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# Antimicrobial Activity of Iranian *Zataria multiflora Boiss*. Essential Oil and Ethanolic Garlic Extract in a Protein-Rich Food

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## Abstract

**Background:** Ready-to-cook breast chicken is susceptible to bacterial changes, which, in turn, adversely affect both food quality and consumers' health.

**Objectives:** This study was intended to investigate the antibacterial potency of *Zataria multiflora Boiss*. essential oil (ZEO) and ethanolic garlic extract (EGE) against four bacterial strains, namely *S. aureus*, *B. licheniformis*, *E. coli* O157:H7, and *S. enteritidis* in ready-to-cook breast chicken.

**Methods:** Initially, minimum inhibitory concentrations (MICs) were measured to define baseline concentrations for in-vivo applications. Afterward, the chicken pieces were contaminated with the given microorganisms before marinating them with ZEO and EGE. Cell viabilities were computed throughout storage at 4°C. A five-scale test was carried out to assess organoleptic features.

**Results and Conclusions:** The findings indicated that the two agents could successfully reduce the bacterial growth although ZEO was more effective than EGE. A better in-vivo antimicrobial performance was observed for ZEO in contrast to EGE, approximately demonstrating a comparable behavior under in-vitro and in-vivo conditions. The concentrations of EGE and ZEO with significant bacterial growth showed lower scores in the sensory survey.

*Keywords:* Garlic, Extract, Essential Oil, Antimicrobial

## 1. Background

The growing demand for convenient foodstuff has led to a rapid growth in the consumption and thus the production of ready-to-eat products (1, 2). Yet, this category of food is prone to microbiological changes, diminishing their quality and affecting consumers' health (3). A myriad of food poisoning cases is being reported on a daily basis because of bacterial growth during the gap time between raw food production/introduction to the market by suppliers and consumption/final preparation by consumers and/or food handlers such as diners and restaurants (4). Both Gram-positive and Gram-negative bacteria have been reported to implicate in this unwanted process (5-7). In this regard, microorganisms such as Bacillus are reported to diminish quality by affecting odor and flavor. Other microorganisms including *Salmonella enteritidis*, *Staphylococcus aureus*, and *E. coli* have been identified as chief culprits in cases of food poisoning when samples are mishandled (8).

There can be seen a global tendency towards preservation methods which are both environmental-friendly and healthy in terms of processing, production, and preservation. Nature-oriented methods have been particularly popular in this respect, with a focus on natural additives that have long been applied in different cultures, in order to not only promote palatability of the treated ingredients but also lower the perishability of foodstuff (8). Consumers opt for organic and natural additives, which are strongly associated with healthy properties (1, 8, 9).

Phytogenic substances, comprising a heterogeneous group of plant-based herbs, spices, and essential oils, are gaining popularity in human food preservation techniques. A prime example includes *Zataria multiflora Boiss*. from the Labiatae family, which is indigenous to Iran, Pakistan, and Afghanistan, and known for their properties, namely palatability improvement, digestive enzymes secretion, nutrient digestibility, antibiotic-like bactericidal effect, and bacteriostatic effects (10). The vernacular name of this compound is Avishan-e-Shirazi, which is especially popular in cuisine, added to yogurt and poultry for the pleasant aroma it creates (11).

Studies showed that *Zataria multiflora Boiss*. could enhance the permeability of the cell membrane, resulting in the release of cell constituents. The apparent antimicrobial efficacy depends on factors such as the extraction

methods, the volume of inoculum, growth phase, culture medium, as well as intrinsic or extrinsic properties of the given food such as pH, fat, protein, water content, antioxidants, preservatives, incubation time/temperature, packaging procedure, and physical structure of food (12-15).

On the other hand, as a strong antibacterial agent, garlic has proven to inhibit both Gram-positive and gram-negative bacteria (16). Garlic (*Allium sativum*) is proven to possess a broad range of antimicrobial features, acting as a growth inhibitor for gram-positive and negative microbes namely *E. coli*, *Salmonella*, *Aeromonas hydrophila*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus*, and *H. pylori* (17, 18). This effect has also been shown to be more intense against *Campylobacter jejuni* (13). Bali et al. reported that garlic has been used to treat samples of chicken sausage, indicating stronger antimicrobial properties in comparison with coriander (19).

## 2. Objectives

To the best of our knowledge, there has not been any research investigating the antimicrobial effects of the two above-mentioned additives (garlic and Avishan-e-Shirazi) in preserving chicken breast meat. Given the long history of culinary application of these herbs in Iran as well as their broad cultural acceptance among the nation, we decided to study *Zataria multiflora Boiss.*'s antimicrobial influences when applied to chicken breast meat as marinade, in comparison with garlic extract as a known preservative in food industry against both gram-positive and gram-negative bacteria. The latter serves as a reference in our qualitative analysis for its widely known culinary and medicinal applications. In this study, we also consider palatability as higher concentrations of both additives can adversely affect this important property of the treated chicken meat.

## 3. Methods

### 3.1. Extraction of ZEO/EGE

The aerial part of *Zataria multiflora Boiss.* was raised in the Medical Plant Farm, Jahad Daneshgah, Islamic Republic of Iran. Fresh garlic, however, was purchased from the local market, Hamadan, Iran. Both plants were dried away from the sun and subsequently powdered using a mixer. The ZEO extraction was carried out applying hydro-distillation with Clevenger apparatus, electro mantle model (20). By the time the distillate was tested and analyzed, it had been maintained in tightly closed dark vials. The garlic extract was obtained using ethanol 96% as described by Nasri et al. (21). Based on gas chromatography-mass spectroscopy, the major constituents of ZEO were carvacrol, thymol, and p-cymene. Moreover, the ethanolic extract was also analyzed for the determination of bioactive components namely allicin, flavonoids, and phenols, which corroborated the findings in the study by Nasri et al. (21) (data not shown). The extract was then maintained in sealed dark vials at 4°C.

### 3.2. Bacterial Strains

The lyophilized ampoules containing *S. aureus* (PTCC 1431) and *B. licheniformis* (ATCC 9789) as well as stock cultures of *E. coli* O157:H7 (ATCC 35218) and *S. enteritidis* (ATCC 13076) were provided by the department of microbiology, faculty of veterinary medicine, University of Tehran. They were grown in Nutrient Broth (Merck, Germany) for 24 hours at 35°C. Afterward, the microorganisms were suspended in sterile saline (0.85% NaCl) to the density of the 0.5 McFarland standard at 625 nm (approximately $1.5 \times 10^8$ CFU/mL of bacterial suspension), from which 2 $\mu$L was taken.

### 3.3. Minimum Inhibitory Concentration

MIC was determined by applying agar dilution method in which Mueller-Hinton agar (MHA; GIBCO Diagnostics, Madison, Wis., USA) served as the culture medium. Instructions were provided by the national committee for clinical laboratory standards (NCCLS) 2006 (22). A range of concentrations for both ZEO (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, and 1 mg/mL) and EGE (0, 1, 2, 3, 4, 5, 6, 7, 8, and 10 mg/mL) was incorporated into MHA. In doing so, we used 1 mL of DMSO and normal saline as solvents to create certain concentrations of ZEO and EGE respectively and separately. The amount of DMSO in the experiment should not exceed 1%. Afterward, 2 $\mu$L ($1.5 \times 10^8$ CFU/mL) of each bacterial inoculum was placed at the center of MHA. The MIC value was read following an overnight incubation at 35°C.

### 3.4. Microbiological Analyses of the Chicken Samples

Initially, ready-to-cook chicken breasts and the marinade (150 mL of extra-virgin olive oil) were purchased from the central kitchen of Avachi chain Restaurant in Tehran. They were then transported to the lab in iceboxes. Tenderized pieces circular in shape were uniformed in terms of both weight and diameter (10 g, 1 cm). Each piece was contaminated with one of the four selected bacteria. In doing so, the population of $1 \times 10^6$ CFU/g was used to inoculate the low-bacterial-load chicken samples (with the initial bacterial load of lower than 3.50 log10 CFU/g, which is in an acceptable range for ready-to-eat foods based on bacterial count standards published by the centre for food safety (23)). We chose one strain of bacteria for contaminating one chicken piece before their immersion in the marinade containing the extracts. To ensure that ZEO and

EGE reach chicken samples homogeneously as well as effectively, olive oil was used. Nevertheless, we had to select a control group to ensure the truth of this assumption. The marinade was 150 mL in quantity, kept in sterile bags. Each bag contained four samples treated with a specified concentration of either ZEO or EGE (33 bags in total). The contaminated pieces were then randomly divided into three groups and subsequently vacuum-packed for storage at 4°C; group A was to be treated with prepared concentrations of ZEO (0.2, 0.3, 0.4, 0.5, 0.8, and 1 mg/mL) whereas group B, serving as reference samples, was treated with different concentrations of EGE (6, 7, 8, and 10 mg/mL). We also considered group C, which included control samples treated with neither ZEO nor EGE. Each bag was unsealed on days 1, 2, 3, and 7 to remove chicken pieces using a sterile forceps for microbial counts. The enumeration of the pathogenic bacteria was carried out in violet Red Bile Lactose agar for *E. coli*, Baird Parker agar for *S. aureus*, Mueller-Hinton agar for *B. licheniformis*, and *Salmonella-Shigella* agar for *S. enteritidis*.

### 3.5. Sensory Evaluation

The ZEO and EGE sensory was evaluated using a five-point scale by 12 trained panelists recruited from the employees of Avachi chain restaurant in terms of flavor, odor, and overall acceptability (24). This test was carried out in a standardized tasting room equipped with individual booths along a wall that divided the room from the preparation area (25, 26). Chicken breasts were soaked in marinades enriched with 0.4, 0.5, 0.8, and 1 mg/mL of ZEO (group A) and 7, 8, and 10 mg/mL of EGE (group B) the night before. Those received neither ZEO nor EGE were considered as group C. They were refrigerated at 4°C prior to broiling. All samples were served in three coded dishes: six judges tested two samples from groups A and B besides one sample from group C, and the other six received one sample from groups A and B and two samples from group C. The panelists were asked to recognize the odd sample and the one they preferred. They were also asked to indicate the degree of difference between the duplicate and odd samples. The five-score evaluation was used to score the difference, encompassing the following items: (1) strongly dislike; (2) slightly dislike; (3) neither like nor dislike; (4) slightly like; (5) strongly like.

### 3.6. Statistical Analysis

All measurements were performed in three replicates. Data were described as the mean ± standard deviation (SD). Considering a normal distribution, one-way ANOVA and Duncan's multiple range tests were initially conducted to determine any significant difference in microbiological counts at P values < 0.05 (SPSS 19.0 software Package, IBM Inc., Chicago IL, USA).

## 4. Results

The extraction yield for ZEO and EGE was reported as 1.56% (v/w) and 23.74% (w/w), respectively. Overall, the in vitro studies (determination of MIC) indicated that ZEO was more effective (10-fold) than EGE in hampering the bacterial growth for the microorganisms used in this study. In other words, ZEO had a MIC value of 0.3 mg/mL in cases of *S. aureus* and *B. licheniformis* and 0.4 mg/mL for *E. coli* and *S. enteritidis*. These values were in a stark contrast with the MIC measurements of EGE, which were reported as 6 mg/mL in case of *B. licheniformis* and 7 mg/mL for the other three microorganisms. The most sensitive bacterium to EGE was *B. licheniformis*. In case of ZEO, both Gram-positive microorganisms were sensitive and equally hindered.

As for in vivo findings from chicken breast samples, the count of *S. aureus* and *B. licheniformis* in control samples (group C) was respectively 6.76 log10 (CFU/g) and 6.83 log10 (CFU/g) on day 1. These values increased to a maximum number of 6.85 log10 (CFU/g) and 6.92 log10 (CFU/g) on day 7 in the absence of both extracts (Tables 1 and 2). Statistical analysis showed that all concentrations of ZEO (group A) and EGE (group B) had significantly decreased the number of *S. aureus* and *B. licheniformis* at 4°C as compared to group C ($P < 0.05$). Using concentrations of ZEO above 0.3 mg/mL (i.e. 0.4, 0.5, 0.8, and 1 mg/mL) could reduce the bacterial population to an acceptable range of below 5 log10 CFU/g. Moreover, the counts of *S. aureus* and *B. licheniformis* at each concentration in group A considerably increased during seven-day storage ($P < 0.05$). Akin to ZEO, a similar pattern was observed in group B when the samples were treated with a minimum concentration of 7 mg/mL (Tables 1 and 2). Furthermore, the interaction between storage time and herbal additives (i.e. ZEO or EGE concentrations) led to notable changes in cell counts ($P < 0.05$).

As indicated in Tables 3 and 4, the cell viability of *E. coli* and *S. enteritidis* decreased as ZEO or EGE was used in comparison with group C ($P < 0.05$). Additionally, the growth of *E. coli* and *S. enteritidis* at each concentration of ZEO or EGE appeared on the sharp decline during storage ($P < 0.05$). Yet, such influences were at a slower pace with respect to *S. aureus* and *B. licheniformis* when treated with ZEO (Tables 3 and 4). In other words, ZEO caused the highest inhibitory effects on *S. aureus* and *B. licheniformis*. This is because the lowest concentration of ZEO required for keeping the counts of *E. coli* and *S. enteritidis* below 5 log10 (CFU/g) in the chicken samples was 0.8 and 0.5 mg/mL, respectively. The minimum concentrations of EGE around 7 mg/mL decreased the cell counts of *E. coli* and *S. enteritidis* within the acceptable range of 5 log10 CFU/g (Tables 3 and 4). Furthermore, there was a statistically significant difference in the growth of *E. coli* and *S. enteritidis* in our samples in response to the interaction of storage time and herbal additive ($P < 0.05$).

As shown in Table 5, the samples treated with 0.4 and

**Table 1.** The Viability of *S. aureus* in Chicken Breast Samples Treated with Different Concentrations of ZEO and EGE on Days 1, 2, 3, and 7[a,b]

| Concentration, mg/mL | Cell Count, Log10, CFU/g | | | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 7 |
| **Positive Control** | | | | |
| 0 | 3.50 ± 0.111 | 3.57 ± 0.354 | 3.60 ± 0.671 | 3.78 ± 0.211 |
| **Negative Control** | | | | |
| 0 | 6.76 ± 0.131d | 6.78 ± 0.005h | 6.81 ± 0.610l | 6.85 ± 0.377l |
| ***Zataria multiflora Boiss*. essential oil** | | | | |
| 0.2 | 5.70 ± 0.445d | 5.78 ± 0.951h | 5.86 ± 0.231l | 5.90 ± 0.700l |
| 0.3 | 5.65 ± 0.780d | 5.79 ± 0.007h | 5.82 ± 0.716l | 5.86 ± 0.114l |
| 0.4 | 3.54 ± 0.715a | 3.72 ± 0.608e | 3.82 ± 0.044i | 3.51 ± 0.619i |
| 0.5 | 3.41 ± 0.006a | 3.65 ± 0.111e | 3.71 ± 0.423i | 3.81 ± 0.005i |
| 0.8 | 3.30 ± 0.483b | 3.52 ± 0.001f | 3.62 ± 0.008j | 3.68 ± 0.602j |
| 1 | 3.11 ± 0.300c | 3.23 ± 0.401g | 3.38 ± 0.615k | 3.49 ± 0.766k |
| **Ethanolic garlic extract** | | | | |
| 6 | 5.15 ± 0.031l | 5.23 ± 0.001i | 5.30 ± 0.368f | 5.40 ± 0.070c |
| 7 | 4.48 ± 0.104k | 4.52 ± 0.232h | 4.54 ± 0.342e | 4.61 ± 0.401b |
| 8 | 3.49 ± 0.555j | 3.58 ± 0.057g | 3.68 ± 0.009d | 3.78 ± 0.011a |
| 10 | 3.00 ± 0.008j | 3.34 ± 0.211g | 3.41 ± 0.013d | 3.53 ± 0.001a |

[a]Values are expressed as mean ± SD.
[b]For each treatment condition, means with different letters in the same column and row indicate a significant difference (P value < 0.05).

**Table 2.** The Viability of *B. licheniformis* in Chicken Breast Samples Treated with Different Concentrations of ZEO and EGE on Days 1, 2, 3, and 7[a,b]

| Concentration, mg/mL | Cell Count, Log10, CFU/g | | | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 7 |
| **Control** | | | | |
| 0 | 6.83 ± 0.002o | 6.86 ± 0.021j | 6.89 ± 0.265o | 6.92 ± 0.415e |
| ***Zataria multiflora Boiss*. essential oil** | | | | |
| 0.2 | 5.45 ± 0.040n | 5.47 ± 0.001i | 5.56 ± 0.010n | 5.59 ± 0.151d |
| 0.3 | 5.12 ± 0.016m | 5.17 ± 0.142h | 5.22 ± 0.003m | 5.26 ± 0.002c |
| 0.4 | 3.86 ± 0.001m | 3.89 ± 0.030h | 3.92 ± 0.114m | 3.94 ± 0.210c |
| 0.5 | 3.59 ± 0.231k | 3.67 ± 0.011f | 3.72 ± 0.212k | 3.77 ± 0.011a |
| 0.8 | 3.31 ± 0.151l | 3.43 ± 0.415g | 3.49 ± 0.007l | 3.55 ± 0.003b |
| 1 | 3.10 ± 0.040l | 3.15 ± 0.202g | 3.26 ± 0.238l | 3.29 ± 0.309b |
| **Ethanolic garlic extract** | | | | |
| 6 | 5.37 ± 0.320i | 5.41 ± 0.025f | 5.54 ± 0.000c | 5.62 ± 0.008c |
| 7 | 4.34 ± 0.008h | 4.38 ± 0.006e | 4.44 ± 0.120b | 4.49 ± 0.121b |
| 8 | 4.33 ± 0.000g | 4.35 ± 0.006d | 4.38 ± 0.008a | 4.46 ± 0.005a |
| 10 | 3.00 ± 0.043g | 3.08 ± 0.000d | 3.19 ± 0.040a | 3.26 ± 0.025a |

[a]Values are expressed as mean ± SD.
[b]For each treatment condition, means with different letters in the same column and row indicate a significant difference (P value < 0.05).

**Table 3.** The Viability of *E. coli* in Chicken Breast Samples Treated with Different Concentrations of ZEO and EGE on Days 1, 2, 3, and 7[a,b]

| Concentration, mg/mL | Cell Count, Log10, CFU/g | | | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 7 |
| **Control** | | | | |
| 0 | 6.17 ± 0.651r | 6.45 ± 0.638l | 6.74 ± 0.001f | 7.05 ± 0.441x |
| ***Zataria multiflora Boiss*. essential oil** | | | | |
| 0.2 | 5.88 ± 0.888o | 5.91 ± 0.112i | 5.95 ± 0.256c | 6.00 ± 0.269u |
| 0.3 | 5.23 ± 0.119p | 5.69 ± 0.347j | 5.78 ± 0.119d | 5.93 ± 0.925v |
| 0.4 | 5.15 ± 0.463q | 5.25 ± 0.176k | 5.49 ± 0.076e | 5.72 ± 0.001w |
| 0.5 | 4.85 ± 0.008m | 4.90 ± 0.864g | 4.94 ± 0.661a | 5.04 ± 0.114s |
| 0.8 | 4.48 ± 0.015m | 4.53 ± 0.008g | 4.58 ± 0.331a | 4.60 ± 0.552s |
| 1 | 4.20 ± 0.455n | 4.25 ± 0.005h | 4.32 ± 0.356b | 4.38 ± 0.011t |
| **Ethanolic garlic extract** | | | | |
| 6 | 5.04 ± 0.006l | 5.18 ± 0.289h | 5.26 ± 0.013d | 5.72 ± 0.022d |
| 7 | 4.61 ± 0.220k | 4.68 ± 0.303g | 4.72 ± 0.119c | 4.89 ± 0.310c |
| 8 | 4.79 ± 0.107j | 4.81 ± 0.001f | 4.85 ± 0.441b | 4.91 ± 0.056b |
| 10 | 3.93 ± 0.006i | 3.94 ± 0.028e | 3.96 ± 0.005a | 3.99 ± 0.189a |

[a]Values are expressed as mean ± SD.
[b]For each treatment condition, means with different letters in the same column and row indicate a significant difference (P value < 0.05).

**Table 4.** The Viability of *S. enteritidis* in Chicken Breast Samples Treated with Different Concentrations of ZEO and EGE on Days 1, 2, 3, and 7[a,b]

| Concentration, mg/mL | Cell Count, Log10, CFU/g | | | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 7 |
| **Control** | | | | |
| 0 | 6.63 ± 0.117e | 6.65 ± 0.856k | 6.67 ± 0.325p | 6.72 ± 0.222u |
| ***Zataria multiflora Boiss*. essential oil** | | | | |
| 0.2 | 6.62 ± 0.151e | 6.65 ± 0.008k | 6.68 ± 0.623p | 6.71 ± 0.005u |
| 0.3 | 5.41 ± 0.001d | 5.48 ± 0.101j | 5.51 ± 0.005o | 5.62 ± 0.922t |
| 0.4 | 5.32 ± 0.006d | 5.43 ± 0.223j | 5.46 ± 0.113o | 5.60 ± 0.188t |
| 0.5 | 4.86 ± 0.700a | 4.90 ± 0.008g | 4.92 ± 0.612l | 4.95 ± 0.581q |
| 0.8 | 4.72 ± 0.354b | 4.76 ± 0.147h | 4.78 ± 0.318m | 4.83 ± 0.001r |
| 1 | 4.60 ± 0.269c | 4.65 ± 0.682i | 4.72 ± 0.004n | 4.79 ± 0.001s |
| **Ethanolic garlic extract** | | | | |
| 6 | 5.00 ± 0.278o | 5.11 ± 0.110k | 5.18 ± 0.002g | 5.30 ± 0.251c |
| 7 | 4.23 ± 0.403p | 4.28 ± 0.360l | 4.36 ± 0.105h | 4.56 ± 0.207d |
| 8 | 4.00 ± 0.001n | 4.18 ± 0.109j | 4.30 ± 0.414f | 4.48 ± 0.313b |
| 10 | 3.71 ± 0.002m | 3.74 ± 0.200i | 3.78 ± 0.171e | 3.83 ± 0.001a |

[a]Values are expressed as mean ± SD.
[b]For each treatment condition, means with different letters in the same column and row indicate a significant difference (P value < 0.05).

**Table 5.** Changes in Odor, Flavor, and Overall Acceptability Scores of Chicken Breast Samples Treated with Different Concentrations of ZEO and EGE[a,b]

| Concentration, mg/mL | Sensory Score | | |
|---|---|---|---|
| | Flavor | Odor | Overall Acceptability |
| **Control** | | | |
| 0 | 3.33 ± 0.000b | 3.50 ± 0.333a | 3.33 ± 0.154ab |
| ***Zataria multiflora Boiss*. essential oil** | | | |
| 0.4 | 4.00 ± 0.333a | 4.00 ± 0.000a | 3.92 ± 0.000a |
| 0.5 | 3.92 ± 0.577a | 3.92 ± 0.554a | 3.92 ± 0.667a |
| 0.8 | 3.83 ± 0.667a | 3.75 ± 0.667a | 3.83 ± 0.333a |
| 1 | 3.00 ± 0.206b | 3.50 ± 0.667a | 3.00 ± 0.000b |
| **Ethanolic garlic extract** | | | |
| 7 | 3.75 ± 0.547ab | 3.67 ± 0.206a | 3.58 ± 0.213ab |
| 8 | 3.83 ± 0.143a | 3.83 ± 0.213a | 3.75 ± 0.828a |
| 10 | 3.33 ± 0.000ab | 3.33 ± 0.000a | 3.33 ± 0.213ab |

[a]Values are expressed as mean ± SD.
[b]For each treatment condition, means with different letters in the same column indicate a significant difference (P value < 0.05).

0.5 mg/mL ZEO were relatively favorable in terms of flavor, odor, and acceptability (approximately scale 4) whereas those treated with 1 mg/mL ZEO showed lower scores in the test of palatability (scale 3) (P > 0.05). Similarly, the samples marinated with the higher concentration of EGE were of lowest desirability for organoleptic properties (P > 0.05).

## 5. Discussion

In this study, we originally selected ready-to-cook chicken breasts as a food model to compare and determine the antimicrobial effectiveness of ZEO and EGE.

MIC measures were initially determined to draw the baseline for the concentrations to be applied to our chicken samples as in vivo microbial behavior differs from in vitro conditions owing to the protective and friendly environment that food provides for the microorganisms it accommodates, which prompts bacterial proliferation at a faster pace (27). The samples in the present study had to be contaminated owing to their negligible initial total microbial count (see the count of the samples referred to as the low bacterial load in Table 1). This also exerts strict control over the study methods as a certain number of the bacteria of any given strain can be provided. It prevents any possible interventional factor to affect the outcome.

Ethanolic garlic extract has been reported to be effective against various bacteria namely *S. aureus*, *E. coli*, *S. typhi*, *B. subtilis*, and *Klebsiella pneumonia* (17, 28-30). Moreover, the present study showed that it was of considerable potency against *B. licheniformis* and *S. enteritidis*.

The most striking feature in our findings regards the inhibitory effects of ZEO on both gram-positive and gram-negative bacteria as compared to EGE. Generally, ZEO showed to be more effective (10-fold) than EGE during in vitro and in vivo circumstances. All concentrations of ZEO were significantly influential in the bacterial growth (*S. aureus*, *B. licheniformis*, *E. coli* O157:H7, and *S. enteritidis*); however, a minimum concentration of 0.5 mg/mL of ZEO can afford to reduce the bacterial counts to the acceptable count of 5 log10 CFU/g. As for EGE, considerably higher concentrations were required to achieve the same inhibitory effects observed for ZEO (7 mg/mL as opposed to 0.5 mg/mL), which clearly indicates the greater relative potency of ZEO.

The findings revealed that the lowest concentration of ZEO (0.4 mg/mL and 0.5 mg/mL for gram-positive and gram-negative bacteria) required for maintaining bacterial count below 5 log10 CFU/g was greater than those of MIC (0.3 mg/mL and 0.4 mg/mL for gram-positive and Gram-negative bacteria). On the contrary, the in vitro and in vivo outcomes were comparable in the case of EGE except for *B. licheniformis*. The disparity between the two human food additives can be explained owing to the differences in extraction efficiencies and delivery of extracts to microbes. Therefore, it deserves to be noted that the antimicrobial potency of ZEO is vehemently affected by the protein source -breast chicken. It has been widely reported that the hydrophobic essential oil constituents are impaired by the time they have been incorporated into food matrix components, namely fat (31, 32), starch (33), and proteins (34, 35). More to the point, the antibacterial effectiveness of essential oils may be also associated with pH, temperature, and the microbial load inoculated for contamination (32, 36, 37). Therefore, a lower performance must be expected during in vivo conditions.

The acceptable range of palatability was determined

since additives can alter human food taste beyond the favorable threshold. To our dismay, the maximum concentrations of EGE and ZEO, which could significantly hamper bacterial growth, failed to score satisfactorily in the palatability survey. It seems that high phenolic content can adversely affect the organoleptic properties of food (38).

Implication: *Zataria multiflora Boiss*. essential oil showed a remarkable potency as a food additive to retard the growth of *S. aureus*, *B. licheniformis*, *E. coli*, and *S. enteritidis* in comparison with ethanolic garlic extract in ready-to-cook breast chicken. Nevertheless, the great amount of the essential oil-fortified marinade showed lower scores on the organoleptic properties of the final chicken product.

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## Footnote

**Conflict of Interests:** We declare that there is no conflict of interest regarding the publication of this paper.

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