

The Evaluation of the Antibacterial and Antioxidant Activity of Tarragon (*Artemisia dracunculus* L.) Essential Oil and Its Chemical Composition

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Background: Food born pathogenic bacteria are the most important agents of infections in humans, and food spoilage also results in economic losses in food industry.

Objectives: The aim of this study was the evaluation of chemical components, total phenolic content, antioxidant and antibacterial activities of *Artemisia dracunculus* essential oil.

Materials and Methods: The essential oil of Tarragon was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS). The antioxidant activity and total phenolic content were evaluated by bleaching of β -carotene and folin ciocalteu methods, respectively. The antibacterial effect of the essential oil was inspected on seven Gram-positive and negative bacteria using the microdilution method.

Results: A total of 19 compounds were identified by GC-FID and GC-MS. The main compounds were methyl chavicol (84.83%), trans-ocimene (3.86%), z- β -ocimene (3.42%), limonene (1.79%) and α -pinene (0.57%). Total phenols were 10.16 ± 0.08 mg/g Gallic acid equivalent. The essential oil showed good antioxidant activity in bleaching of β -carotene method ($50 \pm 1.63\%$). The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for essential oil ranged between 3.8 to 250 mg/mL, respectively.

Conclusions: The essential oil of Tarragon might be replaced by synthetic antioxidant and preservatives in food industry.

Keywords: Gas Chromatography-Mass Spectrometry; Artemisia; Essential Oil; Food Preservatives; Antioxidants

1. Background

Nowadays, academic researchers and food industries search for alternative sources of chemical preservatives as protection agents against lipid oxidation and growth of pathogenic bacteria in food. Familiarly, eugenol, limonene, carvacrol and geraniol have been identified as natural food preservatives with no mutagenic effects (1). However, the utilization of chemical antioxidants with high activity, such as tertiary butyl hydroquinone (TBHQ), can threaten the human health (2). The essential oils of spices and medicinal plants containing phenolic compounds have antioxidant and antibacterial properties that have been widely used in pharmaceutical, sanitary, cosmetic, agricultural and food industries around the world (1, 3).

The presence of bioactive substances in medicinal plants may react with microorganisms and restrain microbial growth. Therefore, their extracts and essential oils are regarded as good candidates for replacing syn-

thetic preservatives. Previous works have demonstrated the antioxidant and antibacterial activities of essential oils on food spoilage and pathogen bacteria (4).

The genus *Artemisia* is a small shrub from the *Asteraceae* family. In Iran, Tarragon (*Artemisia dracunculus* L.) is called "Tarkhon" and as a traditional medicinal plant is used for the treatment of stomach pains, pyrexia, diabetes and parasitic or bacterial infections. The fresh and dried leaves are commonly used in salads, soups and barbecues. Furthermore, its essential oil is used as an aromatic and flavoring agent in food industry (2, 5). In a study conducted in Turkey, using the disc diffusion method, it has been shown that the essential oil of *A. dracunculus* has antibacterial effects and antifungal activities on eleven pathogenic fungi (6).

2. Objectives

The aim of this study was to evaluate the antioxidant

Implication for health policy/practice/research/medical education:

The essence of Tarragon can be used as a natural antibacterial and antioxidant agent in food industry.

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and antibacterial activities of Tarragon (*A. dracunculus* L.) essential oil as well as its chemical composition.

3. Materials and Methods

3.1. Preparation of the Essential oil

Samples of Tarragon (*A. dracunculus*) were collected in November 2011 from Charmahal va Bakhtiari Province of Iran. Voucher specimens of the collected Tarragon were confirmed and deposited at the herbarium of the Medical Plants Research Center of Shahrekord University of Medical Sciences, Iran (No. 235). The dried plants (100 g) were hydro-distilled for 3 hours using a Clevenger type apparatus. The essential oil was then dehydrated over anhydrous sodium sulphate and kept in sealed vials at 4°C (7).

3.2. Essential Oil Analysis by Gas Chromatography and Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil from Tarragon was analyzed using a Younglin Acme 6000 gas chromatography-flame ionization detector (GC-FID) with a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 0.8 mL/minute. The essential oil was diluted in n-pentane (1/1000, v/v) and 1.0 µL was injected in the splitless mode. The primary oven temperature was maintained at 50°C for 5 minutes and then increased up to 240°C at a rate of 3°C/minute. Temperatures of injector and detector were 290°C and 300°C, respectively. Percent of each compound was obtained from the comparison of the area under each curve of GC peaks with the total area under the curves in which correction factors weren't used. For the GC-MS analysis an Agilent 6890 GC system with a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm) fitted with an Agilent HP- 5973 mass selective detector was used. The electron ionization (EI) system with ionization energy of 70 eV and temperature of ion source 220°C was used for GC-MS detection. Other stages were under similar conditions as GC. Mass spectra were scanned between 50 and 550 a. m. u. range.

3.3. Identification of Essential Oil Constituents

The identification of the constituents of the essential oil was achieved by comparing their retention indices (determined with homologous series of n-alkanes C8-C20, under similar conditions) with data reported on authentic compounds in articles, references books as well as standard libraries (Wiley275.L and Wiley7n.L) (8).

3.4. Total Phenolic Contents

Total phenolic content was examined by Folin Ciocalteu

method (9). The result was expressed as mg of Gallic Acid Equivalents/g of the essential oil (GAEs).

3.5. Antioxidant Activity

The total antioxidant capacity of *A. dracunculus* essential oil was assayed using β-carotene and linoleic acid with minor modification (10). A stock solution of β-carotene (0.5 mg) was prepared in 1 mL of chloroform, linoleic acid (25 µL), and Tween-40 (200 mg). Chloroform was completely evaporated using a vacuum evaporator at 50°C and 100 mL of oxygenated water (30 minutes 100 mL/minute) was added under vigorous shaking. From this emulsion, 2.5 mL aliquots were added to test tubes and 350 µL of the essential oil prepared at 4 g/L concentrations was added and incubated for 48 hours at room temperature. For the positive control, the same procedure was repeated with Butylated hydroxytoluene (BHT) and a blank (only 350 µL ethanol). The absorbance of the solutions was measured at 490 nm. Antioxidant activity of the essence was compared with those of the BHT and the blank.

3.6. Test Organism and Antibacterial Assay

Cultures of *Staphylococcus aureus* PTCC (Persian Type Culture Collection) 1189, *Alcaligenes faecalis* PTCC 1624, *Providencia rettgeri* PTCC 1512, *Serratia marcescens* PTCC 1621, *Shigella dysenteriae* PTCC 1188, *Listeria monocytogenes* PTCC 1163 and *Klebsiella oxytoca* 1402 were purchased from Iranian Research Organization for Science and Technology (IROST). The bacterial strain was inoculated in tryptic soy broth (TSB, Merck Ink, Darmstadt, Germany) at 37°C overnight, and 0.5 McFarland Standard was prepared with densities of 1.5×10^8 cfu/mL in phosphate buffered saline (PBS) (11). The microtiter broth method in sterile 96-microwell plates was used for minimum inhibitory concentration (MIC) (12).

The essence obtained was dissolved in dimethyl sulfoxide (DMSO). Further 95 µL Mueller Hinton broth (Merck Ink, Darmstadt, Germany) plus 5 µL bacterial suspensions plus 100 µL serial 2-fold dilution of essential oil (3.8-1000 mg/mL) were added in microwell and incubated at 37°C for 18 hours. Negative control was 195 µL Mueller-Hinton broth plus 5 µL bacterial suspensions and positive control was 95 µL Mueller Hinton broth plus 5 µL bacterial suspensions and 100 µL of chloramphenicol (Sigma-Aldrich Corporation St. Louis, MO, USA) as a positive antimicrobial reference. Bacterial growth was measured by reading the optical density at 450 nm using an ELISA reader (State fax 2100, USA) at 0 and 18 hours post-inoculation. After 18 hours, the MIC value was determined from the first well, without turbidity. The contents of the wells with no growth were spread on Mueller Hinton agar (Merck Ink, Darmstadt, Germany) and incubated at 37°C for 24 hours. The minimum bactericidal concentration (MBC) was identified as the lowest concentration that did not allow bacterial growth. Each experiment was repeated in

triplicates.

4. Results

Nineteen compounds were identified; the main constituents of the essential oil were methyl chavicol (84.83%),

trans-ocimene (3.86%), z- β -ocimene (3.42%), limonene (1.79%) and α -pinene (0.57%). Majority of the compounds in the essential oil were monoterpene hydrocarbons (95.904%) and the lowest levels were related to sesquiterpene hydrocarbons (0.46%). The constituents of essential oil can be observed in Table 1.

Table 1. The Chemical Components of *A. dracunculus* Essential Oil

Number	Retention Time	Kovats Indices	Name	Concentration, %
1	11.23	927	α -pinene	0.57
2	13.31	969	β -pinene	0.107
3	14.14	986	β -myrcene	0.1
4	16.06	1025	Limonene	1.79
5	16.67	1037	z- β -ocimene	3.42
6	17.22	1047	Trans-ocimene	3.86
7	19.06	1084	Terpinene	0.08
8	19.70	1096	Linalool	0.157
9	21.16	1126	Ocimene (allo)	0.16
10	25.26	1226	Methyl chavicol	84.83
11	28.06	1270	Geranial	0.17
12	28.66	1283	Iso bornyl acetate	0.1
13	31.80	1353	Eugenol	0.12
14	32.87	1377	Iso safrole (E)	0.37
15	33.85	1399	Methyl eugenol	0.07
16	37.65	1491	Valencene	0.1
17	38.84	1520	β -sesquiphellandrene	0.07
18	40.43	1561	Cinnamaldehyde (para-methoxy)	0.2
19	40.95	1574	Spathulenol	0.09
-	-	-	Monoterpenes	10.087
-	-	-	Oxygenated monoterpenes	85.817
-	-	-	Sesquiterpenes	0.17
-	-	-	Oxygenated sesquiterpenes	0.29
Total	-	-	-	96.364

In this study the total phenolic content and antioxidant activity was investigated and was compared with synthetic antioxidant BHT. We showed that Tarragon essen-

tial oil inhibited oxidation of linoleic acid in a manner less than BHT (Table 2).

Table 2. Total Phenolic Content, Antioxidant Capacity of *A. dracunculus* Essential Oil

	Total Phenolic Content, mg GAEs ^a /gr	Antioxidant Capacity
Artemisia dracunculus Essential oil	10.16 \pm 0.08	50 \pm 1.63
BHT^a	-	90.6 \pm 3.30

^a Abbreviations: GAEs, gallic Acid Equivalents; BHT, butylated hydroxytoluene

Also the essential oil showed different degrees of inhibi-

tory effect on the growth of tested bacterial strains (Table 3).

Table 3. Antibacterial Activity of *A. dracunculus* Essential Oil

	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Alcaligenes faecalis</i>	<i>Providencia rettgeri</i>	<i>Serratia marcescens</i>	<i>Shigella dysenteriae</i>	<i>Klebsiella oxytoca</i>
MIC ^a , mg/mL	62.4	15.6	15.6	31.2	3.8	3.8	7.8
MBC ^a , mg/mL	250	250	31.2	62.4	7.8	7.8	62.4

^a Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration

5. Discussion

In a similar study conducted in Turkey, the main compounds in *A. dracunculus* essential oil were (Z)-anethole (81.0%), α - β -ocimene (6.5%), (E)- β -ocimene (3.1%), limonene (3.1%) and methyleugenol (1.8%) (6). Also, Ayoughi et al. reported that the predominant compounds in *A. dracunculus* essential oil were (Z)-anethole (51.72%), α - β -ocimene (8.32%), methyleugenol (8.06%), limonene (4.94%) and linalool (4.41%) (2). In the present work, the major compound was methyl chavicol which is a double-bond isomer of anethole. However, in our results methyleugenol (0.07%) was much lower than that of the above studies. The different chemical compositions of the essential oils might be related to harvest time, geographical situation, ground conditions and genetic factors (13, 14).

It has been shown that there is a correlation between antioxidant capacity and antibacterial activity as well as the total phenolic content of the herb extracts or essential oils (9, 15). Furthermore, it has been reported that oxygenated monoterpenes have antioxidant and antimicrobial activities (2). We showed that Tarragon essential oil had antioxidant activity and inhibited oxidation of linoleic acid in a manner less than BHT (Table 2). This can be due to high level of methyl chavicol such as the oxygenated monoterpenes and the total phenolic content. Other reports have demonstrated that linalool, limonene, spathulenol and eugenol have antioxidant activities (2, 16).

The essential oil showed different degrees of inhibitory effect on the growth of tested bacterial strains (Table 3). The Gram negative bacteria especially *S. marcescens* and *Sh. dysenteriae* were the most sensitive, while *L. monocytogenes* and *S. aureus* (Gram positive) were the most resistant bacteria against this essential oil. Previous studies reported antibacterial activity of Tarragon EO against *E. coli*, *S. aureus* (17), *Salmonella typhimurium*, *L. monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* (18). Its results revealed that *E. coli* (17) and *Y. enterocolitica* (18) were more sensitive than other bacteria. Raeisi et al. showed that *S. aureus* had more sensitivity than *E. coli* to Tarragon EO (19). However, some studies have reported that Gram negative bacteria are more resistant than Gram positives, due to restricted diffusion of the hydrophobic compounds through the hydrophilic cell wall structure, such as lipo-polysaccharide (LPS) (20, 21).

Kordali et al. exhibited that the essential oil of *A. dra-*

cunculus with a high level of (Z)-anethole (81%) did not have any effect on *S. aureus* (6). The results of the present study showed that *A. dracunculus* essential oil with high level of methyl chavicol (84.83%) had antibacterial activity against *S. aureus*. The antibacterial activity of the essential oil is dependent upon its major compounds. However some researchers reported higher antibacterial activity of the whole essence than major compounds blended in essence due to antibacterial properties of minor compounds in essential oil (16, 22).

Based on the results of this study, the essence of Tarragon had biological properties as anti-oxidative and antibacterial activities. Also, the major aromatic compound was methyl chavicol. The compounds in the essence may be helpful for prevention of cancer and atherosclerosis, which is related to the inhibition of lipid oxidation. Eventually, it is recommended to perform surveys on this essential oil and prove it as a natural preservative in food models in order to replace synthetic preservatives in foods.

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Authors' Contribution

Reza Sharafati-Chaleshtori and Noordahr Rokni: developed the original idea and the protocol, abstracted and wrote the manuscript. Reza Sharafati-Chaleshtori, Noordahr Rokni, Vadood Razavilar and Mahmoud Rafieian-Kopaei contributed to the development of the protocol, abstracted data, and prepared the manuscript.

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Authors declare that there are not any relevant financial interests related to the material in the manuscript

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