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Study on the antimicrobial effect of *Ziziphoraclinopodioides* essential oil and extract on *Salmonella enterica, Staphylococcus aureus* and *Saccharomyces cerevisiae* in low fat mayonnaise

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ABSTRACT

Ziziphoraclinopodioides is a plant from Lamiaceae family. In this research the antimicrobial effect of *Ziziphoraclinopodioides* essential oil (at 0.05%) and extract (at 0.4%) were studied on *Salmonella enterica*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*in mayonnaise during storage timeat 4°C. Results showed that the antimicrobial effect of *Z.clinopodioides*essential oil was higherthan the extract. This effect increased with increasingthe concentration rate. The *Ziziphoraclinopodioides* essential oil was able to inhibit both gram-positive and gramnegative 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; *Ziziphoraclinopodioides*; *Salmonella enteric*; *Staphylococcus aureus*; *Saccharomyces cerevisiae*.

INTRODUCTION

Mayonnaise is an oil in water (o/w) emulsion[21]. It is traditionally prepared by fully mixing a mixture of egg yolk, vinegar, oil, and spices (especially 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[7]. Mayonnaise has a low pH value (3.7–4.2)[6,17] and water activity (0.93–0.95)[6,12]. By adding acetic acid to prevent growth of vegetative cells of pathogenic microorganisms[17] and potassium sorbate as an antifungal agent[12], microbiological deterioration of mayonnaise is controlled[21].

Essential oils, plant extracts and their constituents are known for anti-bacterial effects. Wild*Kakoty* is a genus of *Ziziphora* and breed of mint. Among the important species of this breed mint, *thyme* and *dried marjoram*, *lavender*, *marjoram* and*Kakoty* can be listed[2]. *Kakoty* is anherbaceous plant; with one year old lifetime, withshort stems, 5-15 cm tall and thin, sharp leaves thatare scattered in many parts of Iran[2]. This plant groves in wild state and in vast areas ofIran like mountainous regions of Alborz, west Iran, Karaj, Pole Jajrud, southwest of Tehran, Dushan tape, Isfahan, khorasan, Damghan, Semnan, Azna, Qom, Hamedan, Baluchestan and northern mountainousregions like Manjil, Azarbijan provinces, especiallyin mountains of Tabriz[2,8]. Four species of plant called *Ziziphoraclinopodioides* (*mountains' Kakoty*) *Ziziphoracapitata*, *Ziziphorapersica* and *Ziziphoratenuir* havebeen identified in Iran[2]. Among thehealing properties of this plant, sputum collection, carminative and stomach reinforcement can belisted. In some areas the dust of its grains mixedwith honey is used to treat dysentery[2]. In different areas, the plant's powder is used as agarnish on yogurt and dairy products[23]. Also, it is used for treatment of diseases of thestomach and as an antiseptic to relieve colds[19]. Despite the heavy use ofplants in the mint family flavors in Iran, systematicresearch have been performed on antibacterial effectsof the *mountains' Kakoty's* extract on pathogenicbacteria. This study aimed to investigate theantibacterial activity of essential oil and ethanolextract of *Ziziphoraclinopodioides* on the log (cfu/g) *salmonella enterica*, *staphylococcus aureus* and *saccharomyces cerevisiae* in low fat mayonnaise.

MATERIALS AND METHODS

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

Microbial strains and culture media

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

Extraction procedure

The extracts obtained by maceration; 200 g of powdered aerial parts of each plant were immersed in 1000 mL of ethanol (Merck, Germany) and water in equal proportions. Samples were mixed for 48 h on a shaker (Heidolph, Germany) at room temperature. The extraction of the plants samples were repeated for 2 more times. The solvent of the extract was evaporated by rotary evaporator (Heidolph, Germany). Dry extracts were stored in sterile and light protected containers and at 4°C[1].

Preparation of microbial suspension

For preparation of such suspension; a 24 hours culture of each bacterium is needed. Hence, 24 hours before the test; the stored cultures were inoculated into nutrient agar medium and incubated for 24 h at 37°C. The colonization of the medium was washed with normal saline solution and bacterial

suspensions were diluted with normal saline and their turbidity was set equivalent to turbidity of standard tube 0.5 McFarland. The test suspension contained 1.5×10^8 CFU/ml[9,11,29].

The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The inoculums were prepared daily and stored at +4˚C until use. Dilutions of the inoculum were cultured on solid medium to verify the absence of contamination and to check the validityof the inoculum[16].

Treatment category

Mayonnaise was prepared using the suggested formula according to GhasrAftabParmincompany as following: water (48g), salt (1.5g), mustard (0.25g), sugar (6g) and white pepper (0.05g) were mixed with fresh whole egg (4g), vinegar (6g) and lemon juice (0.8g) using blender on low velocity for 60 sec; The previous mixture called the aqueous phase. The oil (33g) was slowly added to the system during the first 30 min. In order the evaluate the antimicrobial effect of *Ziziphoraclinopodioides*, 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 (1×10^5CFU/ml) was added to samples and mayonnaise samples were stored at 4˚C.

Determination of inhibition zone

Pre-evaluation of antimicrobial effects of the extracts were performed using Cup-Plate method. The microbial suspensions of each bacterium with turbidity correspond to 0.5 McFarland (1×10^8Microorganisms) were prepared by normal saline, spectrophotometrically and spread thoroughly on the surface of plates filled with MHA (Muller-Hinton Agar, Merck, Germany) medium. Cup-plate method was performed 3 times and the average diameters of inhibition zones for different concentrations were determined[1].

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 ofessential oil and *Ziziphoraclinopodioides* extract against *Salmonella enterica*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* are shown in TABLE 1.

TABLE 1: The mean (±SD) of inhibition zone diameter of essential and extracts in different microorganism (mm)

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

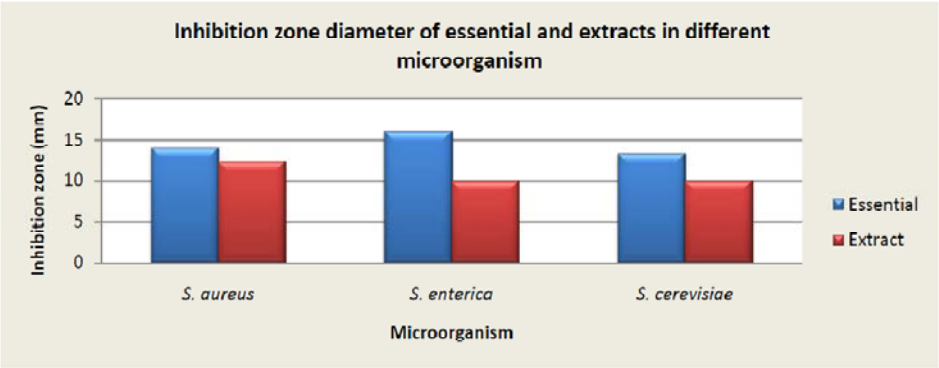

Figure 1: Inhibition zone diameter of essential and extracts for different microorganism (mm)

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

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

Plant		*S.aureus*	*S. enterica*	*S. cerevisiae*
Ziziphoraclinopodioides	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*Ziziphoraclinopodioides* on mayonnaise after 14 day are described in TABLE 3.

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

Plant		*S.aureus*	*S. enterica*	*S. cerevisiae*
Ziziphoraclinopodioides	Essential oil	-	-	-
	Extract	-	+	-

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

The results of this study showed that, in general, with comparison of the inhibitory effect andgermicidal effects of the essence and extract of *mountains' Kakoty,* we can conclude that the essence of this plant compared to its extract and in its low concentrations is able to inhibit the growth of under study bacteria[29]. The essential oils investigated showed better activity against gram-positive than gram-negative bacteria[16]. Our findings show that the antimicrobial effect of essential oil was more than the extract of *Ziziphoraclinopodioides.* Microbial testing results showed that *Ziziphoraclinopodioides* essential oil can be used to inhibit gram-positive andgram-negative bacteria and yeast. *Ziziphoraclinopodioides*extract was not able to control Salmonella, in spite of gram-positive bacteria and yeast.

DISCUSSION

Increasing resistance to antimicrobial agents and finding new and relatively low-risk compounds from different natural plants, oriented researchers to evaluate the effect of plants and their active compounds. On the other hand comparing the antibacterial effect of these plants is important for choosing the most appropriate ones. In this study, the effect of ethanolic extract and *ziziphoraclinopodioides* essential oil of Lamiaceae family against two foodborne bacteria and one yeast were investigated and compared. The plants of genera *Ziziphora*contain many phytochemical substances including terpenoids and phenolics. Chemical compositions of these genera are fairly known at least about two of the most important secondary compounds; volatile oils and phenolic compounds[3,15,28]. Among terpenoids, the phenolic terpenes;thymol and carvacrol, rank highest in importance[4]. According to the results obtained in this study, the antimicrobial effects of the extracts and observed differences may be due to other compounds such as phenolics. On the other hand, total phenolic content determined by Folin-Ciocalteu method is not an absolute measurement of the amount of phenolic materials[14] and it is possible that low-concentration components or interaction between some of the constituents are responsible for the antimicrobial effects. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health-beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores. In general, the differences among the effects of essential oil and extract these plant are

probably related to the amount and type of the phenolic compounds, remaining volatile compounds, trace compounds or maybe interaction between constituents[1].

Onthe other hand some researchers have shownpulegone in *Ziziphoraclinopodioides*withanti-fungus and antibacterial for *Salmonella*bacteria. Another essential substanceis the oil of blue mint bush that affects againstGram-negative bacteria *E.aerogenes*, *K.pneumoniae*, *S.enteritidis*and some Grampositivebacteria as *B.cereus*, *S.aureus*. Similar results were found by Kivane andAlgal in Turkey[24,25]. The inhibitory effect ofhops and *Ziziphoraclinopodioides*aqueous, acetonic and ethanolic extracts were suitable inagar-well diffusion method. All of hop extractswere shown inhibition zone further than*Ziziphoraclinopodioides*extracts between1:10 to 1:1280 of dilutions. Antimicrobialeffect of hops extract on intramacrophage*Brucellaabortus*and *Brucellamelitensis*wereshown by Shapouri and colleagues[10,27].

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

The original composition of the essential oils of mintfamily's plants is Thymol and carvacrol. The stronganti-microbial effect of carvacrol has been expressedby the researcher[18,30].

Ozturk and Ercisli (2006) 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, 4.18% Peperiton. The main component of the essential oils of some the mint family's plants including *Kakoty*, were Pulegone. Pulegone has antibacterial and antifungal properties and is particularly effective for the different isolates of *Salmonella*[5].

According to this study, *Kakoty's* essence showed more antibacterial impact compared to the methanol extract of it and probably this antibacterial activity is more associated with Pulegone which is an essential component of *mountains' Kakoty's* essence. Salehi et al. (2005) studied the antimicrobial effect of *Kakoty's* extract and showed that *mountains' Kakoty's* extract could inhibit the growth of gram-negative bacteria *Klebsiellapneumoniae* and *Escherichia coli*. Besides lack of antibacterial activity observed against *Pseudomonas aeruginosa* in *mountains' Kakoty's* extract in studies of above people are in agreement to the results of the present study[20]. Salehi et al., (2005) also suggested that the extract could inhibit the growth of *Staphylococcus epidermidis* and *Bacillus subtilis*. Ercili and Ozturk's (2006, 2007), also showed that *mountains' Kakoty* extract and *persicaKakoty*were capable to prevent growing awide 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., (1991) the anti-bacterial effect of *Kakoty* essence native for Turkey has been observed on gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*. Salehi, et al. (2005) showed that*mountains' Kakoty* essence could prevent the growth of gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus 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 consistant with the results obtained in this study[13].

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