Sohag University **Faculty of Science**

Study the effect of some medicinal and aromatic plant extracts on the peptic ulcer causing pathogen (*Helicobacter pylori*)

A THESIS

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5. SUMMARY

This work was performed to study microbiological features of *H. pylori*, clinical effects of *H. pylori* on the patients through different symptoms, *H. pylori* infection rates, preferable tests for diagnosis of *H. pylori* in patients, resistance rate of *H. pylori* to antibiotics, effect of some plant extracts on multidrug resistant strains, phytochemical components detection, factors affecting bioactivity of phytochemical component to *H. pylori* and comparison of efficiency of plant extracts with antibiotics.

This study had centralized the following points:

- The clinical features were being diagnosed in two hundred and sixty patients in Sohag and Assiut University Hospitals and some medical laboratories (one hundred and thirty patients for each one): 51.6% patients with abdominal pain (or abdominal discomfort in relation to meals discomfort), 49.1% patients with nausea, and 43.9% patients with heartburn in Sohag University Hospital and some medical labs while 52.2% patients with nausea followed by 48.4% with abdominal pain and heart burn, in Assiut University Hospital and medical laboratories, so the abdominal pain and nausea the most common symptoms in the gastrointestinal disorders.

- The highest rate of gastrointestinal disorders with the abdominal pain, nausea and heartburn among the patients in the rural regions than in the urban regions. In the medical laboratories of Tema (13.8%, 13.8%, 8.5%), Thata (13.1%, 13.8%, 13.1%) and Al-maragha (16.9%, 14.6%, 13.8%), Sohag University Hospital (8.5%, 6.9%, 8.5%) to the abdominal pain, nausea and heartburn respectively. In the medical laboratories of

Sedfa (16.9%, 17.6%), Aboteeg (16.9%, 17.6%) and Assiut university hospital (14.6%, 16.9%) to the abdominal pain, nausea respectively. So the gastrointestinal disorders with their symptoms were the most predominant in some rural regions than in urban regions.

- A total of 210 bacterial isolates were isolated from the 260 stool samples of 260 patients cultured on selective media used for isolation of *H. pylori*. Nine isolates from 13 gastric biopsy samples of 13 patients (suspected in presence of peptic ulcer) in Assiut University hospital. All bacterial isolates were tentatively identified on the basis of morphology, Gram-staining and biochemical tests. The isolates comprised different *H. pylori* strains.

- Thirteen gastric biopsy samples were tested by histological examination and rapid urease test for *H. pylori* detection: Six *H. pylori* isolates were detected in thirteen gastric biopsy samples by histological examination (46.2%) and eight strains by rapid urease test (61.5%).

- Anti *H. pylori* antibodies (IgG, IgA, IgM) were determined quantitatively in two hundred and sixty blood samples of patients in Sohag and Assiut University Hospitals and medical laboratories: in Sohag University Hospital and some medical laboratories, 80.8%, 39.2%, 6.1% of patients were positive to IgG, IgA, IgM antibodies respectively. In Assiut University Hospital and some medical laboratories, 81.5%, 48.5%, 13.8% of patients were positive to IgG, IgA, IgM antibodies respectively.

- *H. pylori* antigens were determined quantitatively in two hundred and sixty stool samples of patients: 66.1% and 76.9% of patients were

positive in Sohag and Assiut University Hospital and some medical laboratories respectively.

- Sensitivity (true positive), accuracy and specificity (true negative) of anti *H. pylori* antibodies and *H. pylori* antigens tests were determined for knowing the best test in detection of infection: in Sohag University Hospital and some medical laboratories, sensitivity of *H. pylori* antibodies IgG, IgA, IgM and antigen tests by quantitative method were 95.9%, 44.9%, 6.1%, 75.5% respectively. Accuracy of *H. pylori* antibodies IgG, IgA, IgM and antigen were 88.5%, 53.1%, 26.9%, 72.3% respectively. In Assiut University Hospital and some medical laboratories Sensitivity of *H. pylori* antibodies IgG, IgA, IgM and antigen were 91.1%, 50.9%, 12.5%, 88.7% respectively. Accuracy of *H. pylori* antibodies IgG, IgA, IgM and antigen were 89.2%, 53.1%, 21.5%, 81.5% respectively so anti *H. pylori* IgG antibodies and *H. pylori* antigens tests were the most characteristic tests for diagnosis of infection.

- *H. pylori* infection rates were different according to anti *H. pylori* antibodies and *H. pylori* antigens tests and bacterial culture (as gold standard) depending on the sensitivity and specificity of test: the infection rate was 72.3% from anti *H. pylori* IgG antibodies test, 33.8% from anti *H. pylori* IgA antibodies test, 4.6% from anti H. pylori IgM antibodies test, 57% from *H. pylori* antigens test in Sohag University Hospital and some medical laboratories. The infection rate was 78.5% from anti *H. pylori* IgG, 43.8% from anti *H. pylori* IgA, 10.8% from anti *H. pylori* IgM, 72.8% from *H. pylori* Ag in Assiut University Hospital and some medical laboratories. The infection rate of *H. pylori* isolated from bacterial culture was 75.4% and 86.1% in patients in Sohag and Assiut University Hospitals and some medical labs respectively.

- Prevalence of infection in rural regions was higher than in urban regions: Prevalence of infection was 21.5%, 20.8%, 23.1%, 10.0%, 30.0%, 28.4%, 27.7% in Tema, Tahta, Al-Maragha and Sohag, Sedfa, Aboteeg, Assiut respectively. This resulted from some factors such as lifestyle and socioeconomic status many environmental parameters.

- The most dominant symptoms in patients who had *H. pylori* infection were abdominal pain (or abdominal discomfort in relation to meals discomfort) and nausea followed by the heart burn: abdominal pain (39.2%), nausea (36.9%), heartburn (21.5%) in Sohag University Hospital and the medical laboratories. In Assiut University Hospital and some medical laboratories, nausea (49.2%) followed by the abdominal pain (40.8%) and heartburn (39.2%).

- *H. pylori* was multi-resistant bacteria to antibiotics in this study: in Sohag University Hospital and some medical laboratories, resistance rates were 93.8%, 86.7%, 58.2%, 79.6%, 91.8%,65.3% and 56.1% to erythromycin, gentamycin, levofloxacin, metronidazole, rifampicin, tetracycline, and clarithromycin respectively. In Assiut University Hospital and some medical laboratories, high resistance rates were shown 81.3%,57.1%,81.3%, 84.8%, 91.1%, 62.5% and 53.6% to erythromycin, furazolidone, gentamycin, metronidazole, rifampicin, tetracycline, and clarithromycin respectively.

- In Sohag and Assiut University Hospitals and some medical laboratories, amoxicillin and ciprofloxacin were the best antibiotics in this study in inhibition of *H. pylori* strains.

- Forty nine *H. pylori* strains were selected as multidrug resistant to evaluate the antibacterial activity of plant extracts: by disc diffusion method, 87.7% and 59.2% of all selected strains were sensitive to *Allium sativum* and *Carum Carvi* and 67.4%, 57.1%, 44.9% of all selected strains were sensitive to *Trigonella Foenum graecum, Moringa oleifera* and *Lawsonia inermis* methanolic extracts. We could conclude that plant extracts proved its superiority over antibiotics and essential oils proved its superiority over methanolic extracts.

- Minimum inhibitory concentration (MIC) was determined to detect the minimal concentration of essential oils at which bacterial inhibition occurs: Approximately one third of *H. pylori* strains (30.6%) inhibited by *Allium sativum* essential oil at the minimum inhibitory concentration MICs (1:32) while 16.3% of these strains were sensitive to *Carum Carvi* essential oil at MICs (1:4) in Sohag and Assiut university hospitals and some medical laboratories, from these results *Allium sativum* essential oil had higher anti-bacterial potency than *Carum Carvi* essential oil against selected multidrug resistant *H. pylori* strains.

- 26.5% strains were inhibited by *Moringa oleifera* methanolic extract at dilution 125 by serial dilution method, 28.5% strains were by *Trigonella Foenum graecum* extract at 250 and 12.2% strains by *Lawsonia inermis* extract in Sohag and Assiut University Hospitals and some medical laboratories, from these results *Moringa oleifera and Trigonella Foenum graecum* methanolic extracts proved their superiority in activity against the strains at different dilutions.

- From phytochemical screening of methanolic extracts: The methanolic extract of *Moringa oleifera* and *Trigonella Foenum graecum*

revealed the presence of various bioactive components of which alkaloid, phenols, flavonoids, tannins, terpenoids, glycosides, steroids, amino acids and proteins were the most prominent.

- Total phenolics content, total flavonoids content and tannins content were determined in methanolic extracts: the highest concentration of total phenols was detected in *Trigonella Foenum graecum* extract (543.3 mg/100g) than *Moringa oleifera* extract (413.76 mg/100g) and *Lawsonia inermis*(293 mg/100g). The highest content of total flavonoids was cleared in *Lawsonia inermis* extract (368.5 mg/100g) than *Trigonella Foenum graecum* extract (5.531 mg/100g) and *Moringa oleifera* extract (3.614 mg/100g). The content of tannins were approximate in *Trigonella Foenum graecum and Moringa oleifera* extracts 0.46% and 0.49% while it was the highest in *Lawsonia inermis* extract 2.67%.

- Sixteen constituents of *Allium sativum* were identified by Gas chromatography-mass spectrometry analysis including: dimethyl disulfide 1.17%, dimethyl sulfide 1.57%, allyl methyl disulfide 1.72%, diallyl disulfide 30.12%, allyl(Z)-1-propenyl disulfide 12.34%, allyl methyl trisulfide 5.11%, 2-vinyl-4-H-1,2 dithine 5.51%, diallyl trisulfide 22.60%, allyl propyl trisulfide 3.82%, diallyl tetrasulfide 7.02%. Five constituents of *Carium carvi* were identified including: myrcene (1.12%), limonene (46.39%), trans-carveol (1.59%), carvone (50.3%) and carveol (0.60%).