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Antimicrobial Activity of *Ziziphora clinopodioides* Essential Oil and Extract on *Salmonella enterica, Staphylococcus aureus* and *Saccharomyces cerevisiae* in Low Fat Mayonnaise

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Abstract

***Ziziphora clinopodioides* is a plant from Lamiaceae family. In this research the antimicrobial activity of *Z. clinopodioides* essential oil (at 0.05%) and extract (at 0.4%) were studied on *Salmonella enterica, Staphylococcus aureus* and *Saccharomyces cerevisiae* in mayonnaise during storage time at 4°C. Results showed that the antimicrobial effect of *Z. clinopodioides* essential oil was higher than the extract. This effect increased with increasing the concentration rate. The *Z. clinopodioides* essential oil was able to inhibit both gram-positive and gram negative bacteria and also the yeast. At the concentrations of *Z. clinopodioides* essential oil and extract we tested in the current study, we observed no unfavorable effect on the sensory parameters in mayonnaise.**

Keywords: ***Mayonnaise; Ziziphora clinopodioides; Salmonella enterica; Staphylococcus aureus; Saccharomyces cerevisia***

Introduction

Mayonnaise is an oil in water (o/w) emulsion [1]. It is traditionally prepared by fully mixing egg yolk, oil, vinegar and spices including mustard to maintain closely packed foam of oil droplets; it may also include salt, sugar or sweeteners, and other optional ingredients. The emulsion is formed by slowly blending oil with a pre-mix that consists of egg yolk, vinegar, and mustard [2]. Mayonnaise has a low pH value (3.7-4.2) [3,4] and water activity (0.93-0.95) [3,5]. By adding acetic acid to prevent growth of vegetative cells of pathogenic microorganisms [4] and potassium sorbate as an antifungal agent [5], microbiological deterioration of mayonnaise is controlled [1].

Plant essential oils and extracts are known for antimicrobial activity. Wild Kakoty is a genus of *Ziziphora* [6] which is one-year-old plant, with short stems (5 cm to 15 cm) and sharp leaves is scattered in many parts of Iran [6] including mountainous regions of Alborz, Karaj, Pole Jajrud, Tehran, Isfahan, Khorasan, Damghan, Semnan, Azna, Qom, Hamedan, Baluchestan and Azarbijan province especially in mountains of Tabriz [6,7]. There have been identified four species of this plant

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including *Ziziphora clinopodioides* (mountains' Kakoty), *Ziziphora capitata*, *Ziziphora persica* and *Ziziphora tenuir* in Iran [6]. Among the healing properties of this plant, sputum collection, carminative and stomach reinforcement can be listed. In some areas the dust of its grains mixed with honey is used to treat dysentery [6]. In different areas, the plant's powder is used as a garnish on yogurt and dairy products [8]. Also, it is used for treatment of diseases of the stomach and as an antiseptic to relieve colds [9]. The aim of this study was to investigate the antibacterial activity of essential oil and ethanolic extract of *Z. clinopodioides* on the log (cfu/g) *Salmonella enterica*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* in low fat mayonnaise.

Materials and Methods

Z. clinopodioides were collected from the suburb of Shirvan city, Khorasan shomali province, Iran. The essential oil of *Z. clinopodioides* was purchased from Iranian Company Magnolia Saveh (Markazi Province, Iran).

Microbial strains and culture media

Lyophilized bacteria; *S. aureus* (ATCC: 13565), *S. enterica* (ATCC: 13076) and *S. cerevisiae* (ATCC: 7754) purchased from Persian Type Culture Collection (PTCC), Tehran-Iran and cultured in specific media (Merck-Germany) and incubated at 37°C for 24 h for bacteria and 25°C for 48 h for yeast.

Extraction procedure

For this purpose, maceration was used. 200 g powdered aerial parts of the plant was immersed in one liter of water: ethanol (50:50) and mixed for 48 h on a shaker (Heidolph-Germany) at room temperature and then the solvent was removed by rotary evaporator (Heidolph-Germany). The obtained dried extracts stored in black containers and at 4°C [10].

Preparation of microbial suspension

For the bacterial suspension, 24 h cultures were needed. For this purpose, the bacterial species were inoculated onto nutrient agar and incubated for 24 h at 37°C. The colonies were washed with normal saline and the bacterial suspensions were diluted to reach turbidity equal to standard tube 0.5 McFarland (equal to 1.5×10^8 CFU/ml) [11-13]. Further dilution was done to obtain a concentration of 1.0×10^5 CFU/ml [14].

Treatment category

Mayonnaise was prepared using the suggested formula according to Ghasr Aftab Parmin company as following: water (48 g), salt (1.5 g), mustard (0.25 g), sugar (6 g) and white pepper (0.05 g) were mixed with fresh whole egg (4 g), vinegar (6 g) and lemon juice (0.8 g) using blender on low velocity for 60 s; The previous mixture called the aqueous phase. The oil (33 g) was slowly added to the system during the first 30 min. In order the evaluate the antimicrobial effect of *Z. clinopodioides*, ethanolic extract (0.4%) and essential oil (0.05%) of *Z. clinopodioides* was added to the oily phase to make final concentration in mayonnaise, microbial suspension (1×10^5 CFU/ml) was added to samples and mayonnaise samples were stored at 4°C.

Determination of inhibition zone

Cup-Plate method was used for this purpose and performed in triplicate on the surface of plates filled with MHA (Muller-Hinton Agar, Merck, Germany) medium and the diameters of clear zones were determined [10].

Statistical analysis

This test was evaluated as a completely randomized factorial design with 3 replicates and the models were shown using Excel.

Results

The inhibitory effect of essential oil and *Z. clinopodioides* extract against *S. enterica*, *S. aureus* and *S. cerevisiae* are shown in TABLE 1 and FIG. 1.

TABLE.1. **The mean ± SD of clear zone diameter of essential and extracts in different microorganism (mm).**

Plant		Concentration (mg/ml)	*S. aureus*	*S. enterica*	*S. cerevisiae*
Z. clinopodioides	**Essential**	50	14 ± 0	16.66 ± 0.44	13.33 ± 0.88
	Extract	50	12.33 ± 0.88	10 ± 0	10 ± 1.33

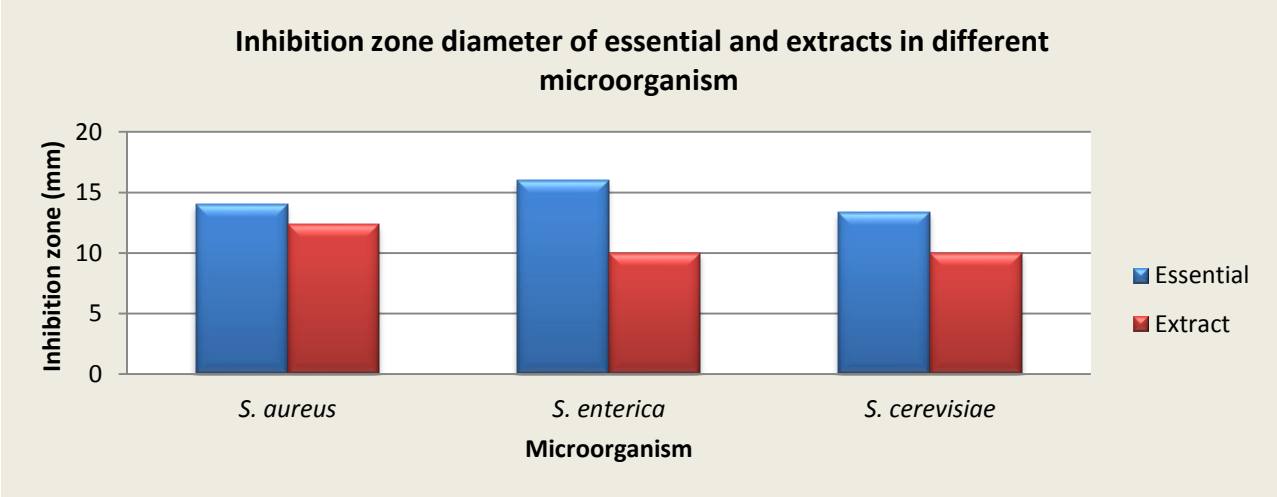

FIG. 1. **Inhibition zone diameter of essential and extracts for different microorganism (mm).**

The result of all antimicrobial activity assays of essential oil and *Z. clinopodioides* extract on mayonnaise after one day are described in TABLE 2.

TABLE. 2. **Antimicrobial effect of essential oil and extract of *Z. clinopodioides* after one day.**

Bacteria	***S. aureus***	***S. enterica***	***S. cerevisiae***
Essential oil	-	+	4.5×10^2
Extract	-	+	10^3

Note: The symbol (+) indicates the growth of microorganism and the sign (-) indicates the absence growth of microorganism.

The result of all antimicrobial activity assays of essential oil and extract of *Z. clinopodioides* on mayonnaise after 14 days are described in TABLE 3.

TABLE. 3. **Antimicrobial activity of essential oil and extract of *Z. clinopodioides* after 14-day.**

Bacteria	*S. aureus*	*S. enterica*	*S. cerevisiae*
Essential oil	-	-	-
Extract	-	+	-

Note: The symbol (+) indicates the growth of microorganism and the sign (-) indicates the absence growth of microorganism.

Results showed antibacterial activity of the essential oil and extract in which and the essential oil was more active against the bacteria [13]. The essential oils investigated showed better activity against gram-positive than gram-negative bacteria [14]. Our findings show that the antimicrobial effect of essential oil was more than the extract of *Z. clinopodioides.* Microbial testing results showed that *Z. clinopodioides* essential oil can be used to inhibit gram-positive and gram-negative bacteria and yeast. *Z. clinopodioides* extract was not able to control *Salmonella*, in spite of gram-positive bacteria and yeast.

Discussion

Increasing the resistance of microbes to antimicrobial agents and also looking for new and safe compounds have led researchers to evaluate plants and their active compounds for their antimicrobial properties. In this study, we assayed ethanolic extract and *Z. clinopodioides* essential oil on two foodborne bacteria and one yeast. *Ziziphora* contains many phytochemical substances such as terpenoids and phenolic compounds [15-17]. Among the terpenoids, thymol and carvacrol, rank highest in importance [18]. Total phenolic content is not an absolute measurement of the amount of phenolic materials [19] and the interaction between some of the existing compounds may be responsible for the effect. It is well-known that phenolic compounds affect nutritional value, taste, aroma, and flavor and also have health-beneficial effects. They also contribute in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent damage by microorganisms and insects. Generally, the differences between the effects of herbal extracts and essential oils are probably related to the type and amount of phenolic compounds [10].

On the other hand, some researchers have shown pulegone in *Z. clinopodioides* with anti-fungus and antibacterial for *Salmonella* bacteria. Another essential substance is the oil of blue mint bush that affects against gram-negative bacteria *E. aerogenes*, *K. pneumoniae*, *S. enteritidis* and some gram positive bacteria as *B. cereus*, *S. aureus* [20,21]. The inhibitory effect of hops and *Z. clinopodioides* aqueous, acetonic and ethanolic extracts were suitable in agar-well diffusion method. All of hop extracts were shown inhibition zone further than *Z. clinopodioides* extracts between 1:10 to 1:1280 of dilutions. Antimicrobial effect of hops extract on *Brucella abortus* and *B. melitensis* were shown in previous studies [22-24].

Represents the efforts of researchers to replace the natural preservatives derived from plant, animal, and microbial sources instead of chemical preservatives. Analysis of essential oils from different plants showed the presence of different combinations.

The original composition of the essential oils of mint family's plants is Thymol and carvacrol. The strong anti-microbial effect of carvacrol has been expressed by the researchers [25,26].

Ozturk and Ercisli [27] showed that the essence of mountains' Kakoty contained 31.86% Pulegone, 12.21% Senion, 10.48% Limonen, 9.13% Menthol, 6.88% beta-pinene, 6.73% Menton, 3.5% Peperitnon and 4.18% Peperiton. The main component

of the essential oils of some the mint family's plants including Kakoty is Pulegone. Pulegone has antibacterial and antifungal properties and is particularly effective for the different isolates of *Salmonella*.

According to our findings, Kakoty's essential oil showed more antibacterial activity compared to methanolic extract which has been probably due to Pulegone which is the main constituent of mountains' Kakoty's essence. Salehi et al. [28] studied the antimicrobial effect of Kakoty's extract and showed that mountains' Kakoty's extract could inhibit the growth of gram-negative bacteria *K. pneumoniae* and *E. coli*. The weak antibacterial activity of the extract against mentioned bacterial species observed in the current study is in agreement to the results of other studies [29]. Yet, Salehi et al. [28] showed that the extract could inhibit the growth of *S. epidermidis* and *B. subtilis*. Ozturk and Ercisli [27] also showed that mountains' Kakoty extract and persica Kakoty were capable to prevent growing a wide range of gram-positive and gram-negative pathogenic bacteria.

The results of this study showed that the mountains' Kakoty essence has good anti-bacterial effect on under test gram-negative bacteria. Based on Baser et al., [7] the anti-bacterial effect of Kakoty essence native for Turkey has been observed on gram-positive bacteria, *S. aureus* and *B. subtilis*. Salehi, et al. [28] showed that mountains' Kakoty essence could prevent the growth of gram-positive bacteria, *B. subtilis* and *S. aureus*. Most studies suggest that the susceptibility of gram-negative bacteria against antibacterial compounds are less than gram-positive ones which may be due to the presence of outer membrane in the structure of their cell walls. Gram-positive bacteria have a large amount of mucopeptide compositions in their cell wall while gram-negative bacteria have only a thin layer of mucopeptide and much of their cell wall's structure are made of lipoprotein and lipopolysaccharide (LPS) and it seems that for this reason they are more resistant to anti-bacterial Substances and these results are consistent with the results obtained in this study [30].

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