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Antimicrobial Activity of Essential oil of Parsley (PetroselinumCrispum) Against FoodPathogenic Bacteria

Fereshteh Karimi, Mohammad Rezaei, Nabi Shariatifar, Mehran Sayadi, Issa Mohammadpourfard, Ebadallahshiri Malekabad and Hassan Jafari

Department of Food Safety and Hygiene, school of public health, Tehran University of Medical Sciences, Tehran, Iran  
Department of Epidemiology and Biostatistics, school of public health, Tehran University of Medical Sciences (TUMS), Tehran, Iran  
General Physician, Arak University of Medical Sciences, Arak, Iran

Abstract: An experiment was conducted to find the antimicrobial activity of the essential oil from leaves and seeds of Parsley. The antimicrobial activity was examined using paper disc diffusion method and by micro dilution technique against five pathogenic bacteria (Escherichia coli, Salmonella, Staphylococcus aureus, Yersinia and Vibrio cholera). The MICs (minimum inhibitory concentrations) of the PetroselinumCrispum seeds and leaves essential oil were 8, 0.25% against S. aureus, 4, 0.125% against V. cholera, 16, 0.5% against Yersinia enterocolitica and 32, 1% against the Salmonella enterica and E. coli, respectively. The results support the high efficacy of essential oils to control pathogenic bacteria and their use in developing new systems to prevent bacterial growth, extend the shelf life and increase the safety of the processed food.

Key words: Antimicrobial activity • Essential oils • Parsley • Seed • Leaf

INTRODUCTION

Food poisoning is yet a worry for people and the food producer notwithstanding the use of diverse preservation ways. Food safety researchers and regulatory organization are ceaselessly concerned with the extend and growing number of illness prevalence caused by some pathogenic and spoilage microorganisms in foods [1]. The increase of infections as a result antibiotic insistance microorganisms has entailed using new and natural antimicrobial substances [2]. One of the methods to keep safe food is used synthetic additives which reduce microbial growth or debar microorganisms and impeded or delay. Additives are harmful for human health particularly monosodium glutamate, aspartame, saccharin, sodiumcyclamate, sulfur, nitrates, nitrites and antibiotics. It brings about headache, nausea, weakness, mental retardation, seizures, cancer and anorexia [3]. Because of the concern about the side effects of conventional preservatives and high attention of people to food safety, people have a better request to the use of natural products as areplacement to customary preservatives in the last few decades.

As a result of these, consumer’s interest in natural products, particularly plant extracts, inclusive their essential oils and essences. Spices and herbs have been added to food for a wide variety of purposes for many thousands of years, for example to enhance the flavor, color and aroma of food. As well as they are also known for their preservative and medicinal value [4]. The antimicrobial activities of plant may be due to a variety of different ingredients, including peptides, unsaturated long chain aldehydes, alkaid components, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble constituents. These plants then appeared as compounds with potentially considerable therapeutic application against human pathogens, including bacteria, fungi or virus [5, 6]. For example the crude methanolic extracts of neem plant have been shown to have strong antibacterial activity [7].

Corresponding Author: Nabi Shariatifar, Department of Food Safety and Hygiene, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Tel: +989125091928.
In this paper, the essential oils from seed and leaf of parsley were screened for their antibacterial activity against five pathogenic bacteria strains.

**MATERIALS AND METHODS**

**Plant Samples:** The different parts of parsley such as seeds, leaves, and stems were purchased from the market. Leaves and stems were washed with distilled water, dried in dark for at least 7 days and then all parts of the plant were crushed to fine powder and stored at room temperature in the dark until used. Seeds were ground in a beaker and then keeping in laboratory until extraction procedure.

**Extraction of Essential Oil:** The seeds and leaves comminuted separately were placed in a hydro-distillation apparatus. For this purpose using a Clevenger apparatus for a minimum of 3h. The essential oils distilled in clean tubes, after the oils were dried over anhydrous sodium sulphate, they were stored in refrigerator until further analysis.

**Microorganisms:** Standard strains of microorganisms used in the present study included Staphylococcus aureus ATCC 25913, Escherichia coli ATCC 8739, Salmonella enterica PTCC 1709, Vibrio cholera PTCC 1611, and Yersinia enterocolitica PTCC 1477 prepared by the Scientific and industrial research center of Iran.

**Antimicrobial Activity Test**

**Assay by Disc Diffusion Method:** The antibacterial activity of the essential oil was appraised by the paper-disk agar diffusion method against the microorganisms. For each test, new cultures from 24h were prepared. And bacterial suspensions were diluted with normal saline, to obtain uniform suspensions of the Bacterial. Tubes were incubated for 30 min at 37°C. Final cell concentrations were about 10^8CFU/ml with reference to the 0.5 McFarland turbidity. The 0.1 ml of inoculum from the prepared culture was conveyed to Mueller-Hinton agar (MHA) medium. The inoculum was spread to surface of plates with a sterile swab. Sterilized filter paper disks (Whatman, 6 mm in diameter) were placed on the surface of the MHB and then by solvent dimethyl sulphoxide (DMSO) was prepared concentration of 10%, 30% and 50% from the essential oil. The sterile discs were impregnated with 10 µl volumes of each concentration.

These plates were incubated aerobically at 37°C for 24h. After incubation, diameters of bacterial growth inhibition zones around the paper disks were measured, recorded and data were analyzed.

**Determination of Minimum Inhibitory Concentration (MIC):** For quantitative tests to determine MIC, serial dilutions from essential oil were made with Mueller-Hinton Broth in a concentration range from 0.5 to 64%. The 96-well plates were prepared by dispensing into each well 100 µl of Mueller Hinton broth (MHB), 100 µl of the essential oil and 10 µl of the inoculum. A positive control (containing 100 µl inoculum and 100 µl MHB) was included on each micro-plate. And then the micro-plates were incubated at 37°C for 24h. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. The experiment was carried out in triplicate.

**Minimum Bactericidal Concentration (MBC) of Essential Oil:** MBC was determined by sub-culturing the 5 µl of test dilution from each well (in the least Dilution not turbidity, was observed) on to a Mueller Hinton Agar (MHA) plates and incubating at 37°C for 24h. The complete absence of growth at applied concentration was considered as the minimum bactericidal concentration.

**RESULTS AND DISCUSSION**

**Determination of MIC and MBC values:** MIC of the essential oil from seeds of parsley varied from 4 to 32%. And MIC of oil from leaves of parsley for pathogenic bacteria ranged from 0.5 to 1%. MBC value of parsley essential oil from seeds was obtained lowest against Vibrio cholera (8%), while it was obtained 16% against Staphylococcus aureus and has shown intermediate effect against Yersinia, While highest (64%) in cases of Escherichia coli and Salmonella. MBC value of the oil from leaves showed inhibitory effect in concentration from 0.125 to 1% (Table 1).

**Inhibition Zone Diameter:** The antibacterial activity of the essential oil from seeds and leaves of parsley was recognized by the presence or absence of inhibition zone and measuring the diameter of the inhibition zone around discs. Essential oil from seed of parsley has shown 11 mm inhibition zone diameter against Staphylococcus aureus, 10 mm against Escherichia coli and Yersinia, 10.5 mm against Vibrio cholera. While low growth inhibition zone diameters...
Table 1: Determination of MIC and MBC value (%) for parsley leaves and seeds essential oil against pathogenic bacterial strains

<table>
<thead>
<tr>
<th>Test</th>
<th>Seed MIC (%)</th>
<th>Leaf MIC (%)</th>
<th>Seed MBC (%)</th>
<th>Leaf MBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>0.25</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>4</td>
<td>0.125</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Yersinia</td>
<td>16</td>
<td>0.5</td>
<td>64</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>32</td>
<td>1</td>
<td>64</td>
<td>0.5</td>
</tr>
<tr>
<td>Salmonella</td>
<td>32</td>
<td>1</td>
<td>64</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 2: comparison of average inhibitory halo diameter (mm) of various bacterial strains for parsley seed essential oil

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Samples</th>
<th>Min</th>
<th>Max</th>
<th>Average ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>10</td>
<td>11.5</td>
<td>11 ± 0.87</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>9.5</td>
<td>11</td>
<td>10 ± 0.87</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>3</td>
<td>9.5</td>
<td>11.5</td>
<td>10 ± 1.32</td>
</tr>
<tr>
<td>Yersinia</td>
<td>3</td>
<td>9</td>
<td>11.5</td>
<td>10 ± 1.32</td>
</tr>
<tr>
<td>Salmonella</td>
<td>3</td>
<td>8.5</td>
<td>10</td>
<td>9 ± 0.87</td>
</tr>
</tbody>
</table>

Table 3: comparison of average inhibitory halo diameter (mm) of various bacterial strains for parsley leaf essential oil

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Samples</th>
<th>Min</th>
<th>Max</th>
<th>Average ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>13.5</td>
<td>15</td>
<td>14.5 ± 0.87</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>13</td>
<td>14</td>
<td>13.5 ± 0.5</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>3</td>
<td>12.5</td>
<td>15</td>
<td>14 ± 1.32</td>
</tr>
<tr>
<td>Yersinia</td>
<td>3</td>
<td>13</td>
<td>15.5</td>
<td>14.5 ± 1.32</td>
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<td>Salmonella</td>
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<td>12.5</td>
<td>12.5</td>
<td>12 ± 0.5</td>
</tr>
</tbody>
</table>

against Salmonella (9mm) (Table 2) and inhibition zone diameter of the essential oil from leaves varied from 12 to 14.5mm (Table 3).

Equal to increment the resistance of microorganisms to antibiotics currently used, Side effects and high cost of synthetic compounds, as a result of this, researchers are looking for natural products.

The results of this study showed that essential oil from the parsley inhibited the growth of five bacteria Escherichia coli, Salmonella, Staphylococcus aureus, Yersinia enterocolitica and Vibrio parahaemolyticus. It is proved by different MIC and MBC values obtained in essential oil when used against each bacterial culture. The MIC value of the essential oil from seed and leaf of parsley were obtained lowest against V.cholera, while highest The MIC value in case of E.coli and Salmonella. And essential oil tested has shown higher MBC values than MIC values against each bacterial strain. MBC value of the essential oil from seed of parsley was found to be lowest (8%) against Vibrio cholera and highest MBC value against E.coli and Salmonella. While lowest and highest MBC value from leaf of parsley was obtained against S.aureus, V.cholera (0.125%) and E.coli (1%).

Further effectiveness of essential oil was determined by agar disc diffusion method and inhibition zone diameters were measured in presence and absence of essential oil. Highest inhibition zone diameters of the essential oils from seed and leaf of parsley were obtained by S.aureus and lowest inhibition zone diameters were obtained by Salmonella.

In another work [8] has been reported that ethanolic extract of parsley seed had inhibitory effect at various concentration (0.1 to 0.4 gr.ml\(^{-1}\)) against Gram negative Br.melitensis, E.coli, P.mirabilis, P.aeruginosa and Gram positive. It was effective in high concentration (0.4 gr.ml\(^{-1}\)) on Salmonella typhi. Ethanolic extract of parsley didn’t inhibit the growth of B.subtilis, B.bronshiseptica and S. aureus. Similarly [9] showed that aqueous extract of the parsley leaf no inhibitory effect at various concentrations on E.coli, Salmonella typhi, S.aureus and other bacteria studied.

However, only the concentration 40 mg/ml was effective on P. aeruginosa. But phenolic extract of the parsley in concentration (>=20 mg.ml\(^{-1}\)) had inhibitory effect against E.coli, Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa. While no inhibitory effect on S.aureus. And methanolic extract of parsley has inhibitory effect against P. aeruginosa and Staph. aureus, Enterococcus and Salmonella typhi. And also ethanolic extract of parsley no inhibitory effect on S.aureus. On the other hand E.coli, Salmonella typhi and Pseudomonas
Aeruginosawere affected by the ethanolic extract of parsley at \( \geq 10 \text{ mg.ml}^{-1} \), while more than ethanolic extract of parsley at the concentration of 500 mg•ml\(^{-1}\) exhibited antibacterial effect against Proteus Mirabilis and in the concentration (\( \geq 100 \text{ mg.ml}^{-1} \)) had inhibitory effect on Staphylococcus aureus, while E.coli, B. Cereus, E. Faecalis, S. Choleraesuis were resistant to all concentrations used [10]. Similar antimicrobial activity is reported in essential oil act of the parsley at a dose of 40 l had inhibitory effect on listeria innocua and it was not active in the inhibition of S. marcescens and P. fluorescens at all added doses[11] According to a report of [12]. Methanolic extract of parsley (petroselinum crispum) leafe had inhibitory effect against Bacillussubtilis, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Psuedomonasaeruginosa and Salmonellalatyphi. The comparison of four resultswiththestudies showed that the essential oils from Seed and leaf of parsley have stronger anti bacterial effect than the extract.

**CONCLUSION**

In the present study, essential oil tested has shown a variable degree of antimicrobial activity on different microorganisms. Therefore using essential oil of parsley as antimicrobial additives in food may be useful and alternative medical therapy for microorganisms which may resist customary treatment. This will suggest a great help in facing the appearance spread of bacteria.

**ACKNOWLEDGMENT**

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