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Original Article

Chemical Composition and Antioxidant Properties and Antimicrobial Effects of *Satureja bachtiarica* Bunge and *Echinophora platyloba* DC. Essential Oils Against *Listeria monocytogenes*

Running title: Biological activities of *Satureja bachtiarica* and *Echinophora platyloba*.

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Abstract

Satureja bachtiarica Bunge and *Echinophora platyloba* DC. are two important indigenous medicinal plants of Iran with high antimicrobial and antioxidant effects. The existing examination was done to assess the chemical composition and antioxidant and antibacterial properties of *S. bachtiarica* and *E. platyloba* essential oils against *Listeria monocytogenes*. Aerial parts of plants were collected and their essential oil were extracted using the water distillation method (Clevenger apparatus). Chemical components were analyzed using the GC-MS. Total phenolic and flavonoid components and DPPH radical scavenging effects of essential oils were analyzed. Antibacterial effects were assessed using the disk diffusion and MIC and MBC values were assessed by the micro broth dilution. Carvacrol (31.25%), thymol (23.50%) and o-cymene (13.87%) were the most frequently identified chemical components in *S. bachtiarica*. Ocimene (44.15%), α-phellandrene (16.80%) and γ-terpinene (8.52%) were the most frequently identified chemical components in the *E. platyloba*. DPPH radical scavenging effects of S. *bachtiarica* and *E. platyloba* were 76.72 ± 2.52% and 64.21 ± 2.11%, respectively. TPC of S. *bachtiarica* and *E. platyloba* were 88.33 ± 1.69and 30.05 ± 1.14 (mg GAE per 100 g dw), respectively. TFC of S. *bachtiarica* and *E. platyloba* were 20.63 ± 1.24 and 17.28 ± 1.07 (mg quercetin per 100 g dw), respectively. Antibacterial effects of *S. bachtiarica* and *E. platyloba* essential oils were dose-dependent (P<0.05). *S. bachtiarica* (40 mg/mL) had the highest diameter of the growth inhibition zone (23.64 ± 0.35 mm). MICs and MBCs of *S. bachtiarica* and *E. platyloba* essential oils were 5 and 20 and 10 and 40 mg/mL, respectively. Both plants are suitable candidate as food preservatives.

Keywords: *Satureja bachtiarica*, *Echinophora platyloba*, Chemical compositions, Antimicrobial effects, Antioxidant effects.

Introduction

Traditional medicine has continued as the major reasonable and simply available source of treatment in the primary health care system. Natural products have played a significant portion in treating and preventing human diseases and also as a favorable additive in foods. Medicinal plants have played an imperative importance in the growth of human culture. Lately, there is an developed attention in natural ingredients displaying antioxidant and antimicrobial characters,

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particularly those of plant origin, that are provided to human and animal organisms as food compounds or as precise pharmaceutics [1].

The genus *Echinophora* (family: Apiaceae or Umbelliferae) is introduced in the plants of Iran by four species of *E. platyloba*, *E. cinerea*, *E. orientalis*, and *E. sibthorpiana*. *E. platyloba* and *E. cinerea* are limited to Iran [2, 3]. *E. platyloba* is a perfumed herb with alternative compound leaves lead to prickles distinguishing of the genus *Echinophora*. *E. platyloba* has steady, bisexual flowers in umbels and two fruits [2, 3]. It is routinely extensive in boost latitudes (1500–2100 m overhead sea level) in Southwest Iran. *E. platyloba* and its essential oil have been applied as antifungal, antiseptic, antimicrobial, and antioxidant agents [4,5]. It is also full from natural preservatives, phenolic and flavonoid components with high antioxidant and antibacterial activities [4,6].

The genus of Satureja (family: Lamiaceae) consists of 30 species of perfumed herb verbosely dispersed in Mediterranean area, Africa, Asia and America [7, 9]. These are yearly semi-bushy perfumed plants that occupy dry, sunlit, pitiless and stony environments. About 12 species of this genus are growing wild in Iran [7, 9]. *Satureja bachtiarica* Bunge (*S. bachtiarica*) is one of the endemic species of this genus in Southwest of Iran [7, 9]. Aerial fragments of some species of *Satureja*are extensively applied as a flavoring agent for abundant type of foodstuffs and also as a old-style herbal medicine for the treatment of gastrointestinal illnesses. *S. bachtiarica* is well-known for its therapeutic characters as an palliative and antiseptic in medicine. It is also valuable for treatment of diverse illnesses, such as muscle pains, spasms, vomiting, dyspepsia, diarrhea and infectious diseases [7, 11]. *S. bachtiarica* and its essence have been applied as antiseptic, antimicrobial, antifungal and antioxidant agents [7, 11]. It is also full from natural preservatives, phenolic and flavonoidcomponents with high antioxidant and antibacterial activities [10, 11].

The development of multidrug–resistant bacteria is a phenomenon of worry to the clinician and the pharmaceutical industry, and is a foremost reason of disappointment in the treatment of infectious diseases all around the world. Listeria monocytogenes (L. monocytogenes) is a Gram-positive, motile, facultative anaerobic and intracellular bacterium with an emergence of antibiotic resistance [12,13]. The pathogen can be originate universal in the food samples and most *L. monocytogenes* infections are developed through consumption of contaminated food. The chief clinical syndromes instigated by *L. monocytogenes* comprise feverish gastroenteritis, systemic and perinatal infections noticeable by infections of the central nervous system like meningitis with or without bacteremia (12, 13). Abortion may happen through the listeriosis in pregnant women [12,13]. Documented data revealed that *L. monocytogenes* strains isolated from human clinical infections and also those of food samples harbored boost incidence of resistance against routinely applied antibacterial agents including penicillins, tetracyclines, aminoglycosides, macrolides, lincosamides, folate inhibitors, fluoroquinolones andphenicols [14, 16].Therefore, it is important to found novel antibacterial substitutions for treatments of cases of human and animal listeriosis.

Throughout the previous decade, curiosity in drugs of herbal origin has been increasing progressively and the consumption of medicinal plants has virtually folded not only in Iran but also in other sites of the world. All plants may not be useful as claimed, or may have more therapeutic properties than are known traditionally. Thus, proper systematic and scientific research is required to explore the exact medicinal potential of plants. Consequently, the existing investigation was done to evaluate chemical composition and antioxidant and antimicrobial effects of *S. bachtiarica* and *E. platyloba* essential oils against *L. monocytogenes* in vitro condition.

Material and Methods

Morals Endorsement

The Research was permitted by the Moral Convention of the College of Veterinary Medicine Shahrekord Branch, Shahrekord, Iran. Corroboration of this research scheme and the certificates correlated to sampling were agreed by the professors of this institute.

Plant materials and Essential Oil Extraction

The aerial sections (up to ~ 5 cm, 200 g) of *S. bachtiarica*and *E. platyloba*were obtained from Chaharmahalva Bakhtiari province through spring of 2017. Plants were identified by expert professor of the field of Agriculture and Medicinal Herbs and

coupon sample was placed at the Herbarium of the Department of Agriculture of Chaharmahalva Bakhtiari province, Iran. The dried aerial sections of *S. bachtiarica* and *E. platyloba*were ground and applied for extraction of essential oil using water distillation method (Clevenger apparatus) [17]. Fleetingly, 100 grams of the ground plant was weighed and moved to a 2-liter flask fixed to the Clevenger. Distilled water (1,500 ml) was added to the flask comprising the powder. Extraction was carried out for 4 h and then the oil was recovered and dehydrated by anhydrous sodium sulfate. The organized essential oil was kept at -20 °C till further operations and additional analysis.

GC-MS Analysis

Chemical and biological components of the S. *bachtiarica* essential oil was determined using an gas chromatograph (Agilent 7890 A, USA) equipped with a HP-5MS 5% phenyl methyl siloxane capillary column (30 m × 0.25 mm × 0.25 μm). Oven temperature was reserved at 60 °C for 4 min firstly, and formerly elevated at the degree of 4 °C/min to 260 °C. Detector and injector temperatures were line up at 290 and 300°C, respectively. Helium was accompanied as gas carrier at a flow degree of 0.8 mL/min, and 0.5 μL specimens were injected physically in the splitless style. Summits area percent's were applied for achieving numerical information. Mass array was determined from *m/z* 50 to 550 amu. Holding directories were restrained for compounds by a homologous types of *n*-alkanes (C5-C24) injected in circumstances equivalent to those for specimens. Chemical components of the essential oil of *E. platyloba* was recognized using the GC-mass analysis with similar device. Helium was applied as transporter gas with a persistent flow degree of 1 ml/min. Temperature of the oven was line up at 50 ºC for 2 min and at that moment adjusted till 70 ºC at a 5 ºC/min degree and lastly heated to 100 ºC at a degree of 20 ºC/min and last of all elevated, at 10 ºC/min degree, to 290 ºC and remain at mentioned temperature for 2 min. Detector and injector temperatures were 300 ºC and 200 ºC, correspondingly. Four microliters of oil was injected. Injection style, split; split relation 1: 100. The MS functional restrictions were as subsequent: 200 ºC interface temperature, 70 eV ionization potential and 50–800 mass array acquisition. Recognition of oil compounds was determined regarding the techniques identified beforehand [18, 20]. DPPH Radical Scavenging Activities of S. bachtiarica and E. platyloba

Antioxidant effects of *S. bachtiarica* and *E. platyloba* essential oil was assessed by DPPH radical scavenging activity determined based on the method described previously [21]. Fifty microliters of essential oil were blended with methanolic solution (1,950 μL, 40 μM) of DPPH radicals. Afterward agitation, the blending was incubated in the dark place for 30 min and the absorbance was restrained at 517 nm using the spectrophotometer (Shimadzu, France). The antioxidant activity was identified based on the succeeding equation [22]:

% DPPH radical scavenging= $[(A_{C(30)}-A_{S(30)}/A_{C(30)})] \times 100$

Where $A_{C\ (30)}$ resembles to methanol and DPPH radical absorbances at t=30 min and $A_{S(30)}$ to sample and DPPH radical absorbances at t=30 min.

Examination of Total Phenolic Content (TPC)

TPC of *S. bachtiarica* and *E. platyloba* essential oils were assessed using Folin-Ciocalteau reagent based on the technique defined beforehand [23]. Essential oils were thinned and blended with Folin-Ciocalteau (2 N) and also sodium carbonate (20%). Assortment was incubated in the dark place for 2 h. Afterward, the absorbance of the combination was determined at 765 nm. The outcomes were stated as equivalent of Gallic acid (mg GAE) per *S. bachtiarica* and *E. platyloba* essential oils (100 g) in dry weight basis (dw).

Analysis of Total Flavonoid Content (TFC)

TFC of *S. bachtiarica* and *E. platyloba* essential oil was determined by the aluminum chloride colorimetric technique according to method described previously [24]. Briefly, 0.25 mL aliquot of the *S. bachtiarica* and *E. platyloba* essential oil was blended with 2 mL of distilled water and 0.15 mL of sodium nitrite (5%) in a laboratory tube. Afterward 5 min, aluminum chloride solution (10%, 0.15 mL) was mixed with previous combination. Afterward 6 min, sodium hydroxide solution (1M, 1 mL) was blended with the combination. Unswervingly, the solution was diluted using distilled water (1.2 mL) and carefully assorted. Absorbance of the last combination was restrained at 510 nm in contradiction of a blank solution using the spectrophotometer (Shimadzu, France).

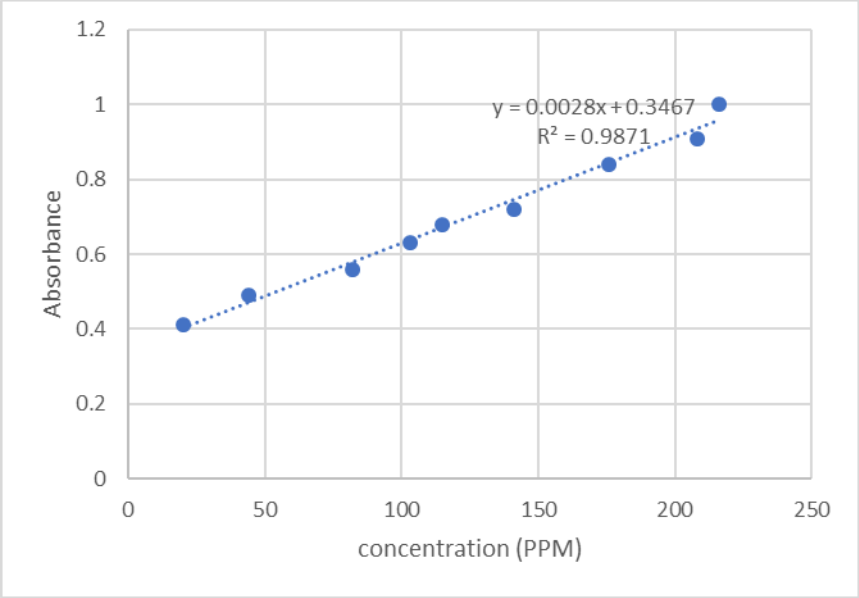

Fig. 1 Standard curves of quercetin (QE).

The concentration of total flavonoid content in the test samples was calculated. Total flavonoid content was stated as milligram quercetin (QE) per essential oil (100 g) in basis of dry weight. Figure 1 represents the standard curves of quercetin (QE).

Bacterial Strains and Growth Conditions

L. monocytogenes (ATCC 15313) were obtained from the Iranian Industrial and Scientific Research Center (Tehran, Iran). Standard cultures were preserved at -80 °C in broth media containing 20% glycerol (Sigma-Aldrich, UK). Tryptic soy broth (TSB, Merck, Germany) with 0.6% yeast extract (Oxoid, Hampshire, UK) was used to culture *L. monocytogenes*. For each experiment, the standard culture of bacterium was melted at room temperature. At that point, 0.01 mL of melted standard culture of the bacterium was inoculated into a 25 mL Erlenmeyer flagon comprising 10 mL of nutrient broth (NB, Merck, Germany), wrapped with a silicone cap and incubated at 37 °C for 24 h in an aerobic condition.

Antimicrobial Effects of *S. bachtiarica* and *E. platyloba* Essential Oil against *L. monocytogenes*

Bacterial inoculates were growth on NB (Merck, Frankfurt, Germany) agar at 37 °C for 24 h; Colonies were added in sterile 0.9% saline and attuned to 0.5 McFarland concentration, which is equal to 10^8 colony-forming units per mL (10^8 CFU/mL). Antimicrobial effects of the *S. bachtiarica* and *E. platyloba* essential oils (0.625 to 40 mg/mL) were tested using the simple disk diffusion method. Sterile paper discs (9 mm in diameter and 250 g m-2) were soaked with 25 µL of pure essential oil and located on plates inoculated with 10^8 suspensions of each culture and incubated at 37 °C for 18-24 h. The inhibition zones diameter, was determined in millimeters, and inhibition was counted regarding the technique defined by Carovic-Stanko *et al.* (2010) [25]. Tests were accompanied in quadruplicate. Antimicrobial susceptibility of bacterial strains was also studied against certain antibiotic disks. For this purpose, the dimeter of the zone of inhibition for each treatments of extract were analyzed. Then, the diameter of the inhibition zones of those that had the highest antimicrobial activities (the highest diameter of the zone of inhibition) were compared with different antibiotic agents. For this purpose, amoxicillin (25 µg/disk), gentamicin (10 µg/disk), cefexime (5 µg/disk), tetracycline (30 µg/disk) and penicillin (10 µg/disk) antibiotic disks were used (Oxoid, Wade RoadBasingstokeHampshireRG24 8PW, UK). Analyses was done according to the method described by an International Institute (CLSI, USA) [26].

Minimum Inhibitory Concentrations (MIC) and Minimum Bacterial Concentrations (MBC)

for evaluation of MIC and MBC, the standard of 0.5 McFarland was line up with a density of 1.5 × 10^8 CFU/mL in phosphate buffered saline solution. The MIC value for *L. monocytogenes* was demonstrated by the micro broth dilution technique. An achieved essential oil was melted in

dimethyl sulfoxide. Serial dilution performance was accompanied to display the MIC of the essential oil at concentrations of 0.625 to 40 mg/mL after 18 h of incubation. Each well had 5 µL of bacterial suspension with 95 µL of Müller-Hinton broth and also 100 µL of serial double dilution of the essences. Müller-Hinton broth (200 µL) and Müller-Hinton broth (195 µL) with 5 µL of the bacterial suspensions were accompanied as positive and negative controls, correspondingly. The MIC value was identified as the first tube deprived of any turbidity. The MBC value was determined as the minimum concentration that displayed no noticeable growth on Müller-Hinton agar media at 37 °C for 24 h [27].

Arithmetical Examination

Achieved data were entered to the Microsoft Excel software (Microsoft Corp., Redmond, WA, USA). Findings were then examined based on the principles of the SPSS 21.0 arithmetical software (SPSS Inc, USA). One-way analysis of variance (ANOVA) test was applied for study the presence of significant differences between data recovered from the existing research.

Results

Chemical Compositions of the *S. bachtiarica* Essential Oil

Table 1 represents the chemical compositions of the *S. bachtiarica* essential oil. GC-mass analysis can find a total of 28 separate chemical components (98.59%) in the essential oil extracted from the *S. bachtiarica*. Findings showed that carvacrol (31.25%), thymol (23.50%), o-cymene (13.87%) and γ-terpinene (10.65%) were the most routinely identified chemical compositions in the essential oil of the *S. bachtiarica*. Frequency of carvacrol acetate (0.089%), α-humulene (0.018%) and cis-pinocamphone (0.12%) were entirely lower than other detected chemical components in the essential oil extracted from the *S. bachtiarica*.

Table 1 GC-MS analysis of chemical compositions of the *Saturejabachtiarica* Bunge essential oil.

No	RT (min)	Chemical components	Frequency (%)
1	5.06	α-thujene	0.15
2	5.226	α-pinene	0.91
3	5.552	camphene	0.97
4	6.216	β-pinene	0.15
5	6.513	myrcene	0.69
6	6.92	α-phellandrene	0.15
7	7.2	α-terpinene	1.65
8	7.429	o-cymene	13.87
9	7.538	Limonene	0.44
10	7.623	1,8-cineole	0.13
11	8.407	γ-terpinene	10.65
12	9.266	Terpinolene	0.25
13	9.58	Linalool L	2.24
14	27.193	Cis-limonene oxide	0.15
15	11.709	Borneol l	4.4
16	11.978	*Cis*-pinocamphone	0.12
17	12.069	Terpineol-4	0.68
18	14.633	Thymol	23.5
19	15.777	Geranylformate	0.22
20	16.086	carvacrol	31.25
21	17.746	Thymol acetate	1.04
22	18.335	Carvacrol acetate	0.089
23	19.846	*Trans*-caryophyllene	3.06
24	20.429	Aromadendrene	0.15
25	20.881	α-humulene	0.018
26	22.506	β-bisabolene	0.18
27	24.566	Spathuleno	0.36
28	24.738	Caryophyllene oxide	1.13
Total			98.597

Chemical Compositions of the *Echinophora platyloba* DC. Essential Oil

Table 2 represents the chemical compositions of the *Echinophora platyloba DC.* essential oil. GC-mass analysis can find a total of 32 separate chemical components (99.17%) in the essential oil extracted from the *Echinophoraplatyloba DC.*. Findings showed that ocimene (44.15%), α-phellandrene (16.80%), γ-terpinene (8.52%) and β-myrcene (6.08%) were the most routinely identified chemical components in the essential oil of the *E. platyloba*. Frequency of 2,3-dimethyl-cyclohexa-1,3-diene (0.06%), and 2-methyle-2-bornene (0.08%) were entirely lower than other detected chemical components in the essential oil extracted from the *E. platyloba*. Chemical and antioxidant properties of S. bachtiarica and E. platyloba essential oils

Table 3 represents theTPC, TFC and DPPH radical scavenging activity of *S. bachtiarica* and *E. platyloba* essential oils. DPPH radical scavenging effects of S. *bachtiarica* and *E. platyloba* essential oils were 76.72 ± 2.52% and 64.21 ± 2.11%, respectively. TPC of S. *bachtiarica* and *E. platyloba* essential oils were 88.33 ± 1.69and 30.05 ± 1.14 (mg GAE per 100 g dw), respectively. TFC of S. *bachtiarica* and *E. platyloba* essential oils were 20.63 ± 1.24 and 17.28 ± 1.07 (mg quercetin per 100 g dw), respectively. Statistically significant differences were seen for the DPPH radical scavenging effects and TPC between S. *bachtiarica* and *E. platyloba* essential oils (P<0.05). Nevertheless, there was no noteworthy arithmetical variance for the TFC between S. *bachtiarica* and *E. platyloba* essential oils (P<0.05).

Table 2 GC-MS analysis of chemical compositions of the *Echinophora platyloba DC.* essential oil.

No	RT (min)	Chemical components	Frequency (%)
1	3.79	2,3-dimethyl-cyclohexa-1,3-diene	0.06
2	5.06	α-thujene	0.15
3	5.226	α-pinene	3.14
4	6.119	sabinen	0.48
5	6.216	β-pinene	0.33
6	6.513	Myrcene	6.08
7	6.92	α-phellandrene	16.8
8	7.429	p-cymene	3.36
9	7.555	β-phellandrene	5.91
10	7.755	Cis-β-ocimene	1.49
11	8.156	*Trans*-β-ocimene	44.15
12	8.407	γ-terpinene	8.52
13	9.266	terpinolene	1.72
14	14.496	2-methyle-2-bornene	0.08
15	11.034	Verbanol	0.19
16	11.503	2,3-dihydro-2,2,6-trimethylbenzalhyde	0.25
17	18.870	Camphenone	0.45
18	13.042	1,3,8-p-menthatriene	0.15
19	12.499	α-terpineol	0.29
20	13.797	Cis-3-Hexenyl benzoate	0.15
21	13.923	Isovaleric acid,3-hexenyl ester	0.17
22	14.221	Carvone	0.32
23	14.107	Dimethoxy-(E)-citral	0.56
24	10.542	1,3,8-p-menthatriene	0.41
25	14.53	Geraniol	0.15
26	15.068	Citral	0.70
27	15.777	Thymol	0.11
28	16.086	Carvacrol	0.11
29	19.348	Methyleugenol	0.20
30	44.170	γ -dodecalactone	1.04
31	22.18	Bicyclogermacrene	0.20
32	26.037	Farnesyl acetone	1.45
Total			99.17

Table 3 Total phenolic and total flavonoid contents and DPPH radical scavenging activity of *Satureja bachtiarica* Bunge and *Echinophor aplatyloba DC.* essential oils.

Essential oil	DPPH radical scavenging effects (%)*	TPC (mg GAE per 100 g dw)	TFC (mg quercetin per 100 g dw)
S. bachtiarica	76.72±2.52 a	88.33±1.69 a	20.63±1.24 a
E. platyloba	64.21±2.11 b	30.05±1.14 b	17.28±1.07 a

*All results were expressed as mean of triplicate tests with standard deviation.
**Dissimilar small leathers in each column shows momentous arithmetical variance (P<0.05).

Table 4 Comparison of the diameter of growth inhibition zone of *Satureja bachtiarica* Bunge and *Echinophora platyloba DC.* essential oils and several antibiotic agents against *L. monocytogenes*.

Essential oils and antibiotics (concentration)		Diameter of the growth inhibition zone (mm)*
	0.625 mg/mL	4.33±0.12 d
	1.25 mg/mL	5.79±0.14 d
	2.5 mg/mL	6.28±0.10 d
S. bachtiarica	5 mg/mL	12.25±0.24 c
	10 mg/mL	14.63±0.31 c
	20 mg/mL	18.92±0.42 b
	40 mg/mL	23.64±0.35 a
	0.625 mg/mL	3.14±0.11 d
	1.25 mg/mL	4.20±0.10 d
	2.5 mg/mL	5.51±0.13 d
E. platyloba	5 mg/mL	10.32±0.18 c
	10 mg/mL	12.41±0.42 c
	20 mg/mL	17.56±0.34 b
	40 mg/mL	21.92±0.30 a
	Amoxicillin (25 µg/disk)	10.71±0.12 c
	Gentamicin (10 µg/disk)	7.15±0.16 d
Antibiotic agents	Cefexime (5 µg/disk)	15.23±0.31 b
	Tetracycline (30 µg/disk)	9.65±0.17 c
	Penicillin (10 µg/disk)	11.39±0.16 c

*All results were expressed as mean of triplicate tests with standard deviation.
**Dissimilar small leathers in each column shows momentous arithmetical variance (P<0.05).

Table 5 MIC and MBC of *Satureja bachtiarica* Bunge and *Echinophora platyloba DC.* essential oils against *L. monocytogenes*.

Essential oil	Minimum inhibitory concentrations (mg/ml)*	Minimum bacterial concentrations (mg/ml)*
S. bachtiarica	5	20
E. platyloba	10	40

Antimicrobial Effects of *S. bachtiarica* and *E. platyloba* Essential Oils

Table 4 represents the comparison of the diameter of growth inhibition zone of *S. bachtiarica* and *E. platyloba* essential oils and several antibiotic agents against *L. monocytogenes*. Findings show that antibacterial effects of *S. bachtiarica* and *E. platyloba* essential oils were dose-dependent. Otherwise, increase in the essential oil concentration caused significant increase in the diameter of the growth inhibition zone of *L. monocytogenes* (P<0.05). *S. bachtiarica* (40 mg/mL) had the highest diameter of the growth inhibition zone (23.64 ± 0.35 mm), while *E. platyloba* (0.625 mg/mL) had the lowest diameter of the growth inhibition zone (3.14 ± 0.11 mm). Cefexime (5 µg/disk) antibiotic agent had the highest diameter of the growth inhibition zone (15.23 ± 0.31 mm), while gentamicin (10 µg/disk) antibiotic agent had the lowest (7.15 ± 0.16 mm). Additionally, diameter of the growth inhibition zone of *L. monocytogenes* treated with *S. bachtiarica* and *E. platyloba* essential oils (at concentrations of 20 mg/mL) was entirely higher than tested antibiotic agents. In keeping with this, *S. bachtiarica* essential oil caused higher diameter of the growth inhibition zone than *E. platyloba* in all concentrations.

Table 5 represents the MIC and MBC values of *S. bachtiarica* and *E. platyloba* essential oils against *L. monocytogenes*. MICs of *S. bachtiarica* and *E. platyloba* essential oils were 5 and 10 mg/mL, respectively. MBCs of *S. bachtiarica* and *E. platyloba* essential oils were 20 and 40 mg/mL, respectively.

Discussion

The contemporary investigation was done to assess the chemical compositions of *S. bachtiarica* and *E. platyloba* essences and study their antioxidant effects and antimicrobial activities against *L. monocytogenes*. We found that ocimene (44.15%), alpha-phellandrene (16.80%), γ-terpinene (8.52%) and beta-myrcene (6.08%) were the foremost routinely identified chemical components in *E. platyloba*. Asghari *et al*. (2003) (28) also stated that β-ocimene (67.90%), 2-furanone (6.20%) and myrcene (6%) were the foremost routinely identified components of *E. platyloba*essence (E)-β-ocimene (49.9%), γ-decalactone(8.40%), α-pinene (6.00%) and linalool (5.6%) were reported as the principle constituents of *E. platyloba*essence [29]. Hassanpouraghdam *et al*. (2009) [20] reported that the foremost routinely identified chemical components in *E. platyloba* essence were monoterpene hydrocarbons (84.80%), oxygenated monoterpenes (4.80%). (Z)-β-ocimene (38.90%), α-phellandrene (24.20%), p-cymene (7.40%), β-phellandrene (6.30%), α-pinene (3.40%), γ-decalactone (1.70%) and linalool (1.20%). Thymol (27.2%), trans-ocimene (20.9%) and carvacrol (7.2%) were also described as the foremost routinely identified components of *E. platyloba* essence from Iran [30]. Some investigations have assessed the chemical composition of *E. platyloba* from diverse portions of Iran. β-ocimene (28%-68%) were conveyed as the first foremost routinely identified components of *E. platyloba* essences from four investigations [28, 31, 32], though the another routinely identified component has been dissimilar in these researches, and furanone (6.20%), α-decalactone (8.40%), γ-phellandrene (24.20%), and δ-3- carene (16.20%) were stated as the second routinely identified components of essencefrom Isfahan [28], Tehran[31], Azarbaijan (Maragheh district) [20], Shahrekord [32], Iran; respectively. Asarone (10.20%), anethole (7.40%), eugenol (6.70%) and dimethyl styrene (6.60%) were the routinely identified components of *E. platyloba*essence from Khorasan, Iran [6]. Additionally, carvacrol, thymol, o-cymene and γ-terpinene were the routinely identified chemical components in *S. bachtiarica*. Moeini *et al*. (2018) (33) described that thymol (65.1%), γ-terpinene (15.0%), β- caryophyllene (4.85%), p-cymene (4.4%), linalool (3.5%) and borneol (3.05%) were also the foremost routinely identified chemical components in the *S. bachtiarica* essence. Sefidkon and Jamzad (2000) [34] expressed the the foremost routinely identified of the *S. bachtiarica* essence were thymol (44.50%), *γ*-terpinene (23.90%), *p*-cymene (7.30%), *β*-caryophyllene (5.30%) and borneol (4.20%). Carvacrol (19.90 - 66.50%) and thymol (0.3 - 19.20%) were also recognized as the foremost routinely chemical components in the *S. bachtiarica* essence in different investigations [35, 36]. Ahanjan *et al.* (2014) [37] reported that the most commonly detected chemical components in the *S. bachtiarica*essence were phenol (37.36%), thyme (22.65%), p-cymene (19.29%), g-terpinene (5.01%), l-linalool (4.92%) and b-caryophyllene (2.19%). Changes in the day length, rainfall, height of the zone, soil type and also weather and climate in different studies are the foremost factors caused differences in the chemical compositions of *S. bachtiarica* and *E. platyloba* essences reported in different investigations.

Phenolic complexes are the major imperative antioxidant components and commonly examined in numerous therapeutic plants for showing their antioxidant aspects. Polyphenols are bioactive subordinate metabolites of therapeutic plant that are extensively existing in typically used foodstuffs with herbal origins. The two major kinds of polyphenols are flavonoids and phenolic acids which act as influential antioxidant factors. The attitude of the antioxidant effect is the accessibility of electrons to counterbalance any kinds of free radicals.Flavonoids are extensive plant subordinate elements, including flavanols, flavones, and shortened tannins. Epidemiological investigation recommend that the use of foods with boost flavonoid content defends against human diseases related to oxidative stress. Free radical scavenging activity of flavonoids has been identified *in vivo* and *in vitro* circumstances. DPPH is a steady free radical, extensively acknowledged as an element for assessing the radical scavenging activities of antioxidant agents. Findings of the existing study were also showed that *S. bachtiarica* and *E. platyloba* essences had high DPPH radical

scavenging activities and also considerable levels of TPC and TFC. High antioxidant effect of *S. bachtiarica* and *E. platyloba* essences is mostly because of the boost content of TFC and TFC. The radical scavenging capacity of *S. bachtiarica* and *E. platyloba* essences might be because of groups of hydroxyl existing in the chemical construction of phenolic compounds that can provide the essential composite as a radical scavenger. High TPC, TFC and DPPH radical scavenging effects of *E. platyloba* essence was also reported by Khazai *et al*. (2011) [38]. They reported that the TPC, TFC and DPPH radical scavenging effects of *E. platyloba* essence were 3.15 ± 0.40 mg/g, 8.15 ± 0.22 mg/g and 73.78 ± 1.36 EC_{50}, respectively [38]. Gokbulut *et al.* (2013) [39] reported that TPC and DPPH radical scavenging effects of *E. platyloba* essence were 1.39 ± 0.09 g GAE/kg plant and 2.84 ± 0.07 EC_{50} g/L, respectively. Our result of antioxidant activity based on the DPPH is also in agreement with the previous findings for different plants previously reported. For example, in the study of Pourmorad *et al*. (2006) [40] on antioxidant activity (IC_{50}) determined by DPPH assay in five Iranian medicinal plants changed from 0.01 to 2.03 mg/mL. TPC of *S. bachtiarica* and *E. platyloba* essences determined were found to be 88.33 ± 1.69 and 30.05 ± 1.14 mg GAE per 100 g dw, respectively. Compared to the TPC of some plants [41] in dry basis, including mueslis (0.20–1.30 mg GAE/g), tubers (0.40 – 6.60 mg GAE/g), and berries (12.40 – 50.80 mg GAE/g), a higher level was found in the analyzed sample. GhasemiPirbalouti *et al*. (2013) [42] reported that TPC, TFC and DPPH radical scavenging effects of *E. platyloba*, *Heracleumlasiopetalum* and *Kelussiaodoratissima* essences were at a ranges of 74.0 – 120.0 TAN/g essence, 7.63 - 14.52 mg/g extract and 2.28-6.58 IC_{50} mg/ml, respectively. The conclusions of the existing enquiry are comparable to those of Gholivand *et al*. (2011) [43] which initiated that the Arctic fractions of *E. platyloba* essence providing the maximum radical scavenging activity compared to additional analyzed sub-fractions and essences. Saei-Dehkordi *et al.* (2012) [30] demonstrated that *E. platyloba* essence revealed boost scavenging and relative antioxidative in DPPH radicals examine which was comparable to our findings. Ghasemi Pirbalouti *et al*. (2014) [44] reported that TPC, TFC and DPPH radical scavenging effects of *S. bachtiarica* essence were 103.00 ± 1.78 TAE/g extract, 10.05 ± 0.13 equivalents/g extract and 3.05 ± 1.11 IC50 mg/mL, respectively. Plander *et al*. (2012) [45] stated that the phenolic and flavonoid contents in numerous essences obtained from the *S. hortensis* had ranges from 0.040 to 0.13 g pyrogallol corresponding/100 g essence and 1.37% to 7.09% w/w, correspondingly. The phenolic gratified of *S.sahendica* essence was 24.8 to 25.6 of GAE/g essence [46]. Terpenoids and polyphenolic components are principally accountable for the biological activities of the genus, predominantly, antioxidant effects. Carvacrol and thymol related to monoterpenoid phenol are the chief elements in the *S. bachtiarica* essence. Otherwise, the attendance of numerous polyphenolic ingredients, particularly flavonoids and phenolic acids, is well recognized in Satureja family [47]. Phenolic and flavonoid complexes exhibited varied organic effects such as anti-atherosclerotic, antimicrobial, anti-carcinogenic, and anti-inflammatory [48]. The alterations between existing outcomes and the preceding surveys may be ascribed to the changes in the sources of the specimens and method of extraction.

Antimicrobial characters of elements of medicinal herbs have been illustrious since early times. Plants describe an outstanding source of novel antimicrobial elements. The final part of our research was conducted on the antibacterial effects of *S. bachtiarica* and *E. platyloba* essences against *L. monocytogenes*. Findings showed high antibacterial effects of these two medicinal plants against *L. monocytogenes* especially at the concentrations above 5 mg/ml. High antibacterial effects of these two medicinal plants and especially *S. bachtiarica* essence is foremostly due to the high contents of TPC and TFC. Additionally, presence of some antimicrobial components in their essences caused significant antibacterial effects against *L. monocytogenes*. For example, carvacrol was found in the essence of both tested medicinal plants and especially *S. bachtiarica*. Carvacrol is an isothymol that inhibited activity of ATPase enzymes; and to cause increasing nonspecific penetrable bacterial cell membrane, so increase microorganism sensitivity to entry extraneous matters [49, 50]. Cis space structure of essential components is another important factor which increase the antimicrobial effects of medicinal plants. Cis space structure around the twofold linkage cause hyper antibacterial activity and hyper reaction group in aliphatic alcohols (like linalool) and phenols (like

thymol) [49, 50]. Numerous investigations stated that the Satureja essences are recognized to exhibits antibacterial characters which may likely because of the presence of considerable points of thymol, carvacrol methyl ether, carvacrol, and terpinene [51]. Additionally, most researches conducted on this field focused on the effects of herbal extracts on cellular membranes, changing their purpose and their construction, instigating swelling and growing their penetrability. Carvacrol and thymol seem to made the bacterial cell membrane penetrable and act otherwise against Gram-positive bacteria [52]. Serrano *et al*. (2011) [53] reported that *S. montana*L. extract had comparatively boost antimicrobial effects against the seven species of pathogenic bacteria. They expressed that the extract subdued the growth of *L. monocytogenes* (MIC of 1.10 µg/mL). The MIC value of another Satureja essence against *L. monocytogenes* was introduced as 1.56 µg/mL [54]. Presence of high phenolic components is the foremost factor causing high antimicrobial effects of the *E. platyloba* essence. Rises in cytoplasmic membrane penetrability seem to be an outcome of the loss of the gradient of cellular pH, proton motive force and reduced ATP contents, resultant in the cell death [55]. Additionally, cymene and carvacrol are two important chemical components in the essence of *E. platyloba*. The biological precursor of carvacrol and cymene, is hydrophobic and origins development of the cytoplasmic membrane. Cymene joins into the cytoplasmic membrane, easing carriage of carvacrol athwart the membrane. Consequently, the antimicrobial effect of carvacrol is augmented by the presence of its ancestor cymene, owing to the labeled synergistic effect [55]. Hashemi *et al*. (2013) [5] reported that MIC values of essence extracted from *E. platyloba* against *L. monocytogenes* and *Staphylococcus aureus* were 6250 and 12500 ppm, respectively. Other scientists reported that carvacrol, limonene, p-cymene, α-pinene, β-myrcene β-pinene, β-caryophyllene, and γ-terpinene are the most significant chemical components in the essence of Echinophora [56, 58]. High antibacterial effect of *E. platyloba* essence against *L. monocytogenes* was also reported from Serbia [59] and Iran [4,6,60]. Antibacterial effect of essences hinge on the kind, combination and concentration of the oil, the kind and concentration of the related microorganism, and the dispensation/storage circumstances. Additionally, compound and concentration of essence depend on environmental condition, plant creation stage, and growth and development stages.

Conclusion

In conclusion, we identified a high antioxidant and antibacterial effects of *S. bachtiarica* and *E. platyloba* essential oils in vitro condition. Findings showed that carvacrol, thymol, o-cymene and γ-terpinene were the foremost routinely identified chemical components in *S. bachtiarica* essential oil. Ocimene, alpha-phellandrene, γ-terpinene and beta-myrcene were the foremost routinely identified chemical components in *E. platyloba* essential oil. Furthermore, high TPC and TFC and considerable DPPH radical scavenging activities were also found for *S. bachtiarica* and *E. platyloba* essential oils. Additionally, both medicinal plants and especially *S. bachtiarica* essential oil harbored considerable antibacterial effects against *L. monocytogenes*. Antibacterial effects of *S. bachtiarica* and *E. platyloba* essential oils in some concentrations were entirely higher than tested antibiotic agents. High antibacterial effects of *S. bachtiarica* and *E. platyloba* essential oils and also their low MIC values against *L. monocytogenes* make them suitable as a good antimicrobial agents especially in food systems.

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