ORIGINAL ARTICLE

In vitro screening of selected Iranian medicinal plants against *Helicobacter pylori*

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Helicobacter pylori infection is virtually always associated with duodenal, peptic and gastric ulcers and promotion of gastrointestinal cancer. Some of medicinal plants traditionally have been used for gastrointestinal problems. In the present work, the inhibitory effect of the essential oils of some medicinal plants was evaluated against clinical isolate of *H. pylori*. *H. pylori* was isolated from gastric biopsy of patients with gastric complications. Agar diffusion and agar dilution methods were used for evaluating the anti-*H. pylori* effect and minimum inhibitory concentration (MIC) determination of tested plants. The results were reported as mean±SD and differences considered significant at a *P* value <0.05. The essential oils of *Cinnamomum zeylanicum* and *Zataria multiflora* demonstrated potent anti-*H. pylori* effect with inhibition zone diameter of 24.8 mm and 23.6 mm, respectively. The MIC of both two essential oils was estimated to be 0.3 µl/ml. The essential oils of *Heracleum persicum, Syzygium aromaticum* and *Citrus aurantium* exhibited more than 88% inhibition in concentration of 0.3 µl/ml. The essential oils of *C. zeylanicum* and *Z. multiflora* might be good candidate for treatment of gastrointestinal disorders caused by *H. pylori* and it is needed to do further study about these essential oils.

Key words: *Cinnamomum zeylanicum,* Essential oil, *Helicobacter pylori*, MIC, *Zataria multiflora*

INTRODUCTION

Among the bacterial chronic infections of the gastrointestinal system, *Helicobacter pylori* is a Gram-negative spiral-shaped bacteria which is associated with ethiopathogenesis of different clinical conditions such as gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[1,2] Clinical experiences in Iran and many other developing countries have demonstrated that eradication rate of *H. pylori* is much lower than that in modern countries, and in spite of using the same treatment regimens, reinfection in short and long terms are much more prone than that reported in industrial countries.[3] The growing of antibiotic resistance and incidence of specific complications are the most problems of current medicines which demands the search for novel compounds, especially from natural sources such as medicinal plants. In Iranian traditional medicine, some of medicinal plants have shown great promise in the treatment of different acid peptic disorders. We aimed to study the *in vitro* anti-*H. pylori* effect of the essential oil of several of most common used of these medicinal plants. The information of these plants is given in Table 1. No scientific evidences have been reported for these plants in the literature.

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MATERIALS AND METHODS

Plant Materials

The plants were collected from different regions of Iran and identified by a senior in Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of medical Sciences, Kerman, Iran. A voucher specimen of the plants was deposited in the Herbarium centre of Faculty of Pharmacy. Two hundred grams of each dried plant was used for the experiment. The essential oil was isolated by hydrodistillation method using cleavenger apparatus. The essential oil was stored in –20°Ct until the tests.

Bacterial Strain and Cultivation

The clinical isolates of *H. pylori* were obtained from the gastric mucosa of patients referred to the hospital for endoscopic evaluation and diagnosed with gastritis, peptic ulcer disease. Three to four colonies of the isolates were dissolved in microtubes containing 1.5 ml of brain heart infusion broth (BHIB) or tryptic soy broth (TSB) with 40% glycerol and freezed at –70°C until the tests. 100 µl of each essential oil was grown on a

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plate containing Brucella agar, 10% defibrinated sheep blood and supplemented with 2 mg vancomycin, 1 mg trimethoprim and 2 mg/l amphotericin B. The plates were incubated in a microaerobic atmosphere, in a jar containing anaerocult C gas pack (Merck, Germany) at 37°C for 5 days. Identification of *H. pylori* was based on Gram's staining, bacterial morphology, oxidase, catalase and urease test.

The inhibitory effect of tested essential oils was studied using agar diffusion method. MIC was determined using agar dilution method against *H. pylori.*

Agar Diffusion Method

One microlitre of the bacterial suspension was adjusted to a McFarland N1. The bacterial suspension was cultured by spreading plate method on Mueller-Hinton agar-5% sheep blood media. The essential oils were used in two concentrations (0.1 and 0.5 µl/ml) and plates were incubated in conditions previously mentioned for 3 to 5 days, and the inhibition zone diameter (IZD) was measured. Clarithromycin (2 µg/ml) was used as positive control.

Determination of MIC Using Agar Dilution Method

Each essential oil was dissolved in 1% dimethylsulfoxide (DMSO) in different concentration and added to Mueller-Hinton agar-5% sheep blood media and incubated 3 to 5 days in microaerophilic atmosphere. The MIC of each sample was determined as the lowest concentration inhibiting visible growth. The experiments were done in triplicate.

Statistical Analysis

The results were reported as mean±SD and strain susceptibility of samples in different samples were studied by ANOVA. Differences considered significant at a P value <0.05.

RESULTS

The information for tested plants has been given in Table 1.

The Results of Agar Diffusion Method

As shown in Figure 1, the results of agar diffusion method show that *C. zeylanicum* essential oil exhibited the most inhibitory effect on *H. pylori*, with the average IZD of 24.8 mm. Z. *multiflora* also with IZD = 23.6 mm potentially inhibited the growth of *H. pylori,* which was comparable with clarithromycin (IZD = 23.3 mm). The essential oils of *M. longifolia, C. aurantium* and *S. zeylanicum* exhibited weak activity against the isolates *H. pylori* and the other plant essential oils exhibited a mean range of IZD of 4.3 to 7.5 mm against *H. pylori* (data not shown). The IZD of essential oils in 0.1 µl/ml was negligible.

Agar Dilution Method

The results of MIC determination of tested essential oils given in Table 2. The essential oils were tested in three different concentrations. These results show that the essential oils of *C. zeylanicum, Z. multiflora* and *H. persicum* with IC_{50}=0.3 µl/ml completely inhibited the growth of *H. pylori*.

The results of inhibitory effect of tested essential oils in three different concentrations have shown in Figure 2. These results show that all essential oils exhibited a concentration-dependent inhibition. The essential oil of *Z. multiflora* has exhibited the most inhibition in all tested concentration. The essential oils of *H. persicum* and *M. longifolia* were active just in highest concentration (0.3 µl/ml). The other essential oils exhibited weak inhibitory effect.

Table 1: The information of medicinal plants which their essential oils have been tested for anti *H. pylori* effect

Common/ folk name	Scientific name	Plant family	Used part
Asafetida	*Ferula asa-fetida*	Apiaceae	Gums
Sour orange	*Citrus aurantium*	Rutaceae	Flowers
Lime	*Citrus aurantium*	Rutaceae	Fruits
Cat parsnip/ Golpar	*Heracleum persicum*	Apiaceae	Fruits
Mohrkhosh	*Zhumeria majdae*	Lamiaceae	Aerial parts
Pennyroyal	*Mentha longifolia*	Lamiaceae	Aerial parts
Yarrow	*Achillea wilhelmsii*	Asteraceae	Aerial parts
Betony	*Stachys lavandulifolia*	Lamiaceae	Flowers
Figwort	*Linaria vulgaris*	Linaceae	Fruits
Cinnamon	*Cinnamomum zeylanicum*	Lauraceae	Dry bark
Avishan-e-shirazi	*Zataria multiflora*	Lamiaceae	Aerial parts
Clove	*Syzygium aromaticum*	Myrtaceae	Budds

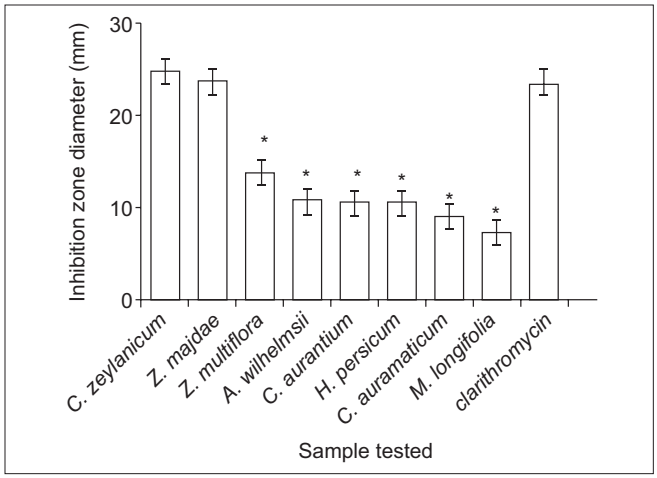

Figure 1: The results of inhibition zone diameter of tested essential oils in concentration of 0.5 µl/ml against *H. pylori* in agar diffusion method in comparison to positive control (clarithromycin). *P<0.001, significantly different from positive control (clarithromycin)

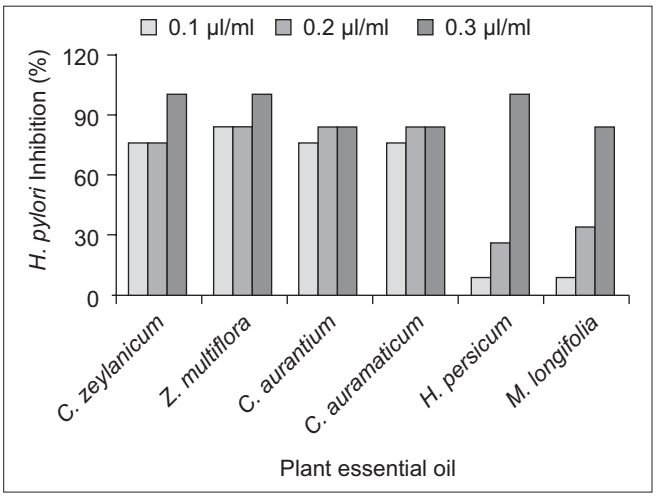

Figure 2: The results of determination of inhibitory effects of tested essential oils against *H. pylori* in three different concentrations in agar diffusion method

Table 2: The results of determination of minimum inhibitory concentration of tested essential oils against *H. pylori* in agar dilution method in comparison to positive control (clarithromycin)

Sample	Minimum inhibitory concentration (µl/ml)
C. zeylanicum	0.3
Z. multiflora	0.3
A. wilhelmsii	ND[1]
C. aurantium	>0.3
H. persicum	0.3
C. aromaticum	>0.3
M. longifolia	>0.3
Clarithromycin	2 µg/ml

[1]Not Determined

DISCUSSION

Gastric cancer involves a multistep mechanism in which *H. pylori* play a vital role. WHO has classified this micro-organism as a class I carcinogen and the eradication of this silent killer with antibiotic combinations has been reported to be beneficial in preventing gastric ailments, especially cancer.[4] Considering the potential of medicinal plants for relieving of different disorders of gastrointestinal, in this study, we aimed to investigate the anti-*H. pylori* activity of the essential oil of several medicinal plants from Iran which are frequently prescribed for gastrointestinal (GI) disorders in traditional system of medicine. The results obtained indicated that some of these essential oils exhibited potent inhibitory effect against *H. pylori*. The diameter of inhibition zone ranged from 4.3 to 24.8 mm. DMSO 10% as solvent showed no inhibition zone.

In agar diffusion method, the most activity was shown *by C. zeylanicum* and *Z. multiflora* essential oils (IZD = 24.8 and 23.6 mm, respectively), which completely was similar to clarithromycin (23.3 mm). The other plant essential oils showed weak and/or no inhibition against the isolates in this method. The results of agar dilution method indicated that among the tested plants, *Z. multiflora, C. zeylanicum*, *C. aurantium* and *C. aromatcum* exhibited high inhibition against *H. pylori.* These essential oils were active in all three used concentrations. *Z. multiflora* and *C. zeylanicum* exhibited 100% inhibition in concentration of 0.3 µl/ml. It is also interesting to note that *H. persicum* and *M. longifolia* potentially inhibited *H. pylori* in concentration of 0.3 µl/ml (100% and 83.3% inhibition respectively), while these essential oils showed weak activity in agar diffusion method [Figure 1]. These differences might be due to nature of active compounds of different essential oils which affect their diffusion to agar media and so their potential of *H. pylori* inhibition. The other tested essential oils showed weak activity in both methods. *Z. multiflora* Boiss. is a popular and medicinal plant native to Iran, Afghanistan and Pakistan. During recent years, the plant has received an increased attention due to its remarkable antibacterial and antioxidant activities.[5,6]

The essential oil of *Z. multiflora* exhibited high anti-*H. pylori* activity in a concentration-dependent manner with 100% inhibition in 0.3 µl/ml concentration. This oil has exhibited the highest inhibitory effect in all three tested concentrations. Previous studies have reported the inhibitory effects of the essential oil of *Z. multiflora* against a wide range of Gram-positive and Gram-negative bacteria, fungi and parasites.[7-11] The analysis of the plant essential oil showed the presence of thymol and carvacrol as major compounds of the oil.[6] The antimicrobial effects of these compounds have been reported.[12-14] This plant traditionally used for relief of gastric pains and irritable bowel syndrome. Moreover, the essential oil of the plant has shown high antioxidant and free radical scavenging effect in *in vivo* studies.[15]

C. zeylanicum was the other active plant which its essential oil exhibited the highest IZD (24.8 mm) against *H. pylori.* This essential oil also showed 100% inhibition in concentration of 0.3 µl/ml. This plant has widely used as condiment in Iranian food diets. The antimicrobial effect of the plant essential oil and its two major components, cinnamic aldehyde and eugenol, has been reported. Several studies also have shown antimicrobial and antifungal properties of *C. zeylanicum*.[16-19] Tabak *et al*. reported high *in vitro* anti-*H. pylori* effects of the plant extract.[20] The antibacterial effect of the essential oil of this plant mostly is due to cinnamic aldehyde which constitutes more than 83% of the oil.[21] The antibacterial and antitumor activities of the plant essential oil have been reported previously.[22] Among the other tested essential oils, *H. persicum* essential oil showed 100% inhibition of *H. pylori* in concentration of 0.3 µl/ml in a concentration-dependent manner. The seeds of *H. persicum* have been widely used as spice in daily diet

of Iranians, especially as a carminative in persons suffering from flatulence and cannot tolerate different legumes and beans. The other tested plants have shown negligible inhibitory effect against *H. pylori*. Generally, our results provide valuable results about some of the medicinal plants having anti-*H. pylori* effects which would be good candidate for more investigations. Our findings also contribute to the better understanding the mode of action of these plants in acid peptic disorders. The other plants might be effective through the other mechanisms. Moreover, it is possible that different mechanisms other than anti-*H. pylori* activity be responsible for therapeutic roles of plants used in gastrointestinal disorders.

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