Original Article

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

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Introduction: In this study, the chemical composition and antimicrobial activity of essential oil of *Ziziphora clinopodiodes* and its potential application as a natural preservative in reducing the indigenous microbial population of hamburger were investigated. **Method:** Essential oil of *Ziziphora clinopodiodes* cultivated in Iran was obtained by Hydro-distillation method (HDM). Chemical composition of the oil was determined by gas chromatography/mass spectrometry (GC-MS) analysis. Antimicrobial activity of the essential oil was checked against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, by using Agar dilution method (ADM). Minimum Inhibitory Concentration (MIC) values of each active oil concentration were determined and its potential application as a natural preservative in reducing the indigenous microbial population of hamburger was investigated. **Results:** The major components were carvacrol (54.31%), thymol (12.51%), octadecane (9.51%) and pulegone (4.88%). The results showed a significant activity against the tested strains (gram-positive and gram-negative bacteria). Addition of essential oil in concentration higher than MIC values reduced the microbial population of hamburgers stored at 25°C, 4°C and -12°C. In samples refrigerated at 4°C, differences between the controls and samples treated with essential oil at MIC values (0.20 and 0.4 mg/ml) were not significant during the first 24 h ($p > 0.05$), but higher concentration of essential oil resulted in about 2 to 3 log reduction in total microorganisms. **Conclusion:** This study showed that the *Ziziphora clinopodiodes* essential oil can be added to the ingredients of foods as the natural antibacterial agent. *J Med Microbiol Infec Dis, 2014, 2 (4): 138-142*

Keywords: Essential oil, *Ziziphora clinopodiodes,* Minimum Inhibitory Concentration.

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 10^3 $CFUg^{-1}$ 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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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 Clevenger type apparatus (AOAC, 1990) for 4 h. The oil sample was stored in glass vials with Teflon-sealed caps at 2 ± 0.5ºC 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 250ºC at a rate of 4ºC/min. Injector and detector temperature were 250ºC and 265ºC, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s and 1 µl of sample dissolved in CH_2Cl_2 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 260ºC 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.

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 4ºC 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 37ºC 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 clinopodiodes* 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).

Table 1. Essential oil composition of *Ziziphora clinopodiodes*

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

Note. *Kovats Index

The essential oil exhibited a broad spectrum antimicrobial activity. MICs of the essential oil were 0.020% (0.20 mg/ml) against *Staphylococcus aureus* and 0.04% (0.4 mg/ml) against *Escherichia coli, Bacillus subtilis, Salmonella typhi, Listeria. monocytogenes, Pseudomonas aeruginosa,* by using ADM [13] (Figure 1).

In samples refrigerated at 4°C (Figure 2), differences between the controls and samples treated with essential oil at MIC values (0.20 and 0.4 mg/ml) were not significant during the first 24 h ($p > 0.05$), but higher concentration of essential oil resulted in about 2 to 3 log reduction in total microorganisms. The inhibitory effect of essential oil was overcome by the microorganisms during prolonged storage time (14 and 28 days) at 4°C.

During storage at -12°C (Figure 3) there were no significant and practical differences between microbial count of controls and samples treated with essential oil at MIC values, but about 1 to 2 and 2.5 to 3.5 log decreases in total microbial counts were observed at higher concentration of essential oil during 24 h and 7 days storage, respectively. 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.

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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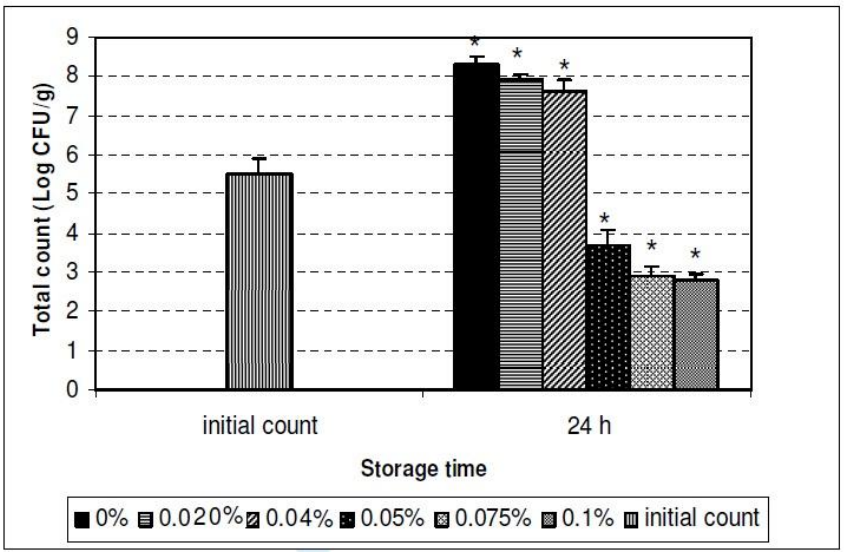

Fig. 1. The effect of different concentration of essential oil of *Ziziphora clinopodiodes* on total microbial counts of hamburgers stored at 25°C.

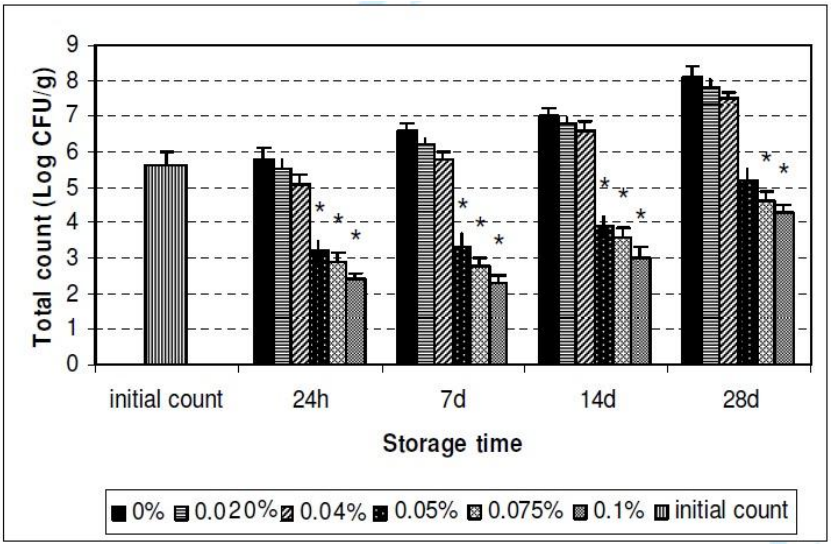

Fig. 2. The antimicrobial activity of essential oil on hamburgers stored at 4°C, during different storage times.

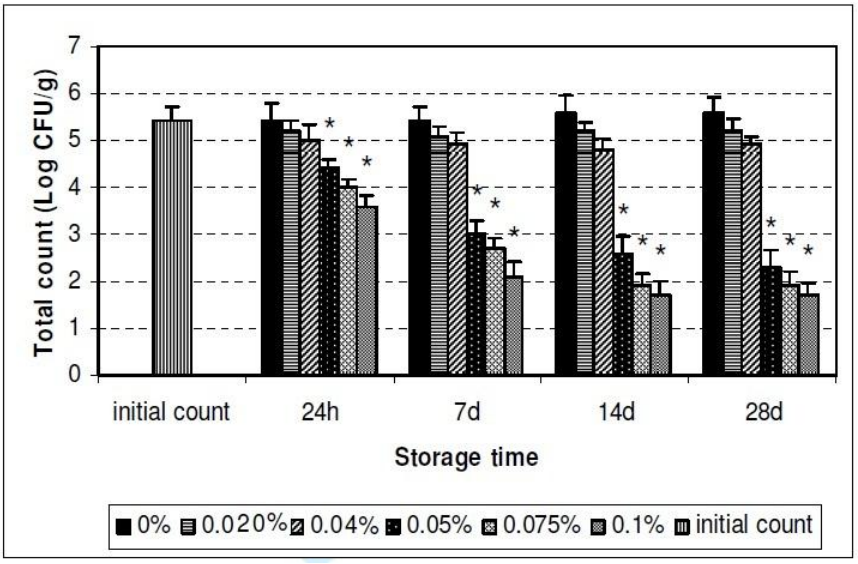

Fig. 3. The antimicrobial activity of essential oil on hamburgers stored at -12°C, during different storage times.

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It may be the cause of plant origin. In this investigation *Ziziphora clinopodiodes* 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, *Enterococcus 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 $CFUg^{-1}$. 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 clinopodiodes* [9]. Thymol acetate percentage was higher in our plant and Alpha-Pinene concentration was more in Chinese *Ziziphora clinopodiodes* [9].

In this study, the required concentration of *Ziziphora clinopodiodes* 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 clinopodiodes* 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.

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