Journal of Biology and Today's World

Journal home page: http://journals.lexispublisher.com/jbtw

Received: 25 March 2016 • Accepted: 01 September 2016

Research

doi:10.15412/J.JBTW.01050803

Phytochemical and Antibacterial Properties of *Echinophora Orientalis* Essential Oil against *Staphylococcus aureus* in Soup

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ABSTRACT

Using of chemical preservatives in food may have harmful effects on human health and reduce food safety; Natural preservatives can be used to improve food safety. *Echinophora Orientalis* is one of the medicinal herbs that traditionally has been used as natural preservative. The objective of the present investigation was to determine the chemical composition and antimicrobial effect of *E. orientalis* essential oil (EO) against *Staphylococcus aureus* in a food model. In order to preparing *E. orientalis* EO, the aerial parts of the plant were collected from Binalud mountain in Nishapur, East of Iran. The EO was extracted using Clevenger and its chemical composition was determined by Gas chromatography–mass spectrometry. Assessment of antibacterial activity of the EO was performed by inoculating the amount of 10^3 cfu/ml *S. aureus* into a certain amount of soup samples. Different concentrations of the EO (6.25, 12.5, 25 µg ml^{-1}) added into the soup samples. The antimicrobial activity of different concentrations of the EO on *S. aureus* was examined in the commercial barley soup kept under fridge condition in 1, 2, 3, 4, 5 days after *S. aureus* inoculation. In total 43 components were identified in *E. orientalis* EO by GC-MS analysis, comprising 99.05% of the volatile oil, of which γ-decalactone (21.15%), β-cis-Ocimene (15.27%), Linalool L (8.82%), Spathulenol (7.74 %), Eugenol methyl ether (6.61%) were the major components. The EO showed strong antimicrobial activity against tested bacteria, so that no bacterial growth was observed in concentrations of 12.5 µg ml^{-1} and 25 µg ml^{-1} five days after bacterial inoculation, but bacterial growth was observed at concentrations of 6.25 µg ml^{-1}. Average growth of bacteria in concentrations of 6.25, within five days counting were respectively 34 and 35 respectively 62.33±4.07, 42.66±3.02, 16±0.81, 1.33±0.65, 0 Cfu/ml ($p<0.05$). Evaluation of the sensory properties showed that concentration of 6.25 µg ml^{-1} of the EO was the most acceptable concentration. It was concluded that *E. orientalis* EO is a strong preservative and a flavoring agent in foods.

Key words: *Echinophora Orientalis*, Essential *oil*, GC-MS, *Staphylococcus aureus*, Soup.

Journal of Biology and Today's World is published by *Lexis Publisher*; Journal p-ISSN 2476-5376; Journal e-ISSN 2322-3308.

1. INTRODUCTION

The occurrence of adverse effects of chemical antimicrobial preservatives and increasing the bacterial resistance to antibiotics and other antimicrobial agents has prompted researchers to carry out extensive studies in order to reducing chemical preservitives and replace them by the natural chemical compounds, especially medicinal plants (1-3). The *Echinophora* plant of the family Apiaceae (umbelliferae) includes 10 species that has been distributed from the Mediterranean area to Iran and Afghanistan. Among ten species, four of them are found in Iran including *E. orientalis*, *E. sibthorpiana, E. cinerea* and *E. platyloba*. Two species including *E. sibthorpiana* and *E.orientalis* are also growing in Anatolia, Turkmenistan, Armenia, Russia, Syria, the Balkans, Crete, Cyprus and Afghanistan (4, 5). *E. orientalis* is a common species in Iran. It is known by local names of *Khosharize*, *Tigh Touragh,Tigh Masti, Koshander, Kouzang, Tanghez or Khousharouze and Kharmoshk*. *E. orientalis*; this plant is an aromatic perennial herbaceous plant, tough and prickly, singular and bottom-branched stems, branches stitched with thick, sturdy and thick, tangled tentacles to name a few highly branched with a height of 30 to 100 cm, white flowers integrated, since the flowering period is from June to July (3, 6). The *Echinophora* EO contains alkaloid compounds and flavonoids. Flavonoids exist abundantly in various

vegetables, fruits, and medicinal plants. The plant and its oil can be used as an antiseptic, antibacterial, antioxidant, antifungal and an ability to inhibit human platelet aggregation and are also used in folk medicine to heal wounds, carminative and digestive properties (2, 7-9). Aerial parts of the plant are used commonly as flavor compounds in dairy products and also used in the preparation “halva” a Turkish sweet, the sweat of these plants create a flavor in home environment and is used as an anti-freeze compound (10, 11). Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and caused by the ingestion of staphylococcal enterotoxins (SEs) that is produced in food by enterotoxigenic strains of *S.aureus*. Food poisoning caused by *S.aureus* is one of the most common disease and in most countries is primarily in terms of toxicity (12). Preservatives are added to food, drugs, paints, environmental samples for reducing the level of deterioration and increasing their shelf life. Despite these advantages, they are harmful and toxic, so, this encourages researchers to find appropriate approaches for reducing the use of chemical preservatives and replacing them with natural preservatives. Natural preservatives allow food manufacturers to supply their products, labeled "natural" or "clean", which indicates the presence of natural components in their products. Manufacturing of these products has difficulties and obstacles, including high levels of using, create undesirable color, flavor stability and reduce the productivity which are due to the low levels of natural anti-bacterial materials in them (13, 14). According to importance of natural preservatives and the role of medicinal plants that are native to Iran, the present study was conducted to assess the antibacterial properties of the EO of *E. orientalis* against *S.aureus* in a soup as a food model.

2. MATERIALS AND METHODS

2.1. Plant

The aerial parts of *E. orientalis* were collected during flowering stage (10th June to 15th August 2015) from Binalud mountains in Nishapur in the Khorasan Razavi, east of Iran and identified by the Herbarium of University of Tabriz, Iran (Figure 1).

Figure 1. *E. orientalis* (Collected from Binalud Mountains in Nishapur in Iran)

Preparation of the EO: According to the method recommended by the European Pharmacopeia, in order to extracting *E. orientalis* EO; dried aerial parts (100 grams) was milled and distilled using a Clevenger apparatus for 3 hours (2),(4). The obtained EO was dewatered using sodium thiosulphate and then kept at 4°C until it was used in the experiment. GC-MS Analysis of the EO: Chemical composition of the extracted EO was measured and quantified using GC and GC-MS (15). The chromatograph (Agilent 6890; Agilent Technologies, Kansas) was equipped with an HP-5MS capillary column (30×0.25 mm ID ×0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 5°C, temperature ramp 5C/min, 240C/min to 300C (holding for 3 min) and injector temperature at 290C. The carrier gas was helium and the split ratio was 0.8 mL/ min. For confirmation of the results, EO was also analyzed by GC-MS (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent, U.K.) and the same capillary column and analytical conditions as above. The MS was run in electron ionization mode with ionization energy of 70 eV (2).

2.2. Antimicrobial activity assay in soup

2.2.1. Bacterial strains

The antimicrobial activity of extracted EO was examined against *S. aureus* ATCC 6538. Lyophilized culture of the organism was obtained from Iranian Research Organization for Science and Technology, Tehran, Iran. Preparation of bacterial suspensions: Concentrations of bacteria were determined using spectrophotometer (Pharmacia LKB-Nova Spacell, Cambridge, UK).

Suspensions of vegetative form of the bacteria were prepared by culturing in BHI broth at 37°C for 24 h. The optical densities (OD) of bacteria cultures were measured at 600 nm to obtain a bacterial concentration of 10^3 Cfu/ml (16). Preparation and inoculation of barley soup: The barley soup (containing carbohydrates as its energy base and some protein, vitamins and minerals) was used as a food model. The barley soup was prepared according to the supplier'sinstruction. The volume of commercial barley soup was 100 ml in a 250-ml flask. The flasks containing barley soup was sterilized by autoclave**.** After cooling, the EO was added in concentrations of 0, 6.25, 12.5 and 25µl ml^{-1} (due to MIC and sensory results) to each flask, and the bacteria was injected into the sterilize flasks at a concentration of 103 cfu/ml (by Superficial cultivation was confirmed). After that, different concentrations of EO (according to the findings of the IMC and sensory evaluation) added to the soup samples. Bacterial growth in each soup sample was examined at 3°C on days 1, 2, 3, 4, and 5, after inoculation in serially diluted samples and superficial cultivation on BHI Agar. All evaluation tests were performed in triplicate (17).

2.3. Sensory Analysis

The organoleptic characteristics of the barley soup comtaining different concentrations of *E.orientalis* EO were evaluated by sensory acceptance tests (18). For this purpose, the prepared soups were divided into seven parts (including an appropriate volume of soup), then the EOs were added at concentrations of 0, 6.25, 12.5and 25µl ml^{-1} to each of flasks. Sensory evaluation was performed by a panel of seven members. Each panelist evaluated the samples by rating them using a 9-point scale (
Table **3**), where 9 = extremely like and 1 = dislike (17, 19).

2.4. Statistical analysis

All data were analyzed by analysis of variance (ANOVA) and Fisher's least significant difference procedure, using the SPSS 17 statistical Software package (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL). The differences were considered significant when $P<0.05$.

3. RESULTS AND DISCUSSION

In the present study, the antibacterial property of *E. orientalis* essential oil (EO) on Staphylococcus aureus (*S. aureus)* at 3°C was investigated in barley soup during 5 days.

Yield of *E. orientalis* EO: The yield of *E. orientalis* EO obtained by hydrodistillation was 0.57% (v/w). This can be compared to the similar studies that found oil yields of 0.55%, 0.7% and 0.67% (13, 20, 21). Chemical composition of the EO: The EO was extracted by the hydrodistillation of the dried aerial parts of *E. orientalis*, and the chemical composition was evaluated by Gas Chromatography Mass Spectrophotometer (GC-MS). The chemical composition and their quantities are summarised in

Table **1**. A total of 43 components were identified in *E. orientalis* EO, which represented 99.05% of the total mass of the extracted EO. The main components of the extracted EO were γ-decalactone (21.15%), β-cis-Ocimene (15.27%), Linalool L (8.82%), Spathulenol (7.74 %**)**, Eugenol methyl ether (6.61%), α–Terpineol (3.68%), α-Pinene (3.19%), 3-Methylenecycloheptene (1.46%), β-Myrcene (1.17%), cis Ocimene (1.36%), Ethanol, 2-methoxyphenyl (1.09), Ethanol, 2-(p-tert-butylphenoxy) (1.21%), β-Pinenol (1.28%), β –Pinene (1.90%), Dodecalactone (1.62%), Isoaromadendrene epoxide (1.89%), Germacrene D (1.58%). Other components were presents in amounts less than 1%. The *E. orientalis* EO could be considered as a source of hydrocarbon monoterpenes, especially the γ-decalactone. The comparison of the EO composition among different species of the genus *Echinophora* revealed a considerable variation in the properties EO.

Table **2** shows chemical components of *Echinophora* EO prepared in the present study and compare them with chemical components of *Echinophora* EO from other studies. In the present study the chemical composition of γ-decalactone as a major component.

Table 1. Chemical composition of *E. Orientalis* EO used in the present study

No	Compound	retention time (RT) (min)	Percentage
1	Cyclopentadien, 1,5,5-Trimethyl	3.48	0.95
2	m-Dimethylbenzene	3.68	0.75
3	3-Methylenecycloheptene	3.78	1.46
4	α-Pinene	4.74	3.19
5	Verbenene	5.09	0.46
6	β-Myrcene	5.69	1.17
7	dl-Limonene	6.40	0.89
8	cis-Ocimene	6.50	1.36
9	β-trans-Ocimene	6.68	15.27
10	trans-p-Mentha-2,8-dienol	7.20	0.96
11	2-Pentene, 3-methyl-	7.51	0.94
12	Linalool L	7.71	8.82
13	1,2,4,4-Tetramethylcyclopentene	7.93	o.61
14	Ethanol, 2-methoxyphenyl	8.01	1.09
15	1,5-dicyano-2,4-dimethyl-2,4-diazapentane	8.19	0.57
16	3a,6-Methano-3ah-inden-5-ol	8.57	0.30
17	1,6-Dimethylhepta-1,3,5-triene	8.72	0.26
18	2-methylenebornane	8.89	0.47
19	α - Terpineol	9.44	3.68
20	Carvotanaceton	10.56	0.40
21	β-(p-tert-Butylphenoxy)ethanol	11.54	1.21
22	Carvacrol	11.77	0.83
23	δ^4 -Carene	12.64	0.41
24	β -Calarene	13.23	0.33
25	5,5,8,8-tetramethyl-cis,exo-tricyclo[4.3.0.0(7,9)..	13.29	0.27
26	3-Benzyl-1,2,4-Triazole	13.46	2.36
27	Benzene, 1,2-dimethoxy-4-(2-propenyl)- (CAS)	13.69	6.61
38	β-Bisabolene	14.70	0.81
29	(R)-γ -decalactone	15.62	21.15
30	γ-lactone	15.88	0.46
31	(1S,4R,5S)-(+)-2(10)-Pinenol	15.99	1.28
32	β –Pinene	16.46	1.90
33	γ. Dodecalactone	16.83	1.62
34	Isoaromadendrene epoxide	17.23	1.89
35	3-Hexen-1-ol benzoate	17.41	2.46
36	β -Spathulenol	17.73	7.74
37	Germacrene D	18.01	1.58
38	Dillapiole	18.40	0.45
39	isospathulenol	18.74	0.42
40	γ-Dodecalactone	19.45	0.72
41	Neophytadiene	22.04	0.22
44	2-Pentadecanone, 6,10,14-trimethyl-	22.15	0.36
43	*trans*-β-Butylene oxide	23.09	0.37
Total			99.05

Table 2. Comparison of the chemical components of *Echinophora* EO used in the present study with those from the other studies

Name plant species	Main components	Region collect plants	References
Echinophora platyloba DC	P-cymene (22.15%) α-pinene (18.52%) β-phellandrene (14.40%) α-phellandrene (9.69%)	Iran	(22)
Echinophora spinosa L.	δ³-carene (60.86 %) α-phellandrene (7.12%) P-cymene (6. 22 %) myrcene (4.82 %) β-phellandrene (2.73 %)	Buljarice Cost, Herceg Novi in Montenegro	(15)
Echinophora platyloba DC	Thymol (27.19%) trans-Ocimene (20.89%) Ocimene(26.51%)	Chaharmahal and Bakhtiari,Iran	(23)
Echinophora platyloba DC	2, 3-Dimethylcyclohexadominant (9.87%) α-pinene(7.69%) γ-dodecanolactone(5.66)	Maragheh city,northwest of Iran	(2)
Echinophora tenuifolia subsp. sibthorpiana	α-phellandrene (43.8%) methyleugenol (28.6%) p-cymene (9.5%) β-phellandrene(7.4)	Sourpi (Magnesia Prefecture), Greece	(4)
Echinophora platyloba DC	(Z)-β-ocimene (26.71%) δ³-carene (16.16%) Limonene (6.59 %)	Isphahan, Iran	(21)
Echinophora platyloba DC	trans-β-ocimene (67.9%) 2-furanone (6.2%) myrcene (6.0%)	Alvand Mountain, Golpaygan-Khomein Road, Iran	(24)
Echinophora lamondiana	δ³-carene (65.9%) α-Phellandrene (12.8%) (Z)-β-ocimene (38.9%)	Malatya, Turkey	(11)
Echinophora platyloba DC	α-phellandrene (24.2%) P-cymene (7.4%) β-phellandrene (6.3%) α-pinene (3.4%)	Northwest Iran (Maragheh district)	(20)
Echinophora sibthorpiana	methyl eugenol (60.40%) p-cymene (11.18%) α-phellandrene(10.23%)	Macedonia, Serbia	(5)
Echinophora Platyloba	trans-β – ocimene (67.9%) 2-furanone (6.2%) myrcene (6%) linalool (3.1%)	Tehran, Iran	(3)
Echinophora orientalis	β-myrcene (32.1%) α-pinene (16.7%) p-cymene (14.34%) γ-decalactone (21.15%)	Eastern Azerbaijan, Iran	(6)
Echinophora orientalis	β-cis-Ocimene (15.27%) Linalool L (8.82%) Spathulenol (7.74 %)E ugenol methyl ether (6.61%)	Binalud mountains in Nishapur ,Iran	The present study

3.1. Antimicrobial activity of Echinophora EO against S. aureus in soup

Results showed that different concentrations of *E. orientalis* EO significantly affected the bacterial growth at 3°C, compared with that of the control group. The EO showed strong antimicrobial activity against *S. aureus* in food model. Each sample was examined for bacterial growth at 3°C on days 1, 2, 3, 4 and 5 after inoculation in serially diluted The contents of flaks and superficial cultivation on BHI Agar with concentrations of 12.5 µg ml^{-1} and 25 µg ml^{-1}. No bacterial growth was observed during the 5 days, but bacterial growth was observed at concentrations of 6.25 µg ml^{-1}. Average growth of bacteria in concentrations of 6.25, within five days counting were respectively 34 and 35 respectively 62.33±4.07, 42.66±3.02, 16±0.81, 1.33±0.65, 0 Cfu/ml ($p<0.05$). Viable count of *S. aureus* was showed affected by different concentrations of *E. orientalis* EO (µg ml^{-1}) and their combinations in barley soup at 3°c in

Table **3**. In addition, two samples as positive and negative controls were also used in the experiment.

Table 3. Survivability of *S. aureus* in soup prepared with *E. orientalis* EO during cold storage

Treatments	EO Concentration (µg ml-1)	Days	Viability S. aureus(Cfu/ml)
1	A	1	0
1	A	2	0
1	A	3	0
1	A	4	0
1	A	5	0
2	B	1	0
2	B	2	0
2	B	3	0
2	B	4	0
2	B	5	0
3	C	1	62.33±4.07^{a*}
3	C	2	42.66±3.02^{b}
3	C	3	16±0.81^{c}
3	C	4	1.33±0.65^{d}
3	C	5	0
4	D	1	248.33±6.23^{e}
4	D	2	545±12.24^{f}
4	D	3	676.66±20.63^{g}
4	D	4	830±21.60^{h}
4	D	5	1075±88.97^{i}
5	E	1	0
5	E	2	0
5	E	3	0
5	E	4	0
5	E	5	0

*Means in the column with different superscript letters are significantly different ($p<0.05$)

A: Concentration of EO 25 µg ml^{-1}
B: Concentration of EO 12.5 µg ml^{-1}
C: Concentration of EO 6.25 µg ml^{-1}
D: Positive control (+): No essential oil
E: Negative control (_): No bacteria& No essential oil

Several studies have been published on the antimicrobial activity of some species of *Echinophora* (2, 3, 5, 15, 22, 23, 25, 26). In study by Gokbulut *et al.* (2013) *E. tenuifolia* EO showed strong antimicrobial activity against *B. cereus* and *Staphylococcus spp* (27). Saei-Dehkordi *et al.* (2012) and Glamočlija *et al* (2011) have reported that *E. platyloba* oil was effective against some tested Gram-positive bacteria, such as *L. monocytogenes*, *S. aureus* and *yeasts* such as *R. mucilaginosa*, *R. rubra*, while *E. spinosa* was the most effective against Gram-negative bacteria including *E. coli*, *P. aeruginosa* and fungus *T. viride* (15, 23). Avijgan *et al.* (2012) studied the antifungal activity of *E. platyloba* ethanol extract on *C. albicans* growth and concluded that it has an inhibitory effect at concentrations above 2 mg ml^{-1} (28).

According to the results of Moghaddam *et al.* (2015) the most sensitive fungi at the highest concentration (800 µl l^{-1}) were *M. phaseolina* and *C. fallax*. However, the most resistant fungi were *C. sacchari* and *A. alternate* in agar dilution and disk diffusion assays, respectively (22). Strong antibacterial effects of the *E. platyloba* ethanol extract on *Alcaligenes faecalis*, and the EO on *Listeria monocytogenes* have been reported by Sharafati-chaleshtori, *et al.* (2012) (26). In a study by Entezari *et al.* (2009), showed that the *Echinophora* methanol extract inhibits the growth of Staphylococcus aureus and *Pseudomonas aeruginosa* (3). In another *in vitro* study conducted by Mileski *et al.* (2014) *E. sibthorpiana* EO showed strong antifungal activity. MIC values for the EO ranged from 0.17-2.70 mg ml^{-1}, and MFC values from 0.34-10.78 mg ml^{-1} and the EO of *E. sibthorpiana* had stronger antibacterial activity than the positive control against (5). According to the obtained data, it can be concluded that the extracted EO has antimicrobial activity of strong against *S. aureus*. In general, the antimicrobial activity can be attributed to high concentrations of phenols and flavonoids in the aerial parts.

3.2. Sensory analysis

Results of sensory Analysis showed that concentration of EO (0.25 µg ml^{-1}) had the most desirable acceptance. The mean values for barley soup sensory acceptance test with various concentrations of the EO are shown in Table 4.

Table 4. The average values of sensory acceptability of barley soup with different concentrations of *E. orientalis* EO

Concentration of EO (µg ml^{-1} soup)	Mean rating ± SD
0	5.55 ±2.35 ^{a*}
6.25	5.11 ±1.79 ^{b}
12.5	3.88 ±3.08 ^{c}
25	3.55 ±1.46 ^{d}

*Means in the column with different superscript letters are significantly different (p<0.05)

Due to high antimicrobial activity and desirable sensory properties, extracted EO can be recommended to be used as an alternative to chemical preservatives.

4. CONCLUSION

Based on antimicrobial and organoleptic effects of *E. orientalis* EO as a natural food additive, its application in combination with other permissible additives in order to reducing the adverse effects of chemical preservatives in food industry is recommended.

ACKNOWLEDGMENT

The Authors would like to thank the dean for research of Qazvin University of Medical Sciences for financial support of the project.

FUNDING/SUPPORT

Not mentioned any Funding/Support by authors.

AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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