Journal of Human, Environment, and Health Promotion. 2019; 5(2): 86-93

Journal of Human, Environment, and Health Promotion

Journal homepage: www.zums.ac.ir/jhehp

The Antibacterial and Antioxidant Effects of Clove (*Syzygium aromaticum*) and Lemon Verbena (*Aloysia citriodora*) Essential Oils

Mahzad Hosseini [a] | **Abdollah Jamshidi** [b*] | **Mojtaba Raeisi** [c] | **Mohammad Azizzadeh** [d]

[a] *Student of Food Hygiene, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
[b] *Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
[c] *Department of Nutrition, Faculty of Health, Golestan University of Medical sciences, Gorgan, Iran.*
[d] *Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*

***Corresponding author:** Abdollah Jamshidi
Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. Postal code: 9187195786.
E-mail address: ajamshid@ferdowsi.um.ac.ir

ARTICLE INFO

Article type:
Original article

Article history:
Received 31 March 2019
Revised 9 June 2019
Accepted 20 June 2019

DOI: 10.29252/jhehp.5.2.7

Keywords:
Essential oil
Syzgium aromaticum
Aloysia Citriodora
Antioxidant activity
Antimicrobial activity

ABSTRACT

Background: The study aimed to investigate the chemical composition, antimicrobial effects, and antioxidant properties of clove and lemon verbena essential oils (EOs).

Methods: The chemical composition of the EOs was identified using gas chromatography/mass spectrometry (GC/MS). In addition, the antibacterial effects of EOs against seven important foodborne bacteria were assessed using the disk-diffusion, agar well-diffusion, and broth microdilution assays. Evaluation of the antioxidant properties of the EOs was carried out using DPPH, β-carotene-linoleic acid bleaching, and reducing power assay.

Results: All the tested bacteria demonstrated susceptibility to EOs, with the highest susceptibility observed in Bacillus cereus to the clove EO in the agar disk-diffusion test. Moreover, *Shigella dysenteriae* was identified as the most sensitive bacterium to the lemon verbena EO. *Salmonella typhimurium* was the most resistant bacterium to both EOs. In the agar well-diffusion test, *Pseudomonas aeruginosa* and *S. typhimurium* had the lowest sensitivity to the clove and lemon verbena EOs, respectively. Although both EOs exhibited significant antioxidant capacity, the lemon verbena EO showed higher antioxidant activity in all the tests, with the exception of β-carotene-linoleic acid bleaching test.

Conclusion: Clove and lemon verbena EOs could be regarded as potential sources of natural antioxidant and antimicrobial agents in food processing.

1. Introduction

Today, increased knowledge regarding the side-effects of chemical preservatives has raised public awareness, giving rise to demands for the use of natural additives in foods [1]. For instance, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are common antioxidants that are suspected to be responsible for liver damage and carcinogenesis.

Considering the consumer demand for natural food preservatives, researchers have been attempting to identify alternative natural and safe sources of edible antioxidants and antimicrobials [2].

Various antimicrobial compounds are found in nature, which are mostly involved in providing defense against numerous microorganisms.

How to cite: Hosseini M, Jamshidi A, Raeisi M, Azizzadeh M. The Antibacterial and Antioxidant Effects of Clove (*Syzygium aromaticum*) and Lemon Verbena (*Aloysia citriodora*) Essential Oils. *J Hum Environ Health Promot.* 2019; 5(2): 86-93.

Natural preservatives are obtained from a wide variety of resources, such as animal enzymes (e.g., lysozyme and lactoferrin), microbial bacteriocins (e.g., nisin and natamycin), organic acids (e.g., sorbic and citric acid), natural polymers (e.g., chitosan), and herbal essential oils (e.g., basil, oregano, and rosemary) [3].

Essential oils (EOs) are aromatic compounds that are extracted from various parts of plants, such as the buds, leaves, seeds, bark, and fruit. EOs have numerous biological properties, including antimicrobial and antioxidant activities, which enables them to increase the shelf life of food products. Traditionally, EOs and their active compounds were used against pathogenic foodborne bacteria, such as Escherichia coli, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and Vibrio [1].

The Aloysia (Lippia) genus belongs to the Verbenaceae family, which contains approximately 200 species that grow in shrubs and small trees and are commonly aromatic. The plant materials and extracts of *A. citriodora*, which is also known as lemon verbena, are used in the formulation of drugs in traditional medicine. Furthermore, these compounds are applied as home remedies for various health problems, such as gastrointestinal and respiratory disorders. The EO obtained from the leaves of this plant has also exhibited remarkable antimicrobial activity [4]. Another genus is the *Syzygium* of the *Myrtaceae* family, which contains approximately 500 species, among which *S. aromaticum* (clove) has the widest application in economy . Clove EO is considered to be a common food additive with significant antifungal properties, as well as antimicrobial and antioxidant effects due to the presence of eugenol and other phenolic compounds [2].

The present study aimed to assess the chemical composition of *A. citriodora* and *S. aromaticum* EOs and investigate their antimicrobial and antioxidant activities in-vitro.

2. Materials and Methods

2.1. Plant Materials

The dried leaves of lemon verbena and dried buds of clove were purchased from the local markets in Gorgan, Iran, and the species were confirmed at Golestan Agricultural and Natural Resources Research and Educational Center in Gorgan, Iran.

2.2. EO Extraction

The dried plant materials were powdered for the extraction of the EOs using a Clevenger apparatus. The dried plant materials were immersed in distilled water, and extraction was performed within 180 minutes, starting from the boiling point of water. Sodium sulfate was used for the dehydration process of the extracted oils. After filtration using 0.22-micrometer filters, the obtained EOs were preserved in dark vials at the temperature of 4 °C for analysis [5].

2.3. GC/MS Analysis

The analysis of the clove and lemon verbena EOs was performed using the Agilent 6890N instrument (San Diego, USA) equipped with an HP-5MS capillary column (30×0.25 mm ID×0.25 mm film thickness). In this process, helium was used as the carrier gas with the flow rate of one milliliter per minute. The column temperature was initially 50 °C, which gradually increased to 120 °C at the rate of 2 °C/min, maintained for three minutes, and finally increased to 300 °C. The procedure was operated at 70 eV [5], and the compounds were identified through the comparison of the retention indices with the authentic samples and mass spectral data available in the library of Wiley-VCH 2001 (Weinheim, Germany) [6].

2.4. Total Phenolic Contents

The Folin-Ciocalteu reagent assay was used to determine the total phenolic contents of the obtained EOs [7]. In brief, 0.5 milliliter of each EO was prepared in methanol at the concentration of 2 mg/ml and mixed with 2.25 milliliters of distilled water and 250 microliters of the Folin-Ciocalteu reagent. Afterwards, the mixture was completely vortexed and allowed to react for five minutes. At the next stage, two milliliters of Na_2CO_3 solution (7.5%) was added to the mixture. After the preservation of the mixture at room temperature for 120 minutes, its absorbance was measured at 760 nanometers using a spectrophotometer (model: LKB Novaspec II; Pharmacia, Cambridge, England). The obtained results were expressed as the milligram of Gallic acid equivalent per gram of the EOs relative to the values obtained based on the standard curve prepared separately using the known concentrations of Gallic acid.

2.5. Antibacterial Effects of the EOs

The antibacterial effects of the EOs were evaluated independently on four gram-negative bacteria (*Escherichia coli* PTCC 1399, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* PTCC 1616, and *Shigella dysenteriae* PTCC 1188) and three gram-positive bacteria (*Staphylococcus aureus* PTCC 1112, *Listeria monocytogenes* PTCC 1298, and *Bacillus cereus* PTCC 1154) using various methods, including the agar disk-diffusion, agar well-diffusion, and microdilution assays. The microorganisms were obtained from the culture collection of the Food and Drug Deputy of Golestan University of Medical Sciences in Gorgan, Iran.

2.5.1. Agar Disk-Diffusion Assay

The agar disk-diffusion assay was performed as described by Moradi et al. (2014). Initially, paper disks (diameter: 6 mm) were impregnated with 10 microliters of each EO. Afterwards, they were placed on plates containing nutrient agar media (Merck, Darmstadt, Germany), which had been previously spread cultured with 0.1-milliliter suspension of the tested microorganisms adjusted to the require concentrations (1.5×10^6 CFU/ml) using the McFarland standard. Following that, the plates were incubated at the temperature of 37 °C for 24 hours. An impregnated disk with 10 microliters of dimethyl sulfoxide (DMSO) was selected as the negative control, and gentamicin and vancomycin antibiotic disks were used as positive controls. Afterwards, the diameters of the growth inhibition zones

were measured in millimeters using a manual caliper (Vernier, Mitutoyo, Japan) [8].

2.5.2. Agar Well-Diffusion Assay

Initially, 100 milliliters of nutrient molten agar (Merck, Darmstadt, Germany) was inoculated with one milliliter of 18-hour broth cultures of the bacteria (1.5×10^6 CFU/ml), which were cooled at the temperature of 45 °C, completely mixed for two minutes, poured into sterile plates, and left to solidify. Following that, four wells were aseptically cut using a sterile cork-borer, and 10 microliters of the EOs were added to the wells. In this process, DMSO was used as the negative control. The plates were incubated at the temperature of 37 °C for 72 hours, and the antimicrobial activity was measured in millimeters using a manual caliper (Vernier, Mitutoyo, Japan) [9].

2.5.3. Determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Using the Broth Microdilution Assay

At this stage, the assay was performed based on the research by Turgis et al. (2012) with slight modifications [10]. Initially, the EOs were dissolved in 10% DMSO to the maximum concentration (10,000 µg/ml), and a twofold serial dilution was prepared within the concentration range of 10-10,000 µg/ml from each concentration. Following that, 125 microliters of the EO solutions was added to wells 1-11 of a 96-well microplate (Sarstedt, Montreal, QC, Canada). Finally, 15 microliters of the media containing 10^6 CFU/ml of the tested microorganisms was added to all the wells, and the final volume in each well was determined to be 140 microliters. Three rows of the microplate were used for each bacterium, with the negative controls (two rows of the microplate) containing 15 microliters of saline solution instead of the cultured bacteria, and the positive controls containing 125 microliters of the growth media and 15 microliters of the working culture bacteria. After incubation at the temperature of 37 °C for 24 hours, the absorbance was recorded at 595 nanometers using the Biotech ELX800O absorbance microplate reader (Biotek Instruments Inc., Winooski, VT, USA).

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent to completely inhibit the growth of bacterial strains, without the microorganisms showing visible growth. Minimum bactericidal concentration (MBC) is defined as the lowest concentration of antimicrobials to destroy a minimum of 99.9% of the initial inoculum as determined by the subculturing of 10 microliters from each well with no visible growth detected in nutrient agar plates and incubation at the temperature of 37 °C for 72 hours [10].

2.6. Antioxidant Activity of the EOs

The antioxidant activity of the extracted EOs was evaluated using three methods, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, β-carotene-linoleic acid bleaching, and reducing power assay.

2.6.1. DPPH Assay

The free radical scavenging activity of the extracted EOs was assessed using the DPPH assay (Sigma-Aldrich, Steinheim, Germany) as previously described by Erkan et al. (2008) with slight modifications [11]. Initially, 50 microliters of each EO within the concentration range of 10-10,000 µg/ml (10, 50, 100, 250, 500, 750, 1,000, 2,500, 5,000, 7,500, and 10,000 µg/ml) and the reference antioxidant (BHT) were added to two milliliters of the methanol solution of DPPH (24 µg/ml). After the shaking of the solutions, they were preserved at room temperature for 60 minutes in the dark. Following that, the absorbance of the mixture was read against a blank sample solution with no antioxidants at 515 nanometers using a spectrophotometer (model: LKB Novaspec II, Pharmacia, Cambridge, England). The formula used to calculate the inhibition rate was as follows:

$$\%\,I = \left[\frac{A_{Blank} - A_{Sample}}{A_{Blank}}\right] \times 100$$

where %I is the inhibition rate of the DPPH radical, and ABlank and ASample represent the absorbance of the blank and sample, respectively. The IC50 value was determined based on the scavenging activity plot, which was drawn in accordance with the concentration of the EOs. It was defined as the total antioxidant activity essential to causing 50% reduction in the initial DPPH radical concentration.

2.6.2. β-carotene-linoleic Acid Bleaching Test

In the present study, the β-carotene bleaching test was performed as described by Miraliakbari and Shahidi (2008) with slight modifications [12]. In a round bottom flask, 0.5 milligram of β-carotene (type I synthetic; Sigma-Aldrich, Steinheim, Germany) was dissolved in one milliliter of chloroform (Sigma-Aldrich, Steinheim, Germany). Afterwards, 20 microliters of linoleic acid (Sigma-Aldrich, Steinheim, Germany) and 200 milligrams of Tween 40 (Sigma-Aldrich, Steinheim, Germany) were added to the solution. The chloroform was removed completely using a rotary evaporator (model: Heidolph Laborota 4003, SchwaBach, Germany) at the temperature if 40°C, followed by the addition of 100 milliliters of distilled and thorough shaking of the mixture. Afterwards, 2.5 milliliters of the aliquots of the solution was pipetted into test tubes containing 350 microliters of the EOs with the concentration of 2 mg/ml.

The same procedure was repeated for BHT (Sigma-Aldrich, Steinheim, Germany) and a blank containing the same volume of DMSO instead of the EOs. The tubes were preserved in a water bath at the temperature of 50 °C, and the absorbance of each tube was measured at 470 nanometers immediately at zero time and after two hours at 20-minute intervals.

The capacity of the EOs against the oxidation of β-carotene was determined using the following formula:

$$\%I = \left[\frac{A_{\beta-carotene\ after\ 2h\ assay}}{A_{initial\ \beta-carotene}}\right] \times 100$$

where %I is the inhibition rate or capacity of the EOs for protection against oxidation, and $A_{\beta\text{-carotene after 2h assay}}$ and $A_{\text{initial }\beta\text{-carotene}}$ indicate the absorbance of the tubes after two hours and at zero time, respectively.

2.6.3. *Reducing Power Assay*

The reducing power of the EOs was analyzed using the method proposed by Oyaizu [13]. Accordingly, the EOs within the concentration range of 10-10,000 µg/ml (10, 50, 100, 250, 500, 750, 1,000, 2,500, 5,000, 7,500, and 10,000 µg/ml) were mixed with 2.5 milliliters of 0.2 phosphate buffer (pH=6.6) and 2.5 milliliters of 1% 0.2 M potassium ferricyanide ($K3Fe[CN]_6$). After 20 minutes of incubation at the temperature of 50 °C, 2.5 milliliters of 10% trichloroacetic acid was added to the mixtures and centrifuged for 10 minutes at 1,036. Finally, 2.5 milliliters of the upper layer was mixed with 2.5 milliliters of distilled water and 2.5 milliliters of 1% ferric chloride solution. The absorbance was measured at 700 nanometers against the blank containing all the reagents (with the exception of the sample EOs) using a double beam UV-Vis spectrophotometer (model: LKB Novaspec II, Pharmacia, Cambridge, England). Higher absorbance values were interpreted as the higher reducing power. In this process, BHT was used as the positive control. The EO fraction concentration providing 0.5 of the absorbance (EC_{50}) was calculated based on the graph absorbance at 700 nanometers against the EO concentrations and compared with the standard antioxidant (BHT).

2.7. Statistical Analysis

Data analysis was performed in SPSS version 16 (SPSS Inc., Chicago, IL, USA), and all the assays were performed in triplicate. Tukey′ s test was used to compare the differences between the mean values obtained from the experiments at the significance level of $P < 0.05$.

3. Results and Discussion

3.1. GC-MS Analysis of the EOs

The obtained EOs from the aerial parts of clove and lemon verbena (leaves and buds) were analyzed using GC/MS, which allowed the identification of approximately 99.79% and 90.91% of the constituents of clove and lemon verbena, respectively. Tables 1 and 2 show the chemical composition of the EOs.

According to the obtained results, the main components of lemon verbena EO were eugenol (14.63%), D-limonene (12.41%), caryophyllene oxide (8.78%), α-curcumene (7.91%), trans-citral (7.44%), β-spathulenol (6.92%), z-citral (5.38%), eucalyptol (5.3%), sulcatone (3.11%), and caryophyllene (3.03 %). The main compounds in the clove EO included eugenol (79.4%), β-caryophyllene (13.36%), eugenol acetate (4.49%), and α-caryophyllene (1.67%). It is also notable that α-copaene was one of the minor components found in both the EOs.

Previous studies have also investigated the phytochemical properties of lemon verbena EO. For instance, Ali et al. (2011) reported the major component of this EO to be citral (14%), while there were also 14 minor compounds, such as eugenol (1.28%) [14]. In another research in this regard, Benelli et al. (2017) reported that the main components of lemon verbena EO were geranial (21%) and neral (16.5%), and the other notable components included limonene (11.4%), 1,8-cineole (7%), ar-curcumene (6.7%), spathulenol (5.8%), and caryophyllene oxide (4.8%) [15].

In the present study, the main compounds of the investigated EOs differed quantitatively and qualitatively compared to the literature. The discrepancy in this regard could be due to several factors, such as the region climate, plant species, distillation conditions, differences in developmental stages, type of soil, and light exposure [16], all of which influence the chemical composition and relative concentration of each constituent in EOs [16]. In order to obtain the same chemical composition, EOs should be extracted in identical conditions and from the same organs of the plants that grow in the same soil in similar climate and are harvested in the same season [17].

Several studies have been focused on the composition of clove EO. For instance, Dehghani et al. (2018) reported the main constituents of clove EO to be eugenol (77.57%), eugenol acetate (10.23%), caryophyllene (7.52%), and α-humulene (1.66%) [18]. Despite minor differences, these findings are consistent with the results of the present study. In another research in this regard, Xie et al. (2015) stated that clove EO was most abundant in compounds such as eugenol (90.6%) and β-caryophyllene (9.4%) [19], which is in congruence with the findings of the current research. Similarly, Fu et al. (2007) reported the relative composition of clove EO, which contained eugenol (68.57%), β-caryophyllene (19%), 2-methoxy-4-[2-propenyl] phenol acetate (10.15%), and α-caryophyllene (1.85%). These findings are inconsistent with the results of the present study.

3.2. Antimicrobial Activity of the EOs

The antimicrobial activity of lemon verbena and clove EOs is challenged against the common gram-negative and gram-positive bacteria that are involved in food poisoning. The objective of the current research was to evaluate the potency of these herbal EOs qualitatively and quantitatively based on the presence or absence of the growth inhibition zone and MIC and MBC values. Tables 3 and 4 show the results regarding the sensitivity of the tested bacteria against lemon verbena and clove EOs based on the agar disk-diffusion and agar well-diffusion assays.

In the agar disk-diffusion method, all the tested microorganisms exhibited significant susceptibility (inhibition zone diameter >10 mm) to the lemon verbena EO.Clove EO, showedinhibitory effects against all the tested bacteria (inhibition zone diameter >10 mm), with the exception of *S. typhimurium* and *P. aeruginosa*. In this test, the most resistant bacteria to the lemon verbena EO were *P. aeruginosa* and *S. typhimurium*, while *S. typhimurium* was slightly more resistant; however, the difference in this regard was not considered significant ($P > 0.05$). According to the results of the present study, the most sensitive

bacteria to the lemon verbena EO were *S. dysenteriae*, *B. cereus*, and *S. aureus*, while *S. dysenteriae* was more sensitive compared to the two other bacteria; however, the difference in this regard was not considered significant ($P > 0.05$).

In case of the clove EO, the most resistant bacteria were similar to the lemon verbena EO, while the most sensitive bacteria were observed to be *B. cereus and S. dysenteriae*, with *B. cereus* being slightly more sensitive; however, no significant difference was denoted in this regard ($P > 0.05$). According to the agar well-diffusion assay, the most resistant bacteria to the clove and lemon verbena EOs were *S. typhimurium* and *P. aeruginosa*, respectively. Furthermore, the most sensitive bacteria to the clove EO were *B. cereus* and *S. dysenteriae*, while the most sensitive bacteria to the lemon verbena EO were *B. cereus* and *S. aureus*, exhibiting the highest susceptibility. Although *B. cereus* was considered to be more susceptible in both cases, no significant difference was denoted in this regard ($P > 0.05$).

Table 1: Chemical Composition of Lemon Verbena Essential Oil Based on GC/MS Analysis

No	Compound name	%	RT (min)
1	alpha.-Pinene	0.62	11.74
2	Sabinene	1.47	13.83
3	Amyl vinyl carbinol	0.26	14.45
4	Sulcatone	3.11	14.69
5	D-Limonene	12.41	16.86
6	Eucalyptol	5.3	17.03
7	Beta –ocimene	0.15	17.7
8	Trans- sabinene hydrate	0.52	19.14
9	l-linalool	0.3	20.63
10	trans-Limonene oxide	0.53	22.57
11	p-Menth-1-en-4-ol	0.37	24.81
12	Terpineol	1.78	25.63
13	trans-Carveole	0.58	26.83
14	Z-Citral	5.38	27.64
15	trans-Carvone	0.55	28.07
16	trans-Citral	7.44	29.07
17	Eugenol	14.63	33.04
18	alpha.-Copaene	0.71	33.58
19	Geranyl acetate	0.82	33.73
20	Caryophyllene	3.03	35.36
21	Alloaromadendrene	0.6	37.3
22	Nerol acetate	0.36	37.58
23	α-Curcumene	7.91	38.14
24	trans-γ-cadinene	0.47	38.82
25	δ-Cadinene	0.93	39.66
26	Eugenol acetate	0.8	39.8
27	Nerolidol	1.29	41.34
28	β-Spathulenol	6.92	42.31
29	Caryophyllene oxide	8.78	42.47
30	Humulane-1,6-dien-3-ol	0.82	43.56
31	Isospathulenol	0.68	44.42
32	.alpha.-Cadinol	1.39	44.77
33	Total Contents	90.91	

Table 2: Chemical Composition of Clove Essential Oil Based on GC/MS Analysis

NO	Compound name	%	RT (min)
1	Methyl salicylate	0.24	25.55
2	Eugenol	79.4	33.39
3	α-Copaene	0.41	33.62
4	β-Caryophyllene	13.36	35.65
5	α-Caryophyllene	1.67	37.14
6	α – Farnesene	0.12	38.95
7	δ-Cadinene	0.1	39.66
8	Eugenol acetate	4.49	39.85
9	Total Contents	99/79	

According to the information in Tables 3 and 4, the lemon verbena EO had greater antimicrobial activity compared to the clove EO ($P < 0.05$), as well as a larger visible inhibition zone clove EO against all tested bacteria. According to the agar disk-diffusion and agar well-diffusion assays, *B. Cereus* and *S. dysenteriae* had the highest sensitivity to the clove EO, while *S. typhimurium* and *P. aeruginosa* exhibited the highest resistance to both the EOs in the agar disk-diffusion test. However, the results of the agar well-diffusion assay indicated that *P. aeruginosa* and *S. typhimurium* had the highest resistance to the clove and lemon verbena EOs, respectively.

In another research in this regard, Hashemi et al. (2018) assessed the antibacterial efficacy of lemon verbena EO extracted by various methods against *B. cereus*, *P. aeruginosa, S. typhimurium*, *S. aureus*, and *E. Coli* O_{157}:H_7, with *B. cereus* and *P. aeruginosa* reported to be the most susceptible and resistant microorganisms, respectively. This is consistent with the results of the present despite the slight differences [20]. *P. aeruginosa* is known to possess high intrinsic resistance to most antimicrobial agents due to its highly restrictive outer membrane barrier [21]. The resistance of this bacterium has been confirmed in the other studies in this regard [21].

In the study conducted by Song et al. (2014), the incorporation of clove EO into composite films reinforced the growth inhibition zone against *E. Coli* O_{157}:H_7 and *L. monocytogenes* compared to the films without the EO [22]. Similarly, Fu et al. (2007) reported the antimicrobial activity of clove EO against *S. epidermidis*, *E. coli* O_{157}:H_7, and *C. albicans*. Moreover, the findings of the mentioned research indicated that clove EO exerted significant antimicrobial effects on all the tested microorganisms [23]. According to the literature, eugenol is the most important active component in clove EO and responsible for its bioactivities [24]. In the present study, the inhibition zone diameters of 12.05, 9.27, 11.96, and 10.38 were observed against *E. coli* O_{157}:H_7, *S. typhimurium*, *S. aureus*, and *L. monocytogenes*, respectively, and the obtained values are lower compared to the previous findings in this regard [25].

In the current research, the agar disk-diffusion and agar well-diffusion assays of the lemon verbena EO could inhibit the growth of all the tested bacteria. On the other hand, Bensabah et al. (2013) have reported that lemon verbena EO has no inhibitory effects on *P. aeruginosa*, and its maximum inhibition effect was observed on *E. coli* O_{157}:H_7 [16]. Furthermore, Ali et al. (2008) examined the activity of lemon verbena EO against six gram-positive and gram-negative bacteria using the agar disk-diffusion method, and the obtained results indicated that *lemon verbena* had no antibacterial activity against *L. monocytogenes*, *Salmonella* spp., and *E. coli* O_{157}:H_7 [26]. These findings are inconsistent with the results of the present study (tables 3 & 4).

The mechanism of the antimicrobial activity of EOs is associated with attacking on the phospholipids that are present in the cell membrane, which increase the permeability and leakage of the cytoplasm or their interactions with the enzymes located on the cell wall. In other words, the phenolic compounds in EOs inhibit the growth of microorganisms by sensitizing the phospholipid bilayer of the cell membrane, thereby increasing its

permeability and causing the leakage of vital intracellular constituents or the impairment of bacterial enzyme systems [24]. The discrepancy observed in the findings in this regard could be due to differences in the type of sample cultures, substance concentrations, and EO extraction methods [25].

According to the current research, the gram-positive bacteria were slightly more susceptible to the clove and lemon verbena EOs compared to the gram-negative bacteria. This phenomenon has also been reported in other studies and could be attributed to the presence of hydrophobic lipopolysaccharides in the outer membrane structure of gram-negative bacteria, which is particularly impermeable to the EO molecules and intrinsic tolerance of the bacteria, as well as the nature and composition of the phytochemical compounds found in herbal EOs [27].

In the present study, the MIC and MBC of lemon verbena and clove EOs were determined against selected pathogens (tables 5 & 6). According to the obtained results, both EOs exhibited bacteriostatic and bactericidal activity against all the examined microorganisms. The MIC of the lemon verbena and clove EOs was within the ranges of 1,250-2,500 and 1250-5000 µg/ml, respectively. However, the MBC of both EOs was equal to or twice higher than the MIC values in all the cases (Table 5).

In a research by Radünz et al. (2018), the MIC of clove EO was reported to be lower than the value obtained in the current research [25], while the reported MIC of lemon verbena EO by Oukerrou et al. (2017) was higher than the value calculated in the present study. The discrepancies in this regard could be due to differences in the type of sample cultures, substance concentrations of substances, and EO extraction methods [4]. Table 5 shows the antibacterial effects of EOs on the tested bacterial strains based on the microdilution method.

Table 3: Antibacterial effect of EOs on tested bacterial strains by agar disk diffusion method (mean ± SD).

	Diameter of inhibition zone (mm)	
	Citriodora	***S. Aromaticum***
E.coli	15.32 ± 0.29^{Aa}	12.05 ± 0.20^{Ba}
S. thyphimurium	11.88 ± 0.14^{Ab}	9.27 ± 0.19^{Bb}
P.aeroginosa	12.38 ± 0.16^{Ab}	9.37 ± 0.15^{Bb}
S.dysenteriae	17.62 ± 0.10^{Ac}	13.12 ± 0.08^{Bc}
S. aureus	16.53 ± 0.38^{Ac}	11.96 ± 0.15^{Ba}
L. monocytogenes	14.54 ± 0.33^{Aa}	10.38 ± 0.19^{Bd}
B. cereus	17.55 ± 0.32^{Ac}	13.25 ± 0.20^{Bc}

The upper-case letters in the same rows show significant differences at P <0.05, and the lower-case letters in the same columns show significant differences at P< 0.05).

Table 4: Antibacterial effect of EOs on tested bacterial strains by agar well diffusion method (mean ± SD).

	Diameter of inhibition zone (mm)	
	Citriodora	***S. Aromaticum***
E.coli	10.83 ± 0.13^{Aa}	8.85 ± 0.17^{Ba}
S. thyphimurium	9.61 ± 0.20^{Ab}	8.45 ± 0.33^{Ba}
P.aeroginosa	11.44 ± 0.16^{Aa}	7.60 ± 0.09^{Bb}
S.dysenteriae	12.97 ± 0.15^{Ac}	9.87 ± 0.08^{Bc}
S. aureus	13.01 ± 0.15^{Ac}	9.51 ± 0.22^{Bc}
L. monocytogenes	11.65 ± 0.15^{Aa}	9.04 ± 0.10^{Ba}
B. cereus	13.24 ± 0.24^{Ac}	10.09 ± 0.13^{Bc}

The upper-case letters in the same rows show significant differences at P<0.05, and the lower-case letters in the same columns show significant differences at P< 0.05).

3.3. Antioxidant Activity of EOs

The Folin-Ciocalteu procedure is considered to be an effectual, rapid method for the estimation of the phenolic contents of herbal EOs and extracts [8]. The current research demonstrated the lemon verbena EO had a more significant phenolic content compared to the clove EO;So the antioxidant activity of this EO could mainly be attributed to its high total phenolic contents; however, the effects of the minor constituents should also be taken into account [8].

DPPH radical scavenging is a common technique for evaluating the capability of herbal extracts to scavenge the free radicals generated from DPPH reagents. According to the information in Table 6, the EO of *A. citriodora* has the greatest free radical scavenging activity, followed by BHT and *S. aromaticum*, respectively. In the present study, the IC_{50} (half maximal inhibitory concentration) for the scavenging of the DPPH radical in clove, lemon verbena, and BHT (positive control) was estimated at 81.18 ± 02, 11.33 ± 01, and 27.43 ± 04, respectively. Furthermore, the lemon verbena EO had the total phenolic content of 816.07 ± 46.81, which indicated higher radical scavenging activity in the DPPH assay compared to the clove EO, confirming its potential application as an electron donor to scavenge free radicals. In this regard, Naser Aldeen et al. (2015) measured the total phenolic content of the dried leaves of lemon verbena, and the obtained values were within the range of 22.83-48.21 (mg GAE. g of dried leaves). Phenol concentration may vary in different plant species due to the genetic, ontogenetic, biotic, and abiotic factors diversely affecting various pant parts [28]. According to the study by Paun et al. (2013), lemon verbena could be used as a natural antioxidant [29].

In the case of β-carotene-linoleic acid assay in the current research, both EOs could effectively inhibit linoleic acid oxidation, and the inhibition values were calculated to be 67.28 ± 0.4% and 51.48 ± 0.33% in the clove and lemon verbena EOs at the concentration of 2 mg/ml, respectively. However, the examined EOs showed lower antioxidant activity compared to the BHT standard, which demonstrated the highest antioxidant activity, followed by the clove and lemon verbena EOs. On the other hand, the study conducted by Farahmandfar et al. (2018) suggested that lemon verbena EO had better function than BHT [30]. Moreover, Raeisi et al. (2016) reported that clove EO had a strong capacity in the preservation of β-carotene, which is highly similar to BHT [8]. The β-carotene bleaching test is similar to an oil-in-water emulsion system; it seems the differences in the solubility of antioxidant compounds influence their activity in this assay [12].

Table 6 shows the ability of the EOs and BHT as the positive control to reduce Fe^{3+} to Fe^{2+}. In this assay, BHT was observed to be most efficient, followed by the lemon verbena and clove EOs, respectively. In this regard, the findings of Gülçin et al. (2012) indicated that clove EO had higher ability to reduce Fe^{3+} to Fe^{2+} compared to BHA and BHT. The reducing capacity of a compound may be considered a significant indicator of its potential antioxidant activity [2].

Table 5: Antibacterial effect of EOs on tested bacterial strains by micro-dilution method

	Citriodora		*S. Aromaticum*	
	MIC	**MBC**	**MIC**	**MBC**
E.coli	2500	2500	5000	10000
S. thyphimurium	1250	2500	2500	5000
P.aeroginosa	2500	5000	5000	10000
S.dysenteriae	1250	2500	1250	2500
S. aureus	1250	2500	2500	5000
L. monocytogenes	2500	5000	2500	2500
B. cereus	2500	2500	5000	5000

Table 6: Antioxidant capacity of tested EOs by different assays (mean ± SD).

Test	***S. aromaticum***	***A. citriodora***	**BHT**
DPPH IC_{50}(µg/ml)	81.18±0.02[a]	11.33±0.01[b]	27.43±0.04[c]
Total phenolic content (mg $_{GAE}$. g $_{EO}$)	128.06±6.78[a]	816.07±46.81[b]	*
β carotene-linoleic acid bleaching test (%)	67.28±0.4[a]	51.48±0.33[b]	91.68±0.15[c]
Reducing power IC_{50}(mg/ml)	7.84±0.21[a]	4.38±0.11[b]	0.86±0.29[c]

The upper-case letters in the same rows show significant differences at $P < 0.05$, and the lower-case letters in the same columns show significant differences at $P < 0.05$).

4. Conclusion

This study provides data on the chemical composition and antioxidant and antimicrobial activities of lemon verbena and clove EOs. According to the results, the tested EOs consisted of various components, some of which were common between the two species with variable proportions. In addition, the clove and lemon verbena EOs exhibited remarkable antimicrobial and antioxidant activities, while the lemon verbena EO proved more efficient owing to its abundant phenolic content, which enhanced its antioxidant activity in all the tests, with the exception of the β-carotene-linoleic acid bleaching test. Therefore, it could be concluded that these EOs are applicable as natural preservatives for the preservation and/or extension of the shelf life of raw and processed food products, while further investigations are recommended on food models to confirm these findings.

Authors' Contributions

M.H., A.J., and M.R., designed the study; M.H., performed the experiments; M.A., analyzed the data. All the authors discussed the results and commented on the final manuscript.

Conflict of Interest

None declared.

Acknowledgments

Hereby, we extend our gratitude to the School of Veterinary Medicine at Ferdowsi University of Mashhad for the financial support of this study (Grant No. 45251). We would also like to than Golestan University of Medical Sciences for assisting us in this research project.

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