

***Echinophora* plant: chemical composition, antioxidant activity, and antimicrobial potency- A mini review**

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Abstract

Background and objective: *Echinophora* is a plant of Umbelliferae family. In Iran, it has four herbaceous, perennial, and aromatic species. *Echinophora platyloba* and *Echinophora cinerea* are two endemic species of Iran. Aerial parts of the plant are used for several purposes. It is used as condiment in buttermilk, yogurt, and pickles. *Echinophora* is also used in traditional medicine for remediation of stomach. Several studies have been done to identify the components with healing properties in *Echinophora*. This review reports and discusses chemical composition, antioxidant potency, and antibacterial properties of *Echinophora*.

Results and conclusion: *Echinophora* essential oil contains several phytochemicals in the class of alkaloids and flavonoids. Among them, α -phellandrene has been introduced as the most abundant. Phenolic compounds of *Echinophora* essential oil are of potent natural antioxidants. *Echinophora* essential oil can also be used as antimicrobial agent in foods. In this regard, carvacrol, linalool, *p*-cymene, pinene, and terpinene are of its antibacterial components. According to the literature, the essential oil could extend the shelf life of mushroom, chicken, fish fillets, and strawberries. Furthermore, due to its antifungal activity, it can be used as a natural preservative in food products such as cheese. In general, with respect to the health benefits of *Echinophora* essential oil, it is of interest in food industry, medicine, pharmaceuticals, and cosmetic industry owing to the phenol contents and considerable antioxidant and antimicrobial properties.

Keywords: Antimicrobial properties, antioxidant properties, chemical compounds, *Echinophora* essential oil

1. Introduction

Plants were used for treatment of diseases by ancient people, through which the term of "medicinal plants" was introduced in different ethnicities and cultures [1]. According to the World Health Organization, 80% of people support the use of herbal medicines in the world [2]. Medicinal plants are popular in populations because they are less expensive than chemical drugs and usually have fewer side effects [1].

Other than therapeutic benefits, medicinal plants are used as flavoring, preservative, and aromatizer [3]. Different parts of medicinal plants such as leaves, stems, and roots are used in various forms of extract, essential oil, and seasoning [4]. Essential oils and extracts contain antioxidant and antibacterial agents with several functionalities. They are included to secondary metabolites of phenolic compounds, flavonols, flavonoids, glycosides, and alkaloids [5].

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Iran has 8500-9500 vascular plant species, of which 2324 species are endemic. Moreover, 29 species are native to Lorestan province (west of Iran) with a lot of plant diversity [6]. There are four aromatic perennial herbaceous species of *Echinophora* in Iran including *E. cinerea* and *E. platyloba* that are endemic, and *E. sibthorpiana* and *E. orientalis* grown in Iran and other countries such as Anatolia, Armenia, Russia, Turkmenistan, Afghanistan, Balkan Peninsula, Crete, Cyprus, and Syria. *E. cinerea* is found in Lorestan at altitudes above 1500 m, especially in Ashtorankuh, Kuh Kala, Garin Kuh, and Sefidkuh. The plant has local names of Khoshrizeh, Tigh Toragh, Kashndar, and Toluq Otto in Iran. In traditional medicine, it is used to treat the stomach [7]. Furthermore, it is used traditionally as seasoning in yogurt and cheese [3].

Echinophora essential oil is colorless or yellowish liquid and shows considerable antimicrobial and antioxidant activities. It is active against bacteria (both gram-negative and gram-positive) as well as yeast [7,8]. Antioxidant role of *E. cinerea* may be due to the activity of α -phellandrene [9]. Food-borne infections and food spoilage yeasts have been effectively inhibited by *E. platyloba* essential oil. Therefore, it can be used as a natural preservative in foods [10]. Use of *Echinophora* essential oil at concentrations of 50 and 100 μ l/l could protect button mushroom from spoilage during storage [11].

Due to the narrow cultivation of *Echinophora*, it is not extensively known globally and no study has reviewed its characteristics. Therefore, chemical composition, antioxidant activity, and antimicrobial properties of various species of *Echinophora* are reviewed in the present work.

2. Chemical compounds

Chemical composition of *Echinophora* plant and its essential oil varies based on geographical region, environmental factors (soil, season, climate, altitude, irrigation, disease, pests, and

pollution), physiological condition (pollinator activity cycle, organ development, type of secretory structure, and mechanical or chemical injuries), manual labor need, genetic factors, and evolution [12-14]. Therefore, there would be different chemical compounds, bioactive properties, and therapeutic effects for plant species.

Main ingredients of *Echinophora* species are listed in Table 1. In some studies, α -phellandrene (an anti-inflammatory, anti-viral, and antibacterial compound) has been introduced as the most abundant chemical in *Echinophora* essential oil followed by limonene, *p*-cymene, α -pinene, carvacrol, and β -myrsen [4,7,15]. Nonetheless, other compounds have been reported in high quantity by other studies [10,16-21]. For example, Rahimi et al. studied chemical compounds of aerial parts of *E. platyloba*. In their study, (*Z*)- β -ocimene (26.71%) was the main ingredient among 29 extracted compounds [20]. In comparison, Saei-Dehkordi et al. reported that thymol, trans-ocimene, carvacrol, and (*E*)-sesquilandulol are major ingredients of *E. platyloba* essential oil [10]. Another study investigated aerial parts of *E. sibthorpiana* species collected from urban area of Tehran. In total, 17 compounds were identified, of which δ -3-carene was reported as the most abundant (32%) [21]. In agreement, Ahmad et al. analyzed *E. sibthorpiana* essential oil in their laboratory and observed similar results. Although, concentration of the major compounds was different in their study. As a result, due to the presence of α -phellandrene and δ -3-carene as fragrant compounds in *E. sibthorpiana* essential oil, it was suggested to be used in perfume and cosmetics [16,22]. What is important is that major components of *Echinophora* species are similar to that of thymus plant. Both species contain linalool, carvacrol, thymol, and *p*-cymene in their essential oils [23,24].

Table 1- Major ingredients of *Echinophora* species

Species	Ingredients (%)	Region	Reference
<i>E. cinerea</i>	α -phellandrene (32.09) Limonene (16.28) <i>p</i> -cymene (10.75) α -pinene (9.79) Carvacrol (3.79) β -myrcene (2.65)	Lorestan, Iran	[7]
<i>E. cinerea</i>	α -phellandrene (40.60) α -pinene (16.50) β -phellandrene (9.80) <i>p</i> -cymene (7.50) Limonene (5.40)	Yasouj, Iran	[15]
<i>E. platyloba</i>	(<i>Z</i>)- β -ocimene (26.71) δ -3-carene (16.16) Limonene (6.59)	Isfahan, Iran	[20]
<i>E. platyloba</i>	Ocimene (26.51) 2,3-dimethyl-cyclohexa-1,3-diene (9.87) α -pinene (7.69) γ -dodecalactone (5.84) Nerolidol (5.66)	Tabriz, Iran	[18]
<i>E. platyloba</i>	Thymol (27.19) Trans-ocimene (20.89) Carvacrol (7.22) E-sesqui-lavandulol (5.59) Geraniol (3.0)	Shahrekord, Iran	[10]
<i>E. platyloba</i>	γ -decalactone (43.96) E- β -ocimene (21.56) <i>Z</i> - β -ocimene (4.23) Methyl eugenol (3.01) Caryophyllene oxide (2.86)	Khorasan Razavi, Iran	[17]
<i>E. platyloba</i>	<i>Z</i> - β -ocimene (38.90) α -phellandrene (24.50) <i>p</i> -cymene (7.40) β -phellandrene (6.30)	Tabriz, Iran	[19]
<i>E. cinerea</i>	α -phellandrene (42.40%–54.87) α -pinene (12.28%–25.54) <i>p</i> -cymene (2.72%-12.05) β -phellandrene (10.29%–11.08)	Shahrekord, Iran	[9]
<i>E. cinerea</i>	α -phellandrene (32.09) Limonene (16.28) <i>p</i> -cymene (10.75) α -pinene (9.79) Carvacrol (3.79) β -Myrcene (2.65)	Lorestan, Iran	[8]
<i>E. sibthorpiana</i>	Methyl eugenol (50.40)	Taleghan, Iran	[16]

	δ -3-carene (17.40) α -phellandrene (16.30) <i>p</i> -cymene (8.30) β -phellandrene (3.70)	
<i>E. sibthorpiana</i>	δ -3-carene (31.90) α -phellandrene (31.0) Methyl eugenol (16.90) β -Phellandrene (5.30)	West Azarbaijan, Iran [21]
<i>E. orientalis</i> (root)	Myristicin (52.90) Terpinolene (24.40) Falcarinol (4.80) Myrcene (3.0)	West Azarbaijan, Iran [22]
<i>E. orientalis</i> (shoot)	Myrcene (20.90) <i>p</i> -cymene (11.50) 1,7-octadiene,3,6-dimethylene (10.0) α -Pinene (7.70)	West Azarbaijan, Iran [22]
<i>E. orientalis</i> (seed)	Spathulenol (10.50) Carotol (9.50) Bicyclogermacrene (4.50) Germacrene D (3.70) A-humulene (3.30)	West Azarbaijan, Iran [22]

3. Antioxidant activity

Free radicals are reactive species that attack vital macromolecules and cause extensive damage to the body leading to many diseases such as cancer, cardiovascular disease, Alzheimer, and Parkinson [25,26]. Synthetic antioxidants are widely used in food industry, but their safety is of concern [27]. Plants are source of phenolic compounds such as phenolic acids, flavonoids, and tannins as natural antioxidant [28]. Antioxidant activity of phenols is directed by several mechanisms including reactive oxygen species (ROS) scavenging, and prevention of ROS formation by chelating the metal ions, enzyme inhibition, transfer of hydrogen atom, transfer of single electron, etc. [29-31]. Indeed, antioxidant activity of plants is directly associated with their phenol content [32-34].

Echinophora plant contains a lot of phenolic compounds with considerable antioxidant activity [35]. *Echinophora* essential oil contains monoterpene hydrocarbons that their antioxidant activity is due to active methyl groups, especially in beta-carotene-linoleic acid system [7,35,36]. Limonene is another antioxidant of *Echinophora*

species [10,13]. Saei-Dehkordi et al. studied antioxidant activity of *E. platyloba* essential oil by DPPH method and β -carotene-linoleic acid bleaching assay. They reported a high antioxidant potency of *E. platyloba* essential oil ($IC_{50} = 49.7 \mu\text{g/ml}$), significantly due to the activity of *trans*-ocimene and limonene [10]. Interestingly, antioxidant activity and chemical composition of three plants of *Heracleum lasiopetalum* Boiss, *Kelussia odoratissima* Mozaff, and *E. platyloba* were assessed by DPPH, FRAP, and ABTS methods. According to results, *E. platyloba* extract had the highest antioxidant potency [36]. Antioxidant activity of *E. cinerea* essential oil and butylated hydroxytoluene (BHT) was assessed by Ghasemi Pirbalouti and Gholipour by DPPH method. In their study, IC_{50} of 1.97-2.25 mg/ml was reported for *E. cinerea* essential oil compared to 0.412 mg/ml for BHT. It showed that *E. cinerea* essential oil has desirable antioxidant activity but it was not as powerful as BHT in suppression of oxidative reactions (Table 2) [9].

Echinophora essential oil could inhibit growth of tumor cells in the lung by apoptosis [8]. Anti-cancer and anti-mutagenic activity of *E. Platyloba* extract by inhibition of fibrosarcoma cells' proliferation were also reported [37]. In addition, wound healing is of functional properties of this plant. It was reported that topical use of *E. platyloba* leaf extract enhanced the restoration phase of skin wound. In fact, *Echinophora* essential oil is a source of terpenes and other phytochemical agents of betulinic acid and ursolic acid able to induce apoptosis. These characteristics can be related to the antioxidant activity of *Echinophora* [35,38].

Natural and synthetic antioxidants are commonly used in foods to increase their shelf life [27]. In study of Safari et al., antioxidant and antimicrobial activity of *Echinophora* extract was

investigated in fish fillets. The authors showed that use of the extract at concentration of 1.5 g/l extended the shelf life of fish fillets to 30 days. Furthermore, peroxide value of the samples containing *Echinophora* extract was significantly lower than extract-free samples [39]. In agreement, Hamzeh-kalkenari et al. reported an extended shelf life for button mushrooms coated with polyethylene-clay nanocomposite edible film containing *E. cinerea* essential oil at concentration of 100 μ l/l [11].

Comparison of antioxidant activity between *Echinophorra* essential oil and *Echinophorra* extract revealed that the essential oil has higher potential of oxidation suppression than the extract [3,30]. It can be related to the more phenolic compounds present in the essential oil.

Table 2- Antioxidant activity (IC₅₀) of *Echinophora* essential oil compared to ascorbic acid and BHT (as reference)

<i>Echinophora</i> essential oil	Ascorbic acid	BHT	Reference
0.74 mg/ml (<i>E. cinerea</i>)	-	50.63 μ g/ml	[7]
1.97-2.25 mg/ml (<i>E. cinerea</i>)	-	0.412 mg/ml	[9]
0.74 μ g/ml (<i>E. cinerea</i>)	-	52.72 μ g/ml	[8]
49.70 \pm 2.30 μ g/ml (<i>E. platyloba</i>)	5.60 \pm 0.45 μ g/ml	18.91 \pm 1.12 μ g/ml	[10]

4. Antibacterial effect

Essential oils contain several antimicrobial agents and are used in treatment of various infections. Antimicrobial activity of essential oils is directed by oxygenated sesquiterpenes and monoterpenoid components (particularly oxygenated monoterpenes). Indeed, they have been introduced as primary antibacterial components in the mixture [5,10,17]. These agents are included to cineole, camphor, linalool, α -pinene, β -pinene, berneol, caron, limonene, thymol, carvacrol, semen, camphene, α -tripeneol, etc. [40]. Among them, thymol and carvacrol have similar structure but they are different in substitution of hydroxyl groups. These two potent antimicrobial agents show synergistic effect in the matrix. Moreover, *p*-cymene (precursor of carvacrol) is a hydrophobic component causing more microbial cell disruption than carvacrol.

However, it does not have significant antibacterial activity alone and shows the highest potential in the presence of carvacrol. Synergism of *p*-cymene and carvacrol against *Bacillus cereus* was reported in rice [41].

Essential oils damage microbial cell membrane irreversibly by penetration into the lipid layer due to hydrophobic nature of the bioactive compounds. As a result, leakage of cytoplasmic materials is occurred, which leads to bacterial loss [14]. Furthermore, herbal essential oils interfere in production of amylase and protease, through which production of toxins and electron transfer chain are affected. It causes microbial coagulation and loss [42]. On the other hand, outer membrane of gram-negative bacteria is composed of amphiphilic lipopolysaccharide layer as a barrier to antimicrobial chemicals and helps the bacteria to tolerate the environmental

destructive factors [43,44]. Indeed, porin proteins in the outer membrane operate as hydrophilic transmembrane channels. Therefore, gram-negative bacteria are resistant to hydrophobic antibiotics and medicines. Although, some hydrophobic agents could pass through porins slowly [45-47], and gram-negative bacteria were susceptible to *Echinophora* essential oil in some cases (Table 3).

Antibacterial activity of *Echinophora* plant and its essential oil is mainly related to hydrocarbon monoterpenes such as carvacrol, linalool, *p*-cymene, α -pinene, and γ -terpinene [5,7,18,48]. Antimicrobial effect of *E. platyloba* essential oil against gram-negative and gram-positive bacteria was studied by Hashemi et al. In their study, growth of gram-negative bacteria was not affected by the essential oil and *Listeria monocytogenes* and *Staphylococcus aureus* were the most sensitive bacteria [18]. Moreover, antimicrobial activity of *E. platyloba* against food-borne microorganisms was investigated by Saei-Dehkordi et al. They reported that *E.*

platyloba essential oil strongly inhibited the yeasts followed by gram-positive bacteria. Gram-negative bacteria showed the lowest sensitivity to the essential oil [10].

Zarali et al. studied inhibitory effect of *E. cinerea* essential oil against *E. coli*, Shigella, *S. aureus*, and *B. cereus* compared to chloramphenicol by disk diffusion method. Among gram-positive bacteria, *E. cinerea* essential oil had the most antagonistic effect against *S. aureus* and the lowest inhibitory effect was observed against *B. cereus*. In addition, *S. dysentery* was the most resistant species [5]. Antibacterial activity of *E. cinerea* essential oil against food-borne pathogens of *L. monocytogenes*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and methicillin-resistant *S. aureus* was also investigated in study of Pass et al. Their results showed a great resistance of *Pseudomonas* to the essential oil [7]. This bacterium has also a high resistance to antibiotics due to production of chromosomal AmpC enzymes and limited drug permeability [7,49].

Table 3- Antibacterial effect of *Echinophora* essential oil

Species	Bacteria	MIC	MBC	Results	Reference
<i>E. cinerea</i>	<i>S. aureus</i>	0.16	0.63	Among gram-positive bacteria, the most antagonistic effect was observed against <i>Staphylococcus</i> , and <i>P. aeruginosa</i> was the most resistant species in both microdilution and disk diffusion methods.	[7] ^a
	<i>P. aeruginosa</i>	87	175		
	Methicillin-resistant <i>S. aureus</i>	2.7	11		
	<i>L. monocytogenes</i>	22	44		
	<i>E. coli</i>	5.5	11		
<i>E. cinerea</i>	<i>S. aureus</i>	> 660	> 660	The essential oil had the lowest effect on <i>S. aureus</i> .	[8] ^b
	<i>P. aeruginosa</i>	> 660	> 660		
	<i>L. monocytogenes</i>	> 660	> 660		
	<i>E. coli</i>	41.25	660		
	<i>S. enterica</i>	41.25	330		
	<i>S. typhi</i>	20.62	330		
	<i>S. epidermidis</i>	165	165		
	<i>S. agalactiae</i>	165	330		
<i>E. faecalis</i>	165	640			

<i>E. orientalis</i>	<i>S. aureus</i>	**	**	Antibacterial effect of the essential oil against <i>S. aureus</i> was investigated in barley soup at 3 °C for 5 days, and concentration of 6.25 µg/ml of the essential oil was the most acceptable concentration for inhibition of this bacterium.	[50]
<i>E. cinerea</i>	<i>E. coli</i>	4.6	18.7	<i>E. coli</i> was the most sensitive species.	[5] ^a
	Shigella	9.3	150		
	<i>S. aureus</i>	9.3	0		
	<i>B. cereus</i>	9.3	0		
<i>E. orientalis</i>	<i>S. aureus</i>	75	125	Use of this essential oil at low concentration successfully inhibited <i>S. aureus</i> without adverse effect on the taste of food.	[50] ^b
<i>E. platyloba</i>	<i>S. typhimurium</i>	896	**	<i>P. aeruginosa</i> was the most resistant and <i>L. monocytogenes</i> was the most sensitive species, respectively.	[10] ^b
	<i>E. coli</i>	896	**		
	<i>P. aeruginosa</i>	1344	**		
	<i>L. monocytogenes</i>	336	**		
	<i>S. aureus</i>	448	**		
	<i>B. cereus</i>	672	**		
<i>E. platyloba</i>	<i>B. subtilis</i>	672	**	The most sensitive bacteria to the essential oil were <i>S. aureus</i> and <i>L. monocytogenes</i> .	[18] ^c
	<i>E. coli</i>	*	*		
	<i>S. aureus</i>	25000	*		
	<i>L. monocytogenes</i>	25000	*		
	<i>S. typhi</i>	*	*		

^a In these studies, MIC and MBC were expressed in mg/ml.

^b In these studies, MIC and MBC were expressed in µg/ml.

^c In these study, MIC and MBC were expressed mg/l.

* When the highest concentration of the essential oil was used, no inhibitory impact was observed.

** MBC and MIC were not calculated.

5. Conclusion

Considering the proven risks of synthetic preservatives and the growing rate of antibiotic resistance in the world, use of herbal preservatives and antimicrobials is of interest. *Echinophora* essential oil contains bioactive compounds affecting the normal function of microbial cells. As discussed in detail, *Echinophora* plant and its essential oils have significant antibacterial, antioxidant, and anticancer properties. The findings reported in the current review will pave the ways in development of novel functional pharmaceutical, food, and cosmetic products.

Although, more *in vivo* and clinical studies are required.

6. Conflict of interest

The authors declare that there is no conflict of interest.

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