Antimicrobial Efficiency of Iranian Ziziphora clinopodiodes Essential Oil on Preservation of Hamburger

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INTRODUCTION

Essential oils can be defined as the volatile materials present in plants. Many spices and herbs have antimicrobial activity due to their essential oil fractions. Phenolic components, present in essential oils, have been known to possess antimicrobial activity and some are classified as generally recognized as safe (GRAS) substances and therefore could be used to prevent post-harvest growth of native and contaminant bacteria [1]. Recent plant extracts and essential oils have been used in a variety of foods, beverages and confectionery products due to their antioxidant and antimicrobial activity [2]. According to World Health Organization (WHO) 65-80% world population relies on traditional medicine to treat various diseases and 50% of all modern clinical drugs are of natural product origin [3]. Hamburger with high nutrient supply and a loosely-packed structure present favorable conditions for microbial growth [4]. The literature states a level of 10⁵ CFUg⁻¹ is seen as acceptable for cooked meat products. It was also reported that about 40% of patties have not been cooked well enough to obtain acceptable reduction in their microbial counts. The required microbial reduction was stated to be 2 log or greater [5]. Avishan baric is the persian name for Ziziphora clinopodiodes, belonging to the family Labiatae, and it is native to Iran. Ziziphora clinopodiodes is used traditionally in food, especially in yoghurt and meat flavoring. There are also commercial pharmaceuticals based on Ziziphora clinopodiodes essential oil [6]. The literature survey could ascertain that in-vitro antimicrobial activities of essential oils are documented but their effectiveness in model food systems is poorly elucidated. Important variables appear when essential oils are applied in food systems such as higher resistance of natural food microflora than laboratory grown bacteria [7]. Previous studies showed that metal contents Ziziphora clinopodiodes depends on the collecting region [8]. It seems that the initial flowering stage and the leaves have the highest antimicrobial activity [9]. The aim of the present study is the investigation of chemical composition and antimicrobial activity of essential oil of Iranian Ziziphora clinopodiodes and its potential application as a natural preservative in hamburger.

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http://jommid.pasteur.ac.ir
**MATERIAL AND METHODS**

**Plant material.** The plants were collected from Karaj (Alborz province, Iran). The dried plant was stored in the herbarium of faculty of pharmacy, Tehran University of Medical Sciences. The sample was ground in a blender to produce a fine powder. The average particle size was 0.4 mm.

**Essential oil extraction.** The oil of the plant (50 g) was obtained by Hydro-distillation method (HDM) in a Clever type apparatus (AOAC, 1990) for 4 h. The oil sample was stored in glass vials with Teflon-sealed caps at 2 ± 0.5°C in the absence of light.

**Gas chromatography (GC).** GC analysis was performed using HP 6890 gas chromatograph equipped with a FID and DB1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed at 250°C at a rate of 4°C/min. Injector and detector temperature were 250°C and 265°C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s and 1 μl of sample dissolved in CH₂Cl₂ was injected.

**Gas chromatography/mass spectrometry (GC-MS) analysis.** GC conditions were the same as reported, and the same column was used. MS conditions were as follows: ionization voltage, 70 eV; ion source temperature was 260°C scan rate, 1 scan/s. Identifications of components of the oil were based on retention indices relative to normal alkanes and computer matching with the Wiley 275 library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in literature.

### Table 1. Essential oil composition of Ziziphora clinopodioides

<table>
<thead>
<tr>
<th>No.</th>
<th>KI</th>
<th>Compound</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>939</td>
<td>Alpha-Pinene</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>1017</td>
<td>Alpha- Terpinene</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>1025</td>
<td>Para-Cymene</td>
<td>0.83</td>
</tr>
<tr>
<td>4</td>
<td>1031</td>
<td>1,8-Cineole</td>
<td>0.85</td>
</tr>
<tr>
<td>5</td>
<td>1060</td>
<td>Gamma-Terpinene</td>
<td>1.06</td>
</tr>
<tr>
<td>6</td>
<td>1073</td>
<td>Para-Mentha-3,8-diene</td>
<td>0.50</td>
</tr>
<tr>
<td>7</td>
<td>1153</td>
<td>Menthone</td>
<td>0.45</td>
</tr>
<tr>
<td>8</td>
<td>1163</td>
<td>Trans-beta-Terpinol</td>
<td>0.54</td>
</tr>
<tr>
<td>9</td>
<td>1162</td>
<td>Isoborne</td>
<td>1.49</td>
</tr>
<tr>
<td>10</td>
<td>1237</td>
<td>Pulegone</td>
<td>4.88</td>
</tr>
<tr>
<td>11</td>
<td>1290</td>
<td>Thymol</td>
<td>12.51</td>
</tr>
<tr>
<td>12</td>
<td>1299</td>
<td>Carvacrol</td>
<td>54.31</td>
</tr>
<tr>
<td>13</td>
<td>1352</td>
<td>Thymol acetate</td>
<td>1.69</td>
</tr>
<tr>
<td>14</td>
<td>1400</td>
<td>Tetradecane</td>
<td>0.21</td>
</tr>
<tr>
<td>15</td>
<td>1409</td>
<td>Caryophyllene</td>
<td>2.19</td>
</tr>
<tr>
<td>16</td>
<td>1500</td>
<td>Pentadecane</td>
<td>0.32</td>
</tr>
<tr>
<td>17</td>
<td>1506</td>
<td>cis-α Bisabolene</td>
<td>0.39</td>
</tr>
<tr>
<td>18</td>
<td>1600</td>
<td>Hexadecane</td>
<td>0.35</td>
</tr>
<tr>
<td>19</td>
<td>1700</td>
<td>Heptadecane</td>
<td>0.35</td>
</tr>
<tr>
<td>20</td>
<td>1800</td>
<td>Octadecane</td>
<td>9.51</td>
</tr>
</tbody>
</table>

Note. *Kovats Index

The essential oil yielded 2.5% essential oil content by HDM. After GC-MS analysis; out of 21 peaks (representing 99.99% of the oil), 20 compounds were identified representing 93.36% of the composition (Table 1).

**Antimicrobial activity.** The microorganism strains were obtained from the Department of Drug and Food Control, College of Pharmacy, Tehran University. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used a stock culture. Antibacterial activity of essential oil was determined; using the Agar dilution method (ADM) and the oil was dissolved in ethanol. The inocula were adjusted photo metrically at 600 nm in the cell density equivalent a 0.5 McFarland standard. Culture medium without oil and others without microorganisms was used in the tests as control [10].

Inoculated plates were incubated at 37°C for 24 h and the Minimum Inhibitory Concentration (MIC) was determined [11]. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing different concentration of the oil was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of the oil inhibiting visible growth of each organism on the agar plate [12]. After all, the antimicrobial efficiency of the essential oil in hamburger was determined. The samples were stored at 25°C, 4°C and -12°C.

### RESULTS

Dried aerial parts of Ziziphora clinopodioides yielded 2.5% essential oil by HDM. After GC-MS analysis; out of 21 peaks (representing 99.99% of the oil), 20 compounds were identified representing 93.36% of the composition (Table 1).

**DISCUSSION**

The major compounds based on HDM were carvacrol (54.31%), thymol (12.51%), octadecane (9.51%) and pulegone (4.88%). In this study, a quantitative and qualitative comparison of essential oil constituents of Ziziphora clinopodioides with the other reported species showed different compositions.

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Fig. 1. The effect of different concentration of essential oil of *Ziziphora clinopodiodes* on total microbial counts of hamburgers stored at 25°C.

Fig. 2. The antimicrobial activity of essential oil on hamburgers stored at 4°C, during different storage times.

Fig. 3. The antimicrobial activity of essential oil on hamburgers stored at -12°C, during different storage times.
It may be the cause of plant origin. In this investigation *Ziziphora clinopodioides* for the first time were collected from Baraghan (15 km North-West of Karaj in Alborz province, Iran), during flowering time in July. Many phytochemical studies have investigated the chemical composition of essential oils of *Labiatae* family plants; from different sources and chemo types as well as its variation in different seasons and during the plant life cycle [14]. Evaluations of the oil composition extracted from different parts of the plant or upon variable environment, cultivation, and/or storage conditions have also been reported [15]. The variation in the chemical composition of the hydro distilled essential oil of thymus, from the aerial parts of different growth stages during the plant vegetative cycle (particularly during flowering) was investigated as well. For better characterization of the summer-winter variations, the oil from plants that were still growing through November/December period was also hydro distilled. Finally, the oils from young and old plant clusters were concomitantly analyzed and compared. The study, therefore, emphasized the importance of choosing the appropriate collection (harvest) period of thyme herbs in order to achieve the highest quality and quantity of the essential oil, whose activity is known to be essentially correlated with the content of phenol components [16].

In Salehi study the efficacy of *Ziziphora* against *S. aureus* ATCC 25923, *Salmonella* epidermidis ATCC 12228, *Bacillus subtilis* ATCC 9372, *Entercococcus faecalis* ATCC 15753, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27852, and *Klebsiella pneumonia* ATCC 3583 was investigated [8]. And he showed that all extracts have Acceptable antimicrobial efficacy [8]. The initial microbial load of untreated hamburgers was 10^5 to 10^6 CFU g⁻¹. During 24 h storage at 25°C about 2 log increase in microbial population of controls and hamburgers treated with 0.020% (0.20 mg/ml) to 0.04% (0.4 mg/ml) of essential oil was observed, but significant (p<0.05) microbial reduction of 1.8, 2.6 and 2.7 log were detected in samples treated with 0.05%, 0.075%, 0.1% (0.5, 0.75 and 1 mg/ml) of essential oil, respectively (Figure 1). Chemical components derived from the Iranian plant differ a little from Chinese *Ziziphora clinopodioides* [9]. Thymol acetate percentage was higher in our plant and Alpha-Pinene concentration was more in Chinese *Ziziphora clinopodioides* [9].

In this study, the required concentration of *Ziziphora clinopodioides* essential oil to produce effective reductions in microbial population of hamburgers with desirable sensory characteristics was evaluated by a group of trained panelists who were blinded to the product being tested. Sensory evaluations demonstrated that there were no significant differences (p<0.05) changes in organoleptic attributes of hamburgers due to essential oil in comparison to the control were observed at 0.75 and 1 mg/ml. The general acceptance scores of the samples decreased by increasing essential oil concentrations and samples treated with 0.75 and 1 mg/ml essential oil were unacceptable by the panelists.

In this study, the antimicrobial efficacy of the *Ziziphora clinopodioides* essential oil was found as a bio preservative of hamburger. The results indicated that the use of essential oil in concentration of more than MIC, resulted in about 2 to 3 log reduction in total microorganisms during prolonged storage time (14 and 28 days) at 25°C and 4°C. We found that freezing of hamburgers could inhibit the microbial proliferation in untreated controls, but freezing of essential oil treated hamburgers might reduce the risk of disease associated with consumption of under cooked hamburgers through significant microbial reduction. The highest total microbial reduction was observed in samples treated with 0.5, 0.75 and 1 mg/ml of essential oil during 14 days at -12°C.

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**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest associated with this manuscript.

**REFERENCES**