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

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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 mucus 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, Tetraeycline, 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.

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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 deaerated; 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) (Khulasi 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% CO₂ for 4 days. Subculture was repeated once to ensure reliable growth (Fabry et al., 1995; Mukherjee et al., 1997; Iolette et al., 1999).

Chemicals: All chemicals used, were of analytical grade and were obtained from Becton Dickinson, E. Merek (Damstadt, FRG), BDH Poole (England), Sigma Chemical Co. (USA) and Oxoid Limited, USA. Amoxicillin, a reference antibiotic was obtained from SmithKline Beecham, P.L.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 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 (10⁶ 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% O₂, 15% CO₂ and 80% N₂) 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, 60 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

<table>
<thead>
<tr>
<th>Plant drugs tested</th>
<th>Aqueous extracts</th>
<th>Ethanol extracts</th>
<th>Ethylacetate extracts</th>
<th>Acetone extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anethum graveolens fruit</td>
<td>12.2</td>
<td>9.5</td>
<td>4.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Cichorium intybus leaf</td>
<td>62.6</td>
<td>19.2</td>
<td>4.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Polygonum viviparum root</td>
<td>61.2</td>
<td>23.9</td>
<td>3.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Plantago ovata seed husk</td>
<td>21.2</td>
<td>1.9</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td></td>
<td></td>
<td></td>
<td>72.8</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td></td>
<td></td>
<td></td>
<td>58.4</td>
</tr>
<tr>
<td>DMP/Blank</td>
<td>0.0</td>
<td></td>
<td></td>
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</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Plant drugs tested</th>
<th>Aqueous extracts</th>
<th>Ethanol extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anethum graveolens fruit</td>
<td>9.7</td>
<td>9.6</td>
</tr>
<tr>
<td>Cichorium intybus leaf</td>
<td>0.6</td>
<td>13.2</td>
</tr>
<tr>
<td>Polygonum viviparum root</td>
<td>1.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Plantago ovata seed husk</td>
<td>10.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>DMP/Blank</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

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

(Conrea, 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 \(O_2^-\), \(H_2O_2\), \(HO^-\) and lipid radicals, such as \(ROO^-\), \(RO^-\) and hydroperoxides, are generated during lipid peroxidation and metabolism (Kwieciecn, 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 to 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


