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Chemical composition, antimicrobial and antioxidant activities of *Echinophora platyloba* essential oil

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Abstract

Objective: *Echinophora platyloba* is traditionally used as antimicrobial agent in order to preserve the home-made products from deterioration. The aim of this study was to evaluate chemical composition of essential oil, antimicrobial and antioxidant activity of *E. platyloba* essential oil from Shahr-E-Kord city, Chaharmahal and Bakhtiari Province.

Materials and methods: chemical composition of *E. platyloba* essential oil by GC and GC-MS techniques and evaluate its antibacterial effect against *Staphylococcus aureus*, *Salmonella enterica* and *Helicobacter pylori* by disc diffusion and micro broth dilution assays. The antioxidant activity of *E. platyloba* essential oil was evaluated by ABST radicals. β-ocimene and α-caryophyllene were the main components of *E. platyloba* essential oil. The essential oil showed the promised antibacterial activity against *S. aureus*, followed by *S. enterica* and *H. pylori*. The antioxidant activity evaluation of essential oil showed the IC_{50} of 0.32 mg/ml that was higher than the IC_{50} of ascorbic acid (0.20 mg/ml).

In conclusion, *E. platyloba* essential oil has β-ocimene and α-caryophyllene chemotype and its use as antioxidant and antibacterial agents is recommended as its traditional uses.

Keywords: *E. platyloba* essential oil, β-ocimene, α-caryophyllene, antimicrobial, antioxidant

Composición química y actividades antimicrobianas y antioxidantes del aceite esencial de *Echinophora platyloba*

Resumen

Objetivo: *Echinophora platyloba* se utiliza tradicionalmente como agente antimicrobiano con el fin de preservar los productos caseros de deterioro. El objetivo de este estudio fue evaluar la composición química del aceite esencial, actividad antimicrobiana y antioxidante del aceite esencial de *E. platyloba* de la provincia de Shahr-e-Kord.

Materiales y métodos: la composición química del aceite esencial de *E. platyloba* mediante técnicas de GC-MS y GC y evaluar su efecto antibacteriano frente a *Staphylococcus aureus*, *Salmonella enterica* y *Helicobacter pylori* por ensayos de difusión en disco y de micro dilución en caldo. La actividad antioxidante del aceite esencial de *E. platyloba* se evaluó por los radicales ABST. β-ocimeno y α-cariofileno eran los componentes principales del aceite esencial de *E. platyloba*. El aceite esencial mostró la actividad antibacteriana prometido contra *S. aureus*, seguido de *S. enterica* y *H. pylori*. El aceite esencial presenta actividad antioxidante del aceite esencial mostró la IC_{50} de 0. 32 mg/ml que fue mayor que la IC50 de ácido ascórbico (0.20 mg/ml).

En conclusión: Aceite esencial de *E. platyloba* ha se recomienda ß-ocimeno y quimiotipo α-cariofileno y su uso como agentes antioxidantes y antibacterianas como sus usos tradicionales.

Palabras clave: Aceite esencial de *E. platyloba*, β-ocimeno, α-cariofileno, antimicrobianos, antioxidantes

Introduction

Echinophora platyloba, a plant from Umbelliferae family is used in Iranian Traditional medicine as antimicrobial agent for protection of home made products from spoilage[1]. According to its traditional uses, there are some studies that evaluate the antimicrobial activities of *E. platyloba* extracts against dermatophytes (*Trichophyton* sp, *Microsporum* sp and *Epidermophyton flucosum*)[2,3], *Candida albicans*[1,2,4,5], *Listeria monocytogenes*, *Alcaligenes faecalis*, *Serratia marscescenes*, *Providencia rettgeri*[6], *Staphylococcus aureus*, and *Pseudomonas aeruginosa*[7]. The antimicrobial activity of *E. platyloba* essential oil against *L. monocytogenes*[8-10], *Bacillus cereus*[9], *B. subtilis*[8,9], *S. aureus*[8,9], *E. coli* $O_{157}H_7$[8,9], *P. aeruginosa*, *Candida sp* (*C. albicans*, *C. tropicalis*),

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Rhodotorula sp (*R. rubra* and *R. mucilaginosa*)[9], and *Aspergillus niger*[8] were confirmed. Other biological activities of *E. platyloba* extracts such as antioxidant[11], anti-parasitic[12], and estrogenic activities[13,14] were confirmed.

Although, there are some studies that evaluate the antimicrobial activity of *E. platyloba* essential oil[8-10,15], but for the first time, we evaluate the antibacterial activity of *E. platyloba* essential oil against *Helicobacter pylori*, *Salmonella enterica* and *Staphylococcus aureus* as the most important pathogenic bacteria.

Furthermore, because geographical locations affect on chemical compositions of essential oils[16], analyses of chemical compositions of *E. platyloba* essential oil is most important for any biological evaluation.

Therefore, we analyze the chemical compositions of *E. platyloba* essential oil, and then its antimicrobial activities against three important pathogens and the antioxidant activity were evaluated.

Materials and methods

Plant Materials, extraction of essential oil by hydrodistillation method

Aerial parts of *Echinophora platyloba* at full flowering stage were collected from Shahr-E-Kord city, Chaharmahal-Va-Bakhtiari province, South-West of Iran in June 2015 and were authenticated and deposited in Agricultural Research Center of Chaharmahal-Va-Bakhtiari province, Iran (Sample no:1376). The samples were grinded and subjected to hydrodistillation by Clevenger type apparatus for 3 h. The separated essential oil was dried and kept in a dark vial at a cold place until the analysis.

Analysis of E. platyloba essential oil by Gas Chromatography (GC) and Gas Chromatography-Mass spectrum (GC-MS)

The chemical composition of *E. platyloba* essential oil was analyzed using GC and GC-MS. The GC apparatus was equipped with agilent technology (HP) 6890 system, capillary column of HP-1MS (30 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 40°C, held for 1 min then raised up to 230 °C at a rate of 3°C/min held for 10 min. Helium was used as a carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. The GC/MS analysis was conducted on a HP 6890 GC system coupled with 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50. The programmed injector and oven temperature was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 275.L, Wiley 7n.1 mass computer library, and NIST (National Institute of Standards and Technology), as well as comparison of the fragmentation pattern of the mass spectra with data published in the literature[17].

Microbial strains

The antibacterial activity evaluation was conducted against *Helicobacter pylori* ATCC 26695, *Salmonella enterica* BAA-708 and *Staphylococcus aureus* ATCC 25923. *H. pylori*, *S. enterica* and *S. aureus* were cultured on sheep blood (5%) supplemented brucella agar and *Xylose lysine* deoxycholate *agar* and Manitol salt agar, respectively. The microbial strains were incubated at 30-35 ºC in suitable conditions. One or two colonies of each strain were suspended in saline solution and their turbidities were adjusted to 0.5 McFarland by Spectrophotometer instruments (1×10^8 CFU/ml).

Evaluation the Antibacterial activity of E. platyloba essential oil

The antibacterial activity evaluation of essential oil was evaluated by disc diffusion assay and micro-broth dilution assay. In disc diffusion assay, the inhibition zone diameters (in millimeter) of *E. platyloba* essential oil were determined by inoculating the above suspended microbial strains on Muller Hinton Agar (Merck) and sheep blood (5%) supplemented Muller hinton Agar by sterile cotton swab. Sterile disks containing 10 µl of essential oil were placed on the inoculated plates. The plates were incubated and then the inhibition zone diameters (mm) were measured and were recorded as means ± standard deviation (SD)[18].

For determining the MIC (minimal inhibitory concentration) and MBC (minimal Bactericidal concentration) values of essential oil in micro-broth dilution assay, the essential oil were dissolved in DMSO and then it was diluted in distilled water. 50 µl of each dilution was added into the wells of 96 micro titer plates. The microbial suspensions were diluted to 10^6 CFU/ml. Then, 50 µl of diluted microbial suspensions were added to each well and were incubated at 37ºC for 24 h. The first wells with no turbidity and the first well without any growth on solid media were determined as MIC and MBC values, respectively[19].

Antioxidant evaluation of E. platyloba essential oil

For evaluating the potency of *E. platyloba* essential oil against ABTS free radicals: A solution containing ABTS (7 mM) in per-sulfate (2.45 mM) (1:1) was prepared. This solution was kept in dark place for 12-16 h and then was diluted to 1:25. Three ml of diluted solution was added to 40 µl of different concentrations of essential oil. After 15 min, the absorbance of solutions was read at 734 nm, the inhibition percent of essential oil were estimated in this way:

$I\% = [A_{blank} - A_{sample}/A_{blank}]\times100$, where A_{blank} was the absorbance of control and A_{sample} is the absorbance of different concentrations of essential oil. Ascorbic acid was used as control[20].

Results and discussion

Chemical composition of E. platyloba essential oil

Twenty one components were identified in *E. platyloba* essential oil that represented 90.6% of total oil composition. β-Ocimene (46.4%), α-phellandrene (12.1%), β-myrcene (8.5%), limonene (6.1%) were the main components of *E. platyloba* essential oil from ChaharMahal-E-Bakhtiari (South-West of Iran), followed by linalool (3.2%), α-pinene (3.0%), terpinolene (2.5%), *p*-cymene (1.8%) and δ-3-carene (1.4%). Thymol and carvacrol were not found in this essential oil (Table 1).

Some studies have evaluated the chemical composition of *E. platyloba* essential oils from different parts of Iran. β-ocimene (28%-68%) were reported as the first main component of *E. platyloba* essential oils from four studies[21-24], while the second main component has been different in these studies, and furanone (6.2%), α-decalactone (8.4%), γ-phellandrene (24.2%), and δ-3-carene (16.2%) were reported as the second main components of essential oil from Golpayegan[21], Tehran[23], northwest (Maragheh district)[22], Shalamzar[24], Iran; respectively. Asarone (10.2%), anethole (7.4 %), eugenol (6.7%) and dimethyl styrene (6.6 %) were the main components of *E. platyloba* essential oil from Torbat-Heydariye (Khorasan Razavi Province, Iran)[8].

Thymol (27.2%), trans-ocimene (20.9%) and carvacrol (7.2%) were reported as the main components of *E. platyloba* essential oil from ChaharMahal VA Bakhtiari province, Iran[9], while thymol and carvacrol was not found in our essential oil from ChaharMahal VA Bakhtiari province. The precise location of sampling from ChaharMahal VA Bakhtiari province has not been determined in Saei-Dehkordi et al study. Geographical location, harvesting time and many other unknown factors can affect on chemical composition of *E. platyloba* essential oil.

Table 1 Chemical composition of *E. platyloba* essential oil by GC and GC-MS

Row	Components	Retention Index	percent
1	α-Thujone	928	0.1
2	α-pinene	936	3.0
3	Sabinene	984	0.58
4	β-pinene	990	0.25
5	β-myrcene	1006	8.5
6	α-phellandrene	10240	12.1
7	δ-3-carene	1028	1.4
8	*p*-cymene	1030	1.8
9	limonene	1040	6.1
10	1,8-Cineole	1047	0.17
11	**β-Ocimene**	1098	46.4
12	y-terpinene	1099	0.26
13	Linalool	1101	3.2
14	Terpinolene	1173	2.5
15	Terpin-4-ol	1182	0.1
16	α-Terpineol	1189	0.6
17	Geraniol	1217	0.8
18	Geranial	1243	0.8
19	Methyl eugenol	1404	0.72
20	Citral	1445	0.7
21	Nerol	1546	0.5
	Total		90.6

The first main component of *E. platyloba* essential oil from our study was according to the essential oils from Golpayegan[21], Tehran[23], northwest (Maragheh district)[22], and Shalamzar[24], different geographical regions of Iran, while the second main component of our essential oil was α-phellandrene, and its isomer (γ-phellandrene) were found only in the *E. platyloba* essential oil from Maragheh district, the northwest of Iran[22].

Therefore, four different chemotypes were reported for *E. platyloba* essential oil until now including 1):β-ocimene, δ-3-carene 2):thymol, trans-ocimene, carvacrol 3):Asarone, anethole, eugenol; 4) β-ocimene, α-caryophyllene.

Antimicrobial activity of E. platyloba essential oil

The antimicrobial activity of *E. platyloba* essential oil against three bacteria including Gram negative (*Salmonella enteric*a, *Helicobacter pylori*), Gram positive (*Staphylococcus aureus*) ones by disc diffusion assay showed the least inhibition zone diameter was for *Helicobacter pylori* (17 mm), followed by *Salmonella enterica* (20 mm). The large inhibition zone diameter was for *S. aureus* (23 mm). 10 µl of *E. platyloba* essential oil had higher inhibition zone diameter than tetracycline as antibiotic.

The antimicrobial evaluation by micro-broth dilution assay against above bacteria had the MIC values between 0.5-1.1 µl/ml and MFC values between 1.25-2.5 µl/ml, respectively. The sensitive bacteria to *E. platyloba* essential oil was *S. aureus* (MIC and MBC values of 0.5 and 1.25 µl/ml), followed by *S. enteric*a (MIC and MBC values of 1 and 2.5 µl/ml). The less sensitive bacteria to *E. platyloba* essential oil were *H. pylori* (MIC and MBC values of 1.1 and 5 µl/ml) (Table 2).

Although, there are three studies that evaluate the anti-*staphylococcal* activity of *E. platyloba* essential oil against *S. aureus*[8-10] but their chemical compositions were different with our study. The essential oil with thymol, *trans*-ocimene and carvacrol had the MIC value of 448 µg/ml[9] and the essential oil with asarone, anethole and eugenol as main components had the MIC of 500 µg/ml against *S. aureus*[8]. MIC value of *E. platyloba* essential oil with ocimene (26.5%), 2,3-Dimethyl-cyclohexa-1,3-diene (9.9%), α-pinene (7.7%) against *S. aureus* was 12500 ppm[10]. The results of two different studies by others showed the chemical composition of *E. platyloba* essential oil had no effect on the antibacterial activity of this oil against *S. aureus* (MIC=500 µg/ml)[8,9].

The antibacterial activity of *E. platyloba* essential oil is related to its main components. Our essential oil was containing α-caryophyllene or α- humulene and β-ocimene as main

Table 2 Antimicrobial activity of *E. platyloba* essential oil

	Disc diffusion (mm)		Micro-broth dilution assay (µl/ml)	
	E. platyloba	Tetracycline	MIC	MBC
Salmonella enterica	20	21	1±0.02[b]	2.5±0.05[b]
Helicobacter pylori	17	15	1.1±0.025[a]	5±0.06[a]
Staphylococcus aureus	23	20	0.5±0.02[c]	1.25±0.05[c]

MIC= Minimal Inhibitory Concentration; MBC= Minimal Bactericidal Concentration
Lower MIC and MBC value indicated higher antimicrobial activity

components showed promised antibacterial activity especially against Gram positive bacteria, *S. aureus*. The antibacterial activity of α-caryophyllene has been confirmed against *S. aureus*[25]. Furthermore, α-caryophyllene showed anti-inflammatory effects[26]. Therefore, the presence of α-caryophyllene in *E. platyloba* may donate the anti-inflammatory effect to this essential oil that make it as suitable candidate for inflammatory- infectious diseases or other inflammatory diseases.

Antioxidant activity of E. platyloba essential oil

The antioxidant evaluation showed the IC_{50} for *E. platyloba* essential oil and ascorbic acid were 32 and 20 ppm, respectively. In fact, the antioxidant activity of *E. platyloba* essential oil was a little lower than ascorbic acid as control (Fig 1).

Today, the free radicals threaten the health, and many scientists persuade the people to use the natural antioxidants[27,28]. Furthermore, the use of chemical synthetic antioxidants in different industries has been limited due to their adverse effects on human health[29].

E. platyloba essential oil with (Z)-β-Ocimene (26.7%), δ -3-carene (16.2%) and limonene (6.6%) as the main components showed the IC_{20} of 1.1 mg/ml in DPPH system[11].

The oil with thymol (27.2%), *trans*-ocimene (20.9%) and carvacrol (7.2%) had the IC_{50} of 50 µg/ml[9].

According to the results, the geographic region seems to be important in the composition of essential oils. As our results and other studies have been shown, the IC_{50} of this essential oil can change and the lower IC_{50} or high antioxidant activity is related to the *E. platyloba* essential oil with higher phenolic compound such as thymol, carvacrol (thymol, *trans*-ocimene and carvacrol chemotype), followed by β-ocimene and α-caryophyllene chemotype (0.3 mg/ml) and then *E. platyloba* essential oil with (Z)-β-Ocimene, δ-3-carene and limonene (IC_{20} 1.1 mg/ml) (Table 3). Therefore, *E. platyloba* essential oil can be used as natural antioxidant in preserving the products and humans from deterioration of free radicals.

Conclusion

E. platyloba essential oil of our study had β-ocimene and α-caryophyllene as the main components; furthermore, three chemotypes were reported for *E. platyloba* essential oil including 1):β-ocimene, δ -3-carene 2):thymol, trans-ocimene, carvacrol 3):Asarone, anethole, eugenol; 4) β-ocimene, α-caryophyllene. In β-ocimene chemotype, the second main component was different in many studies but chemical composition of our study was according to the report of Hassanpouraghdam and his colleagues with different geographical origin[22]. *E. platyloba* essential oil with Z- β-ocimene and α-caryophyllene showed promised antibacterial activity against Gram positive bacteria (*S. aureus*), followed by *Salmonella enterica*. The less sensitive bacteria were anaerobe Gram negative bacteria, *Helicobacter*

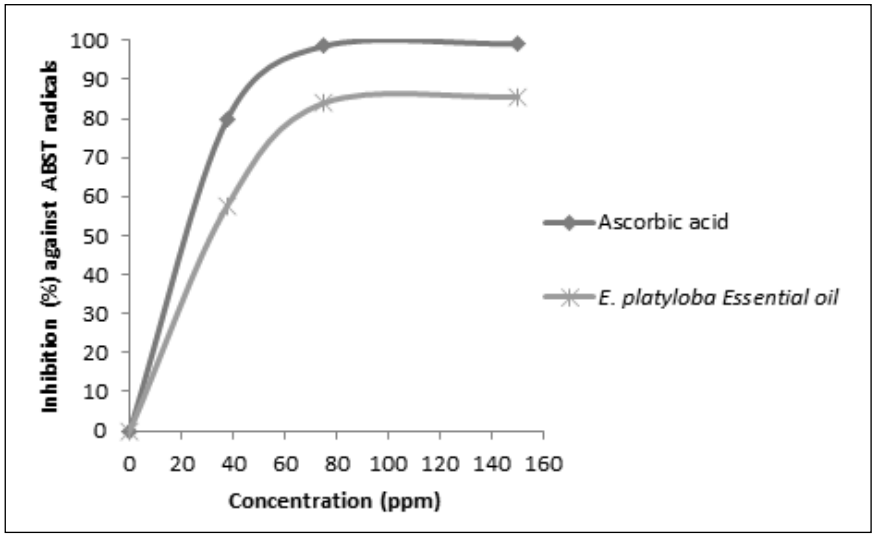

Figura 1. The antioxidant evaluation of *E. platyloba* essential oil by ABTS free radicals

Table 3- The comparison between the results of this study with other study on *of E. platyloba* essential oil

Chemical composition of *E. platyloba* essential oil	Gathering place	Antimicrobial activity	Antioxidant activity (mg/ml)	References
β-Ocimene (46.4%) α-phellandrene (12.1%) β-myrcene (8.5%) limonene (6.1%)	Shahr-E-Kord city, Chaharmahal and Bakhtiari Province	(µl/ml) *S. aureus* =0.5 *S. enterica* =1 *H. pylori* =1.1	IC_{50} =0.32	-
Thymol (27.2%) trans-ocimene (20.9%) carvacrol (7.2%)	ChaharMahal VA Bakhtiari province	mg/ml *S. aureus* =0.448	IC_{50} 0.05	(9)
asarone (10.2%) anethole (7.4%) eugenol (6.7%)	Torbat-Heydariye in Khorasan Razavi province	mg/ml *S. aureus*=0.5	-	(8)
ocimene (26.5%) 2,3-Dimethyl-cyclohexa-1,3-diene (9.9%) α-pinene (7.7%)	Maragheh city northwest of Iran	ppm *S. aureus* 12500	-	(10)
(Z)-β-Ocimene (26.7%) δ -3-carene (16.2%) limonene	Shalamzar, Province of Isphahan, Iran	-	IC_{20} 1.1	(11)
trans-B-ocimene (67.9%) 2-furanone (6.2%) myrcene (6.0%) linalool (3.1%) cis-b -ocimene (2.3%)	Alvand Mountain, Golpaygan-Khomein Road	-	-	(21)
(Z)-β-ocimene (38.9%) α-phellandrene p-cymene (7.4%) β-phellandrene (6.3%) α-pinene (3.4%)	Northwest Iran (Maragheh district)	-	-	(22)
(E)-β-ocimene (49.9%) γ-decalactone (8.4%) α-pinene (6.0%) linalool (5.6%)	Province of Tehran, Iran	-	-	(23)
(Z)-β-ocimene (26.7%) δ -3-carene (16.2%) Limonene (6.6%)	Shalamzar, Province of Isphahan, Iran	-	-	(24)

pylori. Our chemotype showed the low antioxidant activity than thymol, trans-ocimene, and carvacrol chemotype but had higher antioxidant activity than that of *E. platyloba* essential oil with (Z)-β-Ocimene, δ-3-carene and limonene.

Conflict of interests

The authors have no conflict of interests to declare.

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