Anti-microbial Properties of *Valeriana officinalis*, *Satureja bachtiarica* and *Thymus daenensis* Methanolic Extracts against *Helicobacter pylori*

Raheleh Khademian¹, Fatemeh Karimzadeh²*, Parisa Moradi³ and Behvar Asghari⁴

¹Department of Genetic and Plant Breeding, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran.
²Department of Biotechnology, College of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran.
³Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.
⁴Department of Horticultural Sciences Engineering, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Aims:** *Helicobacter pylori* infection spreads widely in the world and has several complications including gastric cancer. The aim of this work is to investigate the antimicrobial properties of *Valeriana officinalis* L., *Satureja bachtiarica* and *Thymus daenensis* extracts against *H. pylori*.

**Methods:** The disk diffusion test was used in this experiment. Methanolic extract was isolated from *Valeriana officinalis* L., *Satureja bachtiarica* and *Thymus daenensis*. Ten clinical isolates of *H. pylori* were used in this experiment, which was obtained at Alzahra hospital, Tehran, Iran from individual patients with gastrointestinal disorders. *H. pylori* strains were identified using the
1. INTRODUCTION

*Helicobacter pylori* infection (*H. pylori*) has a widespread outbreak in various geographical and ethnic areas [1]. So much that, it still has a high prevalence in Iran [2]. This bacterium is the cause of gastritis, gastrointestinal diseases and gastrointestinal ulcers that may lead to dangerous side effects such as, gastrointestinal lymphoma [3,4]. In addition, clinical outcomes and its control are under the influence of environmental factors, bacterial and host colonization [4], thereby, the treatment process is complex. In addition, because of bacterial resistance, three-drug therapy or clarithromycin is not the best choice for medication. Hence, it is essential to find alternative treatment [5]. Medicinal plants on bacteria causing various diseases [6-14], including *H. pylori* have shown positive effects [15]. In this study, we reviewed indigenous plants that have been studied. The genus *Valeriana* belongs to the Valerianaceae family, which contains about 250 species. *Valeriana officinalis* is an important species of this genus that is perennial flowering plant native to Europe and Asia. *V. officinalis* is a source of biologically and pharmacologically active molecules that are classified as monoterpenes and sesquiterpenes [17]. *Thymus daenensis*, a member of the Lamiaceae family, is an aromatic evergreen subshrub with lanceolate leaves, which grows spontaneously in various areas of Iran. The aerial parts of this plant are commonly used as herbal tea, flavoring agents and for medicinal purposes. *T. daenensis* is a rich source of thymol and carvacrol, both of which have been reported to have strong antioxidant activities [18]. *T. daenensis* has a strong antimicrobial properties, but its toxicity and teratogenic effects have not yet been investigated [19]. According to drug resistance of bacteria is due to genotypic and geographical diversity as well as minor complications of herbal medicines [15]. The aim of this work is to investigate the antimicrobial properties of *Valeriana officinalis* L., *Satureja bachtarica* and *Thymus daenensis* extracts against *H. pylori*.

2. MATERIALS AND METHODS

2.1 Preparation of the Plant Extracts

Aerial parts of *Thymus daenensis*, *Valeriana officinalis* L. and *Satureja bachtarica* plants at the flowering stage were collected from the altitudes of Chaharmahal and Bakhtiari Province, Iran. In order to prepare methanolic extract of plants, the powder of dried herbs was poured in Erlenmeyer flasks and methanol was added to the powder. The flasks were kept at room temperature (20-30°C) for two days, then the solutions were filtered. The filtered solutions were condensed in a rotary evaporator at 45°C and their volume reached to 7 ml. The concentrated solutions were added to 5-10 ml methanol and were frozen at -15°C for 24hours, in order to precipitate fat and heavy carbohydrate. The concentrated solutions were added to cold methanol and the mixer was

| Keywords: Valeriana officinalis L.; Satureja bachtarica; Thymus daenensis; Helicobacter pylori. |

Results: The clinical strains showed the highest susceptibility to tetracycline (10%). The inhibition zone diameter was the highest at 10% concentration of methanolic extracts of *Valeriana officinalis* L., *Satureja bachtarica* and *Thymus daenensis* and had the highest growth inhibitory effect at higher concentrations. Conclusion: The methanolic extracts of *Valeriana officinalis* L., *Satureja bachtarica* and *Thymus daenensis* herbs showed high anti-*H. pylori* effects in high concentrations and among them, *Satureja bachtarica* showed the highest antibacterial effect.
2.2 Microbial Strains and Culture Media

Ten clinical isolates of *H. pylori* were used in this experiment, which were obtained at Alzahra hospital, Tehran, Iran from individual patients with gastrointestinal disorders. The isolates were cultured on Brucella agar (Merck, Germany) containing 5–7% sheep blood, 2 mg/l amphotericin, 8 mg/l polymixin-B, and 6 mg/l vancomycin and were kept on the microaerophilic conditions (10% CO2 and 95% humidity) at 37°C for 3–7 days. Clinical isolates of *H. pylori* were identified using Gram staining, bacterial growth (0.5 – 1 mm), oxidase, catalase, urease, nitrate, H2S and hippurate hydrolysis tests and nalidixic sensitivity [21]. To control bacterial quality, *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC25923) in Mueller Hinton Agar (MHA) media was used.

2.3 Microbial Susceptibility Testing

To determine the MIC of the extracts, disk diffusion (Kirby-Bauer) test in agar was used. New suspension cultures that were prepared in saline solution, were adjusted to $1 \times 10^{18}$ (Corresponding to turbidity with OD 0.8 at 600 nm). About 200 µl of bacterial suspensions was placed in 50-ml Mueller Hinton agar disk containing 10% fetal calf serum (Sigma, UK), and incubated under microaerophilic conditions at 37°C for 2 to 5 days. *H. pylori* ATCC26695 was used as a quality control strain. The tests was done three times. The sensitivity of *H. pylori* strains also was determined against the different amounts of antibiotics [22]. The standard values for determining the sensitivity are shown in the Table 1.

2.4 Statistical Analysis

All the data were analyzed using analysis of variance (ANOVA) performed with SPSS 16.0 software. Duncan’s multiple range test was used to distinguish the differences in treatments. Excel software was used to draw figures.

3. RESULTS

Comparison of different strains resistance to different antibiotics, Metronidazole (80%), Tetracycline (10%), Ampicillin (80%) and Clarithromycin (90%) showed that clinical strains were the most sensitive to tetracycline (Table 2). The antibacterial activities of *V. officinalis, S. bachtiarica* and *T. daenensis* methanol extracts were assayed in vitro by disk diffusion (Kirby-Bauer) test against *H. pylori*. The microbial growth inhibition by each plant extract were shown in Fig. 1. According to the results, methanolic extract of *S. bachtiarica* was found to be more active against *H. pylori* than others. Also, different concentrations of this plants

Table 1. Diagnostic standard for antibacterial sensitivity and resistance to antibiotics

<table>
<thead>
<tr>
<th>Antibiotic agents</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10 µg)</td>
<td>≥17</td>
<td>14-12</td>
<td>≤13</td>
</tr>
<tr>
<td>Metronidazole (5 µg)</td>
<td>≥15</td>
<td>12-15</td>
<td>&lt;12</td>
</tr>
<tr>
<td>Erythromycin (5 µg)</td>
<td>≥18</td>
<td>14-17</td>
<td>≤12</td>
</tr>
<tr>
<td>Clarithromycin (2 µg)</td>
<td>≥18</td>
<td>14-17</td>
<td>≤13</td>
</tr>
<tr>
<td>Tetracycline (30 µg)</td>
<td>≥19</td>
<td>15-18</td>
<td>≤14</td>
</tr>
</tbody>
</table>

Table 2. Inhibition zone diameter for each of the ten clinical isolates of *H. pylori* against antibiotic agents

<table>
<thead>
<tr>
<th>Antibiotic agents</th>
<th>HP1*</th>
<th>HP2</th>
<th>HP3</th>
<th>HP4</th>
<th>HP5</th>
<th>HP6</th>
<th>HP7</th>
<th>HP8</th>
<th>HP9</th>
<th>HP10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole (5 µg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tetracycline (30 µg)</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ampicillin (10 µg)</td>
<td>12</td>
<td>0</td>
<td>11</td>
<td>9</td>
<td>0</td>
<td>19</td>
<td>16</td>
<td>0</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td>Erythromycin (5 µg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin (2µg)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Different strains of *H. pylori*
extract had significant effect on bacterium growth. At 10% concentration from Thymus daenensis methanolic extract, the diameter of the zone of inhibition for H. pylori was the highest in comparison to others (Table 3).

In the study of Valeriana officinalis L. extract effect, it was found that the extract of this plant can be inhibitor only at 10% concentration and other concentrations have no effect on the bacteria (Table 3). Also, the study of Satureja bachtiarica extract anti-microbial effect showed that this extract had the highest growth inhibition effect at higher concentrations, such as 5 and 10% (Table 3). Fig. 1 also shows that the diameter of Valeriana officinalis L., Satureja bachtiarica and Thymus daenensis inhibition zone increases with increasing concentration and the effect of these extracts were dose dependent.

4. DISCUSSION

The presence of diverse secondary metabolites in essential oils and extracts of the plants including phenolics, flavonoids, lignans, alkaloids, terpenes and saponins are responsible for the biological properties like anti-bacterial, anti-cancer, anti-oxidant and anti-inflammatory activities. The antibacterial activity of various plants are vary due to difference in type and concentrations of secondary metabolites [23]. The present study was conducted to investigate anti-Helicobacter pylori effects of Valeriana officinalis L., Satureja bachtiarica and Thymus daenensis plants methanolic extracts. The diameter of inhibition zone was the highest at 10% concentration of methanolic extract of Thymus daenensis. In this regard, Moradi et al. also found that Thymus daenensis with compounds like thymol, gamma-terpinene and para-cymene prevents Helicobacter pylori growth, and can be used as an effective treatment [24]. The other Thymus species also showed antibacterial effects. For example, in a study, the effect of two plants, Thymus vulgaris and Eucalyptus globules on H. pylori growth was investigated. The results showed that the essential oils of these plans can decrease the H. pylori specific IgA and IgG titre [25]. The results of eftekhar et al. (2009) study also showed that the essential oil of Thymus caramanicus plant have strong antibacterial properties against clinical strains of H. pylori, and the minimum growth inhibitory concentration was observed in the range of 14.5 - 58.0 μg/ml [26]. In the study of Valeriana officinalis L. extract effect, it was found that the extract of this plant can be inhibitor only at 10% concentration and other concentrations do not have any effect on the bacteria.

No study was found in conjunction with the effect of this plant extract on H. pylori, but similar studies in this regard showed that the essential oil and extracts of the plant were varied according to the type of crop, season and plant age. Major compounds including valerenal,

### Table 3. Antimicrobial effects of different concentrations of methanolic extract of Valeriana officinalis L., Satureja bachtiarica and Thymus daenensis on clinical isolates of H. pylori

<table>
<thead>
<tr>
<th>Medicinal plant</th>
<th>Concentrations</th>
<th>HP1</th>
<th>HP2</th>
<th>HP3</th>
<th>HP4</th>
<th>HP5</th>
<th>HP6</th>
<th>HP7</th>
<th>HP8</th>
<th>HP9</th>
<th>HP10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus daenensis</td>
<td>10%</td>
<td>6</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>17</td>
<td>16</td>
<td>12</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>5</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>12</td>
<td>14</td>
<td>10</td>
<td>16</td>
<td>11</td>
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<tr>
<td></td>
<td>2.5%</td>
<td>4</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>8</td>
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<td>7</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>0.6%</td>
<td>-</td>
<td>5</td>
<td>6</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Valeriana officinalis</td>
<td>10%</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Satureja bachtiarica</td>
<td>10%</td>
<td>17</td>
<td>16</td>
<td>14</td>
<td>15</td>
<td>17</td>
<td>14</td>
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<td>13</td>
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<td>12</td>
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<td></td>
<td>2.5%</td>
<td>8</td>
<td>7</td>
<td>9</td>
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<td>9</td>
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<td>4</td>
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<td>5</td>
<td>5</td>
<td>10</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

*Different strains of H. pylori*
Fig. 1. Antimicrobial effect of different concentration of methanolic extract of Thymus daenensis (1a), Valeriana officinalis.L (1b) and Satureja bachtiarica (1c) on clinical isolates of H. pylori

bornyl acetate, 15-acetoxy valeranone, valerenic acid, and camphene and essential oil in Valeriana officinalis L. have antibacterial properties against Aspergillus niger, Escherichia coli, Staphylococcus aureus and Saccharomyces cerevisiae, but do not have a dramatic effect against Pseudomonas aeruginosa [27].

The results of Wang et al. (2010) study on V. officinalis showed that its essential oil can act as a widespread antibiotic, because it's Minimal Inhibition Concentration (MIC) was reported 62.5 μg/ml to 400 μg/ml. [28]. Another study showed that growth inhibition zone in different species of Valeriana was in the range of 15 to 16 mm and its MIC values were determined about 116 mg/ml and 150 mg / ml [29]. Also, in the study of the effect of Satureja bachtiarica methanol extract it was found that this extract had the highest inhibitory effect at higher concentrations than that of 10% and 5%. Other studies also have shown that Satureja bachtiarica has strong antimicrobial properties. In this regard, a study showed that the minimum growth of Staphylococcus aureus was 1000 mg/l of essential oils and 125 mg/l of this plant's water extract, thus, it showed good antimicrobial properties [30]. Also, an examination of ethanolic extract showed that Satureja bachtiarica exhibits the highest antimicrobial properties at concentrations of 100 and 200 mg/l. [31]. In this regard, another study showed that the MBC of Satureja bachtiarica ethanol extract for Bacillus subtilis and Listeria innocua were 16 and 32 mg/ml, respectively [32]. In a similar study, the extract of this plant at 2 mg / ml concentration had an inhibition effect against Streptococcus pyogenes and Staphylococcus epidermidisat but it did not effect on Pseudomonas aeruginosa [33]. Therefore, these studies confirm that the medicinal herbs have antimicrobial effects. Because of the resistance to chemical drugs, medicinal herbs can be used instead of chemical drugs as well as supplementary medications. It seems that, one of the factors that can prove anti-H. pylori properties of medicinal plants, is their antiulcer and gastroprotective effects [34]. This was achieved with anti-oxidant, anti-inflammatory and anti-histaminic properties of plants. Also, polyphenol compounds in plants are one of the inhibitors of microbial growth.

5. CONCLUSION

Methanolic extract of Valeriana officinalis.L, Satureja bachtiarica and Thymus daenensis
herbs showed anti-
H. pylori
effects at 10% concentration which Satureja bachtiarica has the highest antibacterial effect. But at low concentrations, their antibiotic properties are doubtful, and shows that the antimicrobial function is dose-dependent. It is suggested that in subsequent studies, the active ingredient of plants be isolated and examined. Essential extracts and essential oils of the plants should be investigated separately.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


