpur-Pakistan. All the plant drugs were authenticated and compared with their respective standards in the herbarium maintained by Department of Botany, University of Agriculture, Faisalabad and their samples (AG-102, CI-107, PO-112 and PV-103, respectively) were preserved in the Pharmacognosy laboratory, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

The study was conducted during May 2005-April 2006 at Department of Pharmacy, The Islamia University of Bahawalpur.

Extraction of plant drugs: The shade-dried plant materials were finely powdered to the mesh size 200 before their experimental use (Habtemariam, 1998). Aqueous extract of AG (12.65 g kg^{-1}), CI (10.47 g kg^{-1}) and PV (10.12 g kg^{-1}) were prepared by maceration; 1.0 kg of the respective powder was soaked in about 1.5 L of distilled water, separately for 24 h with occasional shaking. The extracts were decanted; remaining materials were re-extracted in the distilled water twice, similarly. The respective combined extracts were dried completely by Rotavapour at 37°C. While 100 g of PO was soaked in 1.0 L of distilled water to make its aqueous extract (08.76 g kg^{-1}) by above mentioned method. The ethanol extract of AG (13.43 g kg^{-1}), CI (12.11 g kg^{-1}), PV (10.85 g kg^{-1}) and PO (05.36 g kg^{-1}), ethylacetate extract of AG (11.74 g kg^{-1}), CI (09.84 g kg^{-1}), PV (4.51 g kg^{-1}) and PO (08.43 g kg^{-1}) and acetone extract of AG (02.34 g kg^{-1}), CI (10.62 g kg^{-1}), PV (11.73 g kg^{-1}) and PO (10.25 g kg^{-1}) were also prepared by the same method (Wang and Huang, 2005b).

Inoculant: HP bacterial strain was isolated from biopsy specimens of *H. pylori*-positive patients (human) with gastric ulcer (antrum and body) (Khulusi *et al.*, 1996; Bermejo *et al.*, 2003). The organism was identified and characterized by morphological features and by chemical methods (Gardazi Clinical Laboratory, Bahawalpur-Pakistan). The organism was identified as *H. pylori* by Gram's stain appearance and a positive urease test. The isolates were held frozen at -70°C in skim milk (Remel, Lenexa, KS, USA) and 17% glycerol (Fisher Scientific, Fairlawn, NJ, USA), sub-cultured once on to 5% sheep blood agar plates (Remel) and incubated at 37°C in 10% CO_2 for 4 days. Subculture was repeated once to ensure reliable growth (Fabry *et al.*, 1995; Mukherjee *et al.*, 1997; Joette *et al.*, 1999).

Chemicals: All chemicals used, were of analytical grade and were obtained from Becton Dickinson, E. Merck (Darmstadt, FRG), BDH Poole (England), Sigma Chemical Co. (USA) and Oxoid Limited, USA. Amoxicillin, a reference antibiotic was obtained from SmithKline Beecham, P.I.C, Brantford, England. Clarithromycin, another reference antibiotic was taken from Abbott Laboratories (Pakistan) Ltd., Landhi, Karachi, Pakistan.

Determination of anti-*Helicobacter pylori* activities: Anti-HP (*in vitro)* activity of the extracts of AG, CI, PO and PV powders (10 mg mL^{-1}), Amoxicillin and Clarithromycin (10 mg mL^{-1} each) in sterile N, N-dimethyl formamide (DMF), were tested according to the method of Beil *et al.* (1995).

The inocula was prepared by suspending organism in sterile brain heart infusion broth (Oxoid Limited, USA) supplemented by 7.5% heat inactivated calf serum and adjusting the turbidity to that of a 2.0 McFarland standard (10^6 cfu mL^{-1} by prior colony count of a representative strain). The test extracts, Amoxicillin, Clarithromycin and sterile DMF were added to 5 mL of broth, separately and incubated under micro-aerobic conditions (5% O_2, 15% CO_2 and 80% N_2) at 37°C for 72 h. The turbidity of brain heart infusion broths under trial were read at 600 nm against the blank (Beil *et al.*, 1995).

MICs were determined for the experimental drugs and MIC was defined as the lowest concentration of test agent at which no visible growth or only a faint haze occurred (National Committee for Clinical Laboratory Standards, 1997). All procedures were performed in duplicate.

The results showed by the test extracts were compared with the *in vitro* anti-HP activities of amoxicillin and clarithromycin (Mukherjee *et al.*, 1997; Couladis *et al.*, 2000).

RESULTS AND DISCUSSION

The MICs of aqueous and ethanol extracts of AG were 9.7 and 9.6 mg mL^{-1}, respectively. Aqueous and ethanol extracts of CI showed 0.6 and 12.3 mg mL^{-1}, PV; 1.4, 4.9 mg mL^{-1}, PO; 10.5, 10 mg mL^{-1} MICs, respectively. Ethylacetate and acetone extracts of plant drugs under test did not show any significant anti-HP activity (Table 1 and 2).

The stomach has been reported to be a sterile organ and without lymphoid tissues. But the gastric infection caused by *Helicobacter pylori* (HP) has been observed to lead an important inflammatory response, gastritis. Two types of topographic evolution of the gastritis have been shown: antral gastritis which can lead to ulcer disease and pangastritis which can lead to gastric carcinoma

Table 1: Anti-bacterial activity (*In vitro*) of aqueous, ethanol, ethylacetate and acetone extracts of *Anethum graveolens* fruit, *Cichorium intybus* leaf, *Polygonum viviparum* root and *Plantago ovata* seed husk, Amoxicillin, Clarithromycin against *Helicobacter pylori*

	% age of inhibition of *H. pylori* growth			
Plant drugs tested	Aqueous extracts	Ethanol extracts	Ethylacetate extracts	Acetone extracts
Anethum graveolens fruit	12.2	9.5	4.7	2.3
Cichorium intybns leaf	62.6	19.2	4.1	4.2
Polygonum viviparum root	61.2	23.9	3.4	4.1
Plantago ovata seed husk	21.2	1.9	1.4	1.2
Amoxicillin			72.8	
Clarithromycin			98.4	
DMF/Blank			0.0	

DMF: N, N-dimethyl formamide, Concentration (w/v) of all the test agents used; 10 mg mL^{-1} in DMF

Table 2: MICs of Amoxicillin, Clarithromycin aqueous and ethanol extracts of *Anethum graveolens* fruit, *Cichorium intybns* leaf, *Polygonum viviparum* root and *Plantago ovata* seed husk for *Helicobacter pylori*

	MICs (μg mL^{-1})	
Plant drugs tested	Aqueous extracts	Ethanol extracts
Anethum graveolens fruit	9.7	9.6
Cichorium intybns leaf	0.6	13.2
Polygonum viviparum root	1.4	4.9
Plantago ovata seed husk	10.5	10.0
Amoxicillin	0.4	
Clarithromycin	0.3	
DMF/Blank	0.0	

DMF: N, N-dimethyl formamide, Concentration (w/v) of all the test agents used; 10 mg mL^{-1} in DMF

(Correa, 1997; Megraud, 2003). Therefore, anti-HP screening of different drugs having anti-ulcerogenic efficacies has considered important.

In the present study aqueous, ethanol, ethylacetate and acetone extracts of four potential anti-ulcer plant drugs were used for *in vitro* screening of anti-HP activity by the method of Beil *et al.* (1995). The most potent anti-HP activity was demonstrated by aqueous extract of *Cichorium intybus* leaf (CI); 62.6% and ethanol, ethyl acetate, acetone extracts of CI showed 19.2, 4.1, 4.2% inhibition of HP growth, respectively. *Polygonum viviparum* root (PV) was also found potent growth inhibitor of HP, its aqueous, ethanol, ethylacetate and acetone extracts showed 61.2, 23.9, 3.4 and 4.1% inhibition. Aqueous, ethanol, ethylacetate and acetone extracts of *Plantago ovata* seed husk (PO) and *Anethum graveolens* fruit (AG) exhibited 21.2, 1.9, 1.4, 1.2 and 1.2, 9.5, 4.7, 2.3% HP growth inhibitory effects, respectively (Table 1).

The data pointed out aqueous extract of CI was most potent while aqueous extract of AG least potent anti-HP. Ethanol extract of PV was more potent HP growth inhibitor in comparison to ethanol extracts of CI, AG and PO. However, ethylacetate and acetone extracts of AG, CI, PV and PO showed very little (less than 5%) inhibitory effects against HP-growth. The antibacterial activities of CI and AG found comparable to Amoxicillin (72.8% inhibition), a reference antibiotic but less than Clarithromycin (98.4% inhibition), another reference antibiotic (Table 1). Therefore, minimum inhibitory concentrations (MICs) of aqueous and ethanol extracts were determined; AG showed 9.7 and 9.6 mg mL^{-1} CI; 0.6, 12.3, PV; 1.4, 4.9 and PO; 10.5, 10.0 mg mL^{-1} MICs, respectively. The MIC of aqueous extract of CI was found lowest, followed, in ascending order, by the aqueous extracts of PV, AG and PO. Similarly ethanol extract of PV yielded minimum MIC, followed by ethanol extracts of AG, PO and highest with CI extract (Table 2). The reference antibiotics i.e., Amoxicillin and Clarithromycin also exhibited very small MICs (0.4, 0.3 mg mL^{-1}, respectively). MICs of aqueous extract of CI and PV were comparable with the reference antibiotic used (Table 2). The anti-HP activity of indigenous plant drugs tested was in accord to the findings of Wang and Huang (2005b).

The test drugs have already been reported to be the potent inhibitors of lipid peroxidation due to their strong antagonistic activities against indomethacin in rats (Rifat-uz-Zaman *et al.*, 2002, 2004, 2005, 2006). Yoshikawa *et al.* (1993) have been indicated that the lipid peroxidation induced by oxygen radicals plays an important role in the pathogenesis of indomethacin-induced gastric mucosal changes as well as in gastric injuries. Moon *et al.* (2002) have further shown that oxygen free radicals serve as second messengers in pro-inflammatory signal transduction pathways. The oxygen active species, such as O_2^{-1}, H_2O_2, HO^{-1} and lipid radicals, such as ROO^{-1}, RO^{-1} and hydroperoxides, are generated during lipid peroxidation and metabolism (Kwiecien *et al.*, 2001).

It is conceivable therefore, that the under trial drugs i.e., aqueous and ethanol extracts of CI and PV due to potent and AG and PO due to the moderate potent anti-HP activities might ultimately prove be the preferred and curative anti-ulcer agent because of their suspected antioxidant and scavenging free radicals, in addition to the anti-HP activities.

REFERENCES

Beil, W., C. Birkholz and K.F. Sewing, 1995. Effects of flavonoids on parietal cell acid secretion, gastric mucosal prostaglandin production and *H. pylori* growth. Arzneium-Forsch/Drug Res., 45: 697-700.

Bermejo, F., D. Boixeda, D.A.C. Martin, R.A. Lopez-San, V. Defarges, H. Hernandez Ranz, M. Vazquez and P.A. Garcia, 2003. Healing of *Helicobacter pylori* gastric ulcers: Only eradication matters. Rev. Esp. Enferm Dig., 95: 625-628, 621-624.

Choudhary, A.M., I. Roberts, A. Nagar, S. Tabrez and T. Gupta, 2001. *Helicobacter pylori*-related gastric outlet obstruction: Is there a role for medical treatment? J. Clin. Gastroenterol., 32: 272-273.

Correa, P., 1997. *Helicobacter pylori* as a pathogen and carcinogen. J. Physiol. Pharmacol., 48: 19-24.

Couladis, M., A. Tanimanidis, O. Tzakou and I.B.H. Chinou, 2000. Essential oil of *Phlomis lanata* growing in Greece: Chemical composition and antimicrobial activity. Planta Medica, 66: 670-672.

Czesnikiewicz-Guzik, M., E. Karczewska, W. Bielanski, T.J. Guzik, P. Kapera, A. Targosz, S.J. Konturek and B. Loster, 2004. Association of the presence the *Helicobacter pylori* in the oral cavity and in the stomach. J. Physiol. Pharmacol., 55: 105-115.

Fabry, W., P. Okemo, W.E. Mwatha, S.C. Chabra and R. Ansorg, 1995. Susceptibility of *Helicobacter pylori* and *Candida* spp. to the African plant *Terminalia spinosa*. Arzneium-Forsch/Drug Res., 46: 539-540.

Habtemariam, S., 1998. Cistifolin: An integrin-dependent cell adhesion blocker from the anti-rheumatic herbal drug, Gravel root (Rhizome of *Eupatorium purpureum*). Planta Medica, 64: 683-685.

Hopkins, A.M., C. McDonnell, N.P. Breslin C.A. O'Morain and A.W. Baird, 2002. Omeprazole increases permeability across isolated rat gastric mucosa pre-treated with an acid secretagogue. J. Pharm. Pharmacol., 54: 341-347.

Joette, M., S.R. Meyer, L. Susan, T. Pendland, P. Kanyok and H.D. Larry, 1999. *In vitro* synergy of paromomycin with metronidazole alone or metronidazole plus hydroxymetronidazole against *Helicobacter pylori*. J. Antimicrob. Chemother., 43: 403-406.

Khulusi, S., S. Badve, P. Patel, R. Lloyd, J.M. Marrero, C. Finlayson, M.A. Mendall and T.C. Northfield, 1996. Pathogenesis of gastric metaplasia of the human duodenum: role of *Helicobacter pylori*, gastric acid and ulceration. Gastroenterology, 110: 452-458.

Kwiecien, S., T. Brzozowski, P.C. Konturek, S.J. Konturek, M. Pawlik, R. Pajdo, D. Drozdowicz, A. Ptak and E.G. Hahn, 2001. Effect of central and peripheral actions of histamine and its metabolite N-alpha methyl histamine on gastric secretion and acute gastric lesions. J. Physiol. Pharmacol., 52: 625-638.

Malfertheiner, P., J. Dent, L. Zeijlon, P. Sipponen, V. Veldhuyzen, S.J. Zanten, C.F. Burman, T. Lind, M. Wrangstadh, O.E. Bayerd and J. Lonovics, 2000. Impact of *Helicobacter pylori* eradication on heartburn in patients with gastric or duodenal ulcer disease-results from a randomized trial programme. Aliment. Pharmacol. Ther., 16: 1431-1442.

Manes, G., J.E. Dominguez-Munoz, G. Uomo, J. Labenz, A. Hackelsberger and P. Malfertheiner, 1998. Increased risk for *Helicobacter pylori* recurrence by continuous acid suppression: A randomized controlled study. Ital. J. Gastroenterol. Hepatol., 30: 34-35.

Megraud, F., 2003. *Helicobacter pylori*, gastric inflammation and its consequences. Ann. Pharm. Fr., 61: 282-287.

Moon, B.Y., K. Sa-Ouk and P.C. Boon, 2002. Enzyme-like activity of glycated cross-linked proteins in free radical generation. Ann. New York Acad Sci., 899: 168-181.

Mukherjee, P.K., S. Bhattacharya, K. Saha, S.N. Giri, M. Pal and B.P. Saha, 1997. Anti-bacterial evaluation of *Drymaria cordata* wild (Fam. Caryophyllaceae) extract. Phytother. Res., II: 249-250.

National Committee for Clinical Laboratory Standards, 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th Edn., Approved Standard M7-A4. NCCLS, Wayne, PA.

Podolski, J.L., 1996. Recent advances in peptic ulcer disease: *H. pylori* infection and its treatment. Gastroenterol. Nursing, 19: 128-136.

Rifat-uz-Zaman, M.S. Akhtar and M.S. Khan, 2002. Evaluation of acid buffering and pepsin binding properties of Ispaghula (*Plantago ovata*, Forsk) and its extracts. Hamdard, XLV: 32-36.

Rifat-uz-Zaman, M.S. Akhtar and M.S. Khan, 2004. Preliminary evaluation of *Anethum graveolens* fruit in indomethacin-ulcer induced rats. J. Biol. Sci., 4: 151-156.

Rifat-uz-Zaman, M.S. Akhtar and M.S. Khan, 2005. Protective effects of *Polygonum viviparum* L. root and its extracts against lipid-peroxidation induced by Indomethacin in Rats. Intl. J. Pharmacol., 1: 224-228.

Rifat-uz-Zaman, M.S. Akhtar and M.S. Khan, 2006. Anti-ulcerogenic screening of *Cichorium intybus* L. leaf in indomethacin treated rats. Intl. J. Pharmacol., 2: 166-170.

Wang, Y.C. and T.L. Huang, 2005a. Anti-*Helicobacter pylori* activity of *Plumbago zeylanica* L. FEMS Immunol. Med. Microbiol., 43: 407-412.

Wang, Y.C. and T.L. Huang, 2005b. Screening of anti-*Helicobacter pylori* herbs deriving from Taiwanese folk medicinal plants. FEMS Immunol. Med. Microbiol., 43: 295-300.

Yoshikawa, T., Y. Naito, S. Nakamura, S. Nishimura, T. Kaneko, S. Iinuma, S. Takahashi, M. Kondo and K. Yamasaki, 1993. Effect of Rebamipide on lipid peroxidation and gastric mucosal injury induced by indomethacin in rats. Arzneium-Forsch/Drug Res., 43: 1327-1230.