ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 10:4 (2016) 426-436

records of natural products

Antimicrobial Activities of Essential Oils and Hexane Extracts of Two Turkish Spice Plants, *Cymbocarpum erythraeum* (DC.) Boiss. and *Echinophora tenuifolia* L. Against Foodborne Microorganisms

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(Received January 13, 2014; Revised August 25, 2015; Accepted October 14, 2015)

Abstract: The hydrodistillated essential oils and hexane extracts of two spice plants, *Cymbocarpum erythraeum* (DC.) Boiss. and *Echinophora tenuifolia* L. were analyzed by GC and GC-MS. *C. erythraeum* oil is rich in aliphatic aldehydes, alcohols and acids and (*E*)-2-decenal (26.1%), (*E*)-2-decen-1-ol (15.7%), (*E*)-2-dodecenal (13.2%) and decanal (7.8%) were the predominant components. However, ethyl palmitate (16.4%), 2-decenoic acid (14.1%) and (*E*)-2-dodecenal (5.2%) were the major components of the hexane extract of *C. erythraeum*. *E. tenuifolia* oil contained mainly methyl eugenol (53.0%), *p*-cymene (17.0%) and α-phellandrene (13.2%). The hexane extract displayed a different chemical composition, and *n*-tricosane (75.0%) and *n*-pentacosane (7.6%) were found to be the major compounds. The oils showed antimicrobial activity against various microorganisms and they were more active against the tested fungal species as compared with bacteria. The growths of important food-borne pathogens, *Salmonella*, *Staphylococcus aureus* and *Escherichia coli* were also inhibited by the oils. However, hexane extract of *C. erythraeum* was showed weak antibacterial activity against limited number of tested bacteria. The current results showed that the essential oils of *C. erythraeum* and *E. tenuifolia* can be used in food preservation.

Keywords: Antimicrobial; Psychrotrophilic bacteria; *Cymbocarpum erytraeum*; *Echinophora tenuifolia*; (*E*)-2-decenal; essential oil; hexane extracts; methyl eugenol.

1. Introduction

Microorganisms play a critical role in food spoilage. Food-borne illness has been a great concern to public health for years and the control of pathogens may reduce these food-borne diesease outbreaks. There is a worldwide trend to explore new alternatives to control food-borne diseases. In recent years, the food industry is facing an increased pressure from consumers for using chemical preservatives to prevent the growth of food-borne pathogens. To reduce or eliminate synthetic additives from foods is a current demand worldwide. A new approach to prevent the proliferation of

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microorganisms or protect foods from oxidation is the use of essential oils, plant extracts and plant metabolites as preservatives [1,2]. In this context, aromatic plants have emerged as effective agents to provide microbiological safety of foods [2-4]. In the literature, there are numerous reports on antimicrobial activities of various plant essential oils and extracts against important food-borne microorganisms [5-12].

The genus *Cymbocarpum* is one of the most important genera of the Apiaceae family and widespread throughout the world. There are about four species of this genus in Turkish flora [13-15]. The species of *Cymbocarpum* genus are known in Anatolia as "Aşotu", "Tüysüz aşotu", "Öz aşotu" and "Kızıl aşotu". *Cymbocarpum erythraeum* is low, glabrous, foetid annuals; stems 10-30 cm, often branched from base. Basal leaves soon withering; petiole long abruptly dilated into sheath, lamina 10-15x10-15 mm, bipinnate, ultimate segments 3-8x0.5 mm, linear, acute. Cauline leaves similar but smaller, subtended by expanded membranous margined sheath. Umbel rays 9-11, unequal, glabrous. Bracts and bracteoles are linear, acute, recurved, not marginated. Flowers white-pink; petals c. 1 mm. Fruit 3-3.5x2.5 mm, ovoid, glabrous, ridge filiform, slightly dilated at margins [13].

On the other hand, another species that occur in the flora of Turkey is *Echinophora tenuifolia*. The genus *Echinophora* (Umbelliferae or Apiaceae) is represented in the flora of Turkey by six species including three endemic species [12,16,17]. *Echinophora s*pecies grow naturally in low rainfall areas of Anatolia [12-14, 17] and used in folk medicine to heal wounds and to treat gastric ulcer due to its antimicrobial, carminative and digestive properties [14, 16-18]. *E. tenuifolia* is a greyish-pubescent perennial herb, to 20-50 cm tall, with yellow petals and grows in dry places in Anatolia [16,17]. In Turkey and Iran, fresh and dried aerial parts of some of these species are added to cheese and yoghurt for flavouring [16,17].

Besides distillation methods, volatile components from plant samples may also be isolated using extraction method with the nonpolar organic solvents hexane, benzene, and diethyl ether. The extraction procedure is carried out at low temperatures. The odors of these extracts are very similar to the characteristic odours of plant samples. However, in distillation methods, chemical compositions of essential oils may change during distillation because of high temperature. Therefore, comparison of hydrodistillation and hexane extraction methods in terms of chemical composition may provide significant contributions to the literature.

The objective of this study was to determine the chemical composition of the essential oils and the n-hexane extracts isolated from the aerial parts of *Cymbocarpum erythraeum* (DC.) Boiss and *Echinophora tenuifolia* L. and the antimicrobial activity of the essential oils and hexane extract against a large variety of food-borne microorganisms.

2. Materials and Methods

2.1. Plant Material

Cymbocarpum erythraeum (DC.) Boiss aerial parts were collected from Palandöken Mountain in Erzurum (Turkey) during flowering time at July 2008. Furthermore, the aerial parts of *Echinophora tenuifolia* L. were collected from Gaziantep region (Turkey) at flowering stage, August 2008. The plant samples were identified Dr. Y. Kaya, Department of Biology, Faculty of Art and Science, Ataturk University, Erzurum (Turkey). The voucher specimens are deposited the herbarium of Ataturk University (ATA-9874 and ATA-9875, respectively). Plant parts were air-dried at room temperature in a shady place and kept from direct light, then ground in a grinder with a 2-mm-diameter mesh. These materials were used for the isolation of essential oil analysis as well as hexane extraction, and then the fractions were tested for the antimicrobial activity.

2.2. Chemicals and Standards

The hexane, used to prepare the hexane extract and the anhydrous sodium sulphate used to dry the essential oils were obtained from Riedel-de Haen (Seelze, Germany). Nutrient agar and nutrient broth, Sabouraud's dextrose broth, and potato dextrose agar (PDA) were obtained from Merck (Darmstadt, Germany). OFX5, OFX10, KF30, TE30, AMC30, SAM20 and AZM15 were obtained from Oxoid (Basingstoke, Hampshire, UK). Dimethyl sulfoxide (DMSO) for microwell dilution assay, amphotericin B (catalog number A 4888), and Tween 20 used for minimum inhibitory concentration

(MIC) agar dilution assay were obtained from Sigma (St. Louis, MO, USA). Pure chemicals used for gas chromatography (GC)-mass spectrometry (MS) were obtained from Sigma, Fluka (Buchs, SG, Switzerland), Aldrich (Madison, WI, USA), and Merck.

2.3. Preparation of Hexane Extracts

The dried and powdered leaves (50 g) were extracted by macerating with hexane (5 × 100 mL) at room temperature. The extract was filtered using Whatman filter paper (No. 1) (Maidstone, Kent, UK) and then concentrated under reduced pressure at 40°C using a rotary evaporator (BUCHI Labortechnik AG, Flawil, Switzerland). The extract (350 mg, yield 0.9%) was stored in a freezer (NuAire Inc., Plymouth, MN, USA) at –80°C until further tests.

2.4. Isolation of the essential oils

Air-dried plant materials of *C. erythraeum* and *E. tenuifolia* were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus (Ildam Ltd., Ankara, Turkey). The essential oil yields of the plant samples were 0.55 and 1.60 % (w/w), respectively. The essential oils were dried over anhydrous sodium sulfate and, after filtration, stored at 4°C until tested and analyzed.

2.5. GC Analysis Conditions

The analysis of the essential oils and hexane extracts was performed using a Thermo Finnigan Trace GC/A1300 (EI) (San Jose, CA, USA) equipped with an SGE/BPX5 MS capillary column (30 m × 0.25 mm i.d.; film thickness, 0.25 μm) (Scientific Instrument Services Inc., Ringoes, NJ, USA). Helium was the carrier gas, at a flow rate of 1 mL/min. The detector and injector temperature were set at 220°C. The program used was 50–150°C at a rate of 3°C/min, holding isothermal for 10 min and finally raising to 250°C at 10°C/min. Diluted samples [1:100 (v/v) in methylene chloride] of 1.0 μL were injected manually and in the splitless mode. Quantitative data of the oils and hexane extracts was obtained from flame ionization detector area percentage data (Table 1).

2.6. GC-MS Analysis Conditions

The analyses of the essential oils and hexane extracts were performed using a Thermo Finnigan Trace GC/Trace DSQ/A1300 (E.I quadrapole) (San Jose, CA, USA) equipped with an SGE-BPX5 MS capillary column (30 m × 0.25 mm i.d.; film thickness, 0.25 μm) (Scientific Instrument Services Inc., Ringoes, NJ, USA). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium, at a flow rate of 1 mL/minute, was the carrier gas. Injection and MS transfer line temperatures were set at 220°C and 290°C, respectively. The program used was 50–150°C at a rate of 3°C/minute, holding isothermal for 10 minutes, and finally raising to 250°C at 10°C/minute. Diluted samples (1:100 [v/v] in methylene chloride) of 1.0 μL were injected manually and in the splitless mode.

The components were identified based on the comparison of their relative retention indices and mass spectra with those of standards, the Wiley7N and TRLIB library data of the GC-MS system, and literature data [19]. The results were also confirmed by comparison of the compound's elution order with their relative retention indices on nonpolar phases reported in the literature [19].

2.7. Antimicrobial Activity

The essential oil and hexane extract of *C. erythraeum* and essential oil of *E. tenuifolia* were tested individually against a range of food-borne microorganisms. Microorganisms were provided by the Food Microbiology Laboratory, Department of Food Engineering, Faculty of Agriculture, Atatürk University. The identification of the microorganisms used in the present study was confirmed by the Microbial Identification System (Sherlock Microbial Identification System version 4.0, MIDI Inc., Newark, DE, USA), API (BioMerieux, Craponne, France), BIOLOG (MicroStation™ ID System, Biolog Inc., Hayward, CA, USA), and classical identification test from *Bergey's Manual of Determinative Bacteriology* [20].

2.7.1. Disk Diffusion Assays

The essential oils and hexane extract were dissolved in DMSO to a final concentration of 30 mg/mL. Antimicrobial tests were carried out by the disk diffusion method [21] using 100 µL of suspension containing 10^8 colony-forming units/mL bacteria, 10^6 colony-forming units /mL yeast, and 10^4 spores/mL fungi spread on nutrient agar, Sabouraud-2% dextrose agar, and PDA medium, respectively. The disks (6 mm in diameter) impregnated with 10 µL of the sample solutions (300 µg per disk) were placed on the inoculated agar. DMSO without essential oils and hexane extract was used as negative control. OFX (5 µg ofloxacin per disk) (5OFX10 (10 µg ofloxacin per disk); SAM20 (10 µg of sulbactam+10 µg of ampicillin per disk), AMC30 (20 µg of amoxicillin + 10 µg of clavulanic asit per disk), KF30 (30 µg of cephalothin per disk), AZM15 (15 µg of azithromycin per disk), TE30 (30 µg of tetracycline per disk) were used as positive reference standards to determine the sensitivity of bacterial strains tested in this study. Amphotericin B (20 µg per disk) was used for yeast and fungi as the positive control. The inoculated plates were incubated at 32°C for 24 hours for mesophilic bacteria, 20°C for 48 hours for psychophylics, 30°C for 48 hours for the yeast, and at room temperature for 72 hours for fungi isolates [22]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated three times.

2.7.2. Microwell Dilution Assays

The MIC values were determined for the bacterial and yeast strains that were sensitive to the essential oil in the disk diffusion assay. The inoculation of the strains was prepared from 12-hour broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The oils and the extract, dissolved in DMSO, were first diluted to the highest concentration (500 µg/mL) to be tested, and then serial twofold dilutions were made to obtain a concentration range from 7.8 to 500 µg/mL in 10-mL sterile test tubes containing nutrient broth, or Sabouraud-2% dextrose broth. The MIC values of the essential oils and hexane extract against bacterial and yeast strains were determined on the basis of a microwell dilution method [9,10,23] with some modifications. The 96-well plates were prepared by dispensing 95 µL of broth and 5 µL of the inoculum into each well. An 100 µL from the stock solutions of the oils and hexane extract prepared at the 500 µg/mL concentration was added into the first wells. Then, 100 µL from the serial dilutions was transferred into the six consecutive wells. The last well containing 195 µL of nutrient broth without compound and 5 µL of the inoculums on each strip was used as a negative control. The final volume in each well was 200 µL. Clarithromycin and Amphotericin B at a concentration range of 500–7.8 µg/mL was prepared in nutrient broth and Sabouraud-2% dextrose broth, and used as positive controls. The plate was covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker (MS2-Minishaker, IKA, Labortechnik, Staufen, Germany) at 300 rpm for 20 seconds and then incubated at appropriate temperatures for 24 hours. Microbial growth in each medium was determined by reading the respective absorbance at 600 nm using the ELx800 universal micro plate reader (Biotek Instrument Inc., Highland Park, VT, USA). The oil tested in this study was screened three times against each organism.

2.7.3. MIC Agar Dilution Assays

The agar dilution method, as described previously [10] was used to determine the MIC values of the fungal isolates. The essential oils and hexane extract of the plant samples were added aseptically to sterile molten PDA (Potato Dextrose Agar) medium, containing Tween 20 (0.5%, v/v), at the appropriate volume to produce the concentration range of 7.8–500 µg/mL. The resulting PDA media were immediately poured into Petri plates after vortex-mixing. The plates were spot-inoculated with 5 µL (10^4 spores/mL) of each fungal isolate. Amphotericin B was used as a reference antifungal drug. The inoculated plates were incubated at room temperature for 72 hours. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil at which the absence of growth was recorded. Each test was repeated at least three times.

2.8. Statistical Analysis

In order to determine whether there is a statistically significant difference among the obtained results from inhibition zone assays, variance analyses were carried out using SPSS version 10.0 software package (SPSS, Chicago, IL, USA). Differences between means were tested by the Duncan test, and values with $p< 0.05$ were considered significantly different.

3. Results and Discussion

3.1. Chemical composition and antimicrobial activities of the essential oil and hexane extract of Cymbocarpum erythraeum

The chemical compositions of the essential oil and hexane extract of *C. erythraeum* were summarized in Table 1. This table shows that the essential oil is rich in aliphatic compounds, aliphatic aldehydes, alcohols and acids. (*E*)-2-Decenal (26.10%), (*E*)-2-decen-1-ol (15.7%), (*E*)-2-dodecenal (13.2%), decanal (7.8%), undecanoic acid (5.3%), decanoic acid (4.5%), pentadecanal (3.2%) and (*Z*)-10-pentadecen-1-ol (3.2%) were the predominant components of the *C. erythraeum* essential oil. The oil contains mainly aliphatic compounds and aliphatic aldehydes, alcohols and acids representing 61.6%, 19.5% and 13.3%, respectively. The oil contained low amounts of monoterpenes (2.5%) (Table 1). However, the GC-MS analyses results of the hexane extract showed that ethyl palmitate (16.4%), 2-decenoic acid (14.1%), (*E*)-2-dodecenal (5.2%), nonanoic acid (4.7%), *n*-heneicosane (4.3%) and decanoic acid (4.2%) were found to be predominant components of the hexane extract. This is a first report of the chemical composition of the hexane extract of *C. erythraeum*. Plants biosynthesize various secondary metabolites which possesses various polarities. The secondary metabolites can be extracted from plant samples using various organic solvents. Our results indicated that the hexane extract contains less volatile compounds, aliphatic esters, hydrocarbons and acids such as ethyl palmitate, methyl palmitate, ethyl stearate, heneicosane, hexadecanoic acid besides some volatile compounds (Table 1). The hydrocarbons and acids in the hexane extract are less volatile compounds. However, these less polar compounds extracted with nonpolar organic solvents such as hexane, petroleum ether, chloroform, methylene chloride, etc. Previous reports showed that the main class of the compounds was found in the *Cymbocarpum* genus to be aliphatic aldehydes [24-26]. It has been reported that 2-dodecenal (45.5%), (*E*)-2-decenal (17.9%), α-pinene (8.4%) and (*E*)-2-tridecanal (8.35%) were the main components of Iranian *C. anethoides* oil [25]. It has been documented that the essential oil isolated from the aerial parts of *C. erythraeum* growing in Palandoken mountain contained mainly (*E*)-2-decenal (52.2%), (2*E*)-dodecenal (15.8%), 8,14-cedranediol (8.5%), *n*-tetradecanol (5.5%) and (*E*)-4-decenal (5.1%) [26]. In the current study, (*E*)-2-decenal (26.1%) and (*E*)-2-dodecenal (13.2%) were found to be predominant compounds in the essential oil of *C. erythraeum.* Similarly, the hydrodistillation oils of crushed fruits and herbal parts of endemic *Cymbocarpum wiedemannii* from Turkey were analyzed and (*E*)-2-decenal (39.3% and 31.7%), (*E*)-2-dodecenal (16% and 11.4%), (*E*)-2-tetradecenal (8.8% and 4.1%), and 2-decenoic acid (4.5% and 19-9%), decanoic acid (2% and 8.7%) and 2-dodecenoic acid (1.6% and 6.6%) were found to be main constituents in the fruit and herb oils, respectively [24]. An another study, the hydrodistillation essential oil of *C. erythraeum* (DC.) Boiss harvested from Erzincan region of Turkish flora was dominated by fatty alcohols and aldehydes which accounted for 73.10% and 24.64%, respectively and it contained myristyl alcohol (73.10%) as predominant component [11].

The antimicrobial activities of the oil and hexane extract of *C. erythraeum* against 24 bacteria, 3 fungi and 3 yeasts were assayed by evaluating the presence of inhibition zones, zone diameter, and MIC values (Tables 2 and 3). The essential oil showed antimicrobial activity against all tested bacteria with inhibition zones between 28.3 and 9.4 mm, except *Yersinia enterocolitica*. As can be seen from Table 4 the oil showed high antifungal activity as compared with antibacterial activity, the growths of all tested fungi were potently inhibited by the oil. *Aspergillus niger* was the most sensitive microorganism against the oil. The oil was more active against gram-positive bacteria (mean inhibition zone=16.8) as compared with gram-negative bacteria (mean inhibition zone=12.6).

Table 1. Chemical composition of essential oil and *n*-hexane extract of *C. erythraeum* (DC.) Boiss.

RI[a]	Components	Oil (%)	Extract (%)	Identification methods
1014	Octanal	1.3	-	GC, MS, RI
1034	*p*-Cymene	t	-	GC, MS, RI
1042	1,8-Cineole	t	-	GC, MS, RI
1081	*cis*-Linalool oxide (furanoid)	t	-	GC, MS, RI
1091	Heptanoic acid	-	t	GC, MS, RI
1100	(*E*,*E*)-2,4-Octadienal	2.5	-	MS, RI
1106	Linalool	2.0	-	GC, MS, RI
1113	Nonanal	2.3	t	GC, MS, RI
1153	Camphor	0.5	-	GC, MS, RI
1172	*n*-Nonanol	0.6	-	GC, MS, RI
1175	Octanoic acid	t	t	GC, MS, RI
1184	Naphthalene	1.5	-	GC, MS, RI
1190	α-Terpineol	t	-	GC, MS, RI
1197	Decanal	7.8	4.0	GC, MS, RI
1262	(*E*)-2-Decenal	26.1	1.1	GC, MS, RI
1268	(*E*)-2-Decen-1-ol	15.7	t	GC, MS, RI
1267	Nonanoic acid	2.5	4.7	GC, MS, RI
1288	2-*n*-Octyl furan	t	1.6	MS, RI
1296	Carvacrol	-	t	GC, MS, RI
1313	Undecanal	1.5	t	GC, MS, RI
1368	Decanoic acid	4.5	4.1	MS, RI
1392	Ethyl decanoate	-	t	GC, MS, RI
1419	Dodecanal	2.7	4.6	MS, RI
1428	2-Decenoic acid	0.2	14.1	MS, RI
1440	Undecanoic acid	5.3	3.8	GC, MS, RI
1481	(*E*)-2-Dodecenal	13.2	5.2	GC, MS, RI
1563	Lauric acid	0.8	t	GC, MS, RI
1574	Spathulenol	t	-	GC, MS, RI
1579	Caryophyllene oxide	t	-	GC, MS, RI
1610	Tetradecanal	1.0	0.3	GC, MS, RI
1677	Pentadecanal	3.2	t	GC, MS, RI
1844	(*Z*,*Z*)-Farnesyl acetone	-	1.9	GC, MS, RI
1908	Methyl palmitate	-	4.5	GC, MS, RI
1923	Hexadecanoic acid	-	3.6	GC, MS, RI
1963	Ethyl palmitate	-	16.4	GC, MS, RI
1974	5-Octadecenal	t	-	GC, MS, RI
1976	(*Z*)-10-Pentadecen-1-ol	3.2	-	GC, MS, RI
2000	*n*-Eicosane	-	1.6	GC, MS, RI
2012	Ethyl stearate	-	3.2	GC, MS, RI
2100	*n*-Heneicosane	-	4.3	GC, MS, RI
2300	*n*-Tricosane	-	1.9	GC, MS, RI
2600	*n*-Hexacosane	-	2.5	GC, MS, RI
Grouped components (%)				
Aliphatics				
	Aldehydes		61.6	15.2
	Alcohols		19.5	t
	Acids		13.3	30.3
	Esters		-	24.1
Monoterpene hydrocarbons			t	-
Oxygenated monoterpenes			2.5	t
Sesquiterpene hydrocarbons			-	-
Oxygenated sesquiterpenes			t	1.9
Others			1.5	11.9
Total			**98.4**	**83.4**

[a] Retention index relative to *n*-alkanes (C_8-C_{28}) on SGE-BPX5 capillary column. GC: co-injection with standards; MS; tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data [17]; RI: identification based on comparison of retention index with those of published data [17]; t: trace (less than 0.1%).

These results showed that the structure of the cell wall of the microorganisms is an important factor on the antibacterial effect of the oil. The current results also showed that the oil was active against important food-borne pathogens such as *Salmonella aureus*, *Staphylococcus* and *E. coli*. However, the hexane extract was showed weak antibacterial activity exhibiting high MIC values against limited number of the tested bacteria in comparison to those of the oil. Nevertheless, the hexane extract was most active against all tested fungal and yeast species. According to our literature survey, this is also first report on the antimicrobial activities of the essential oil and hexane extract of *C. erythraeum*.

Table 2. Antibacterial activity of the essential oil and hexane extract of *C. erythraeum* (DC.) Boiss.

Test Bacteria	The essential oil		Hexane extract		Antibiotics	
	DD[a]	MIC[b]	DD[a]	MIC[b]	DD[c]	MIC[d]
Acinetobacter lwoffi BC 2819	22.0±0.2	7.8±0.0	-	-	18(OFX10)	7.8
Alcaligenes faecalis BC 0452	15.8±0.4	7.8±0.0	-	-	20(OFX10)	7.8
Bacillıs cereus BC 6830	12.7±0.4	250±0.0			14(SAM20)	7.8
Bacillus subtilis BC 5211	20.5±0.7	7.8±0.0	16.1±0.3	7.8±0.0	36(AMC30)	7.8
Enterobacter cloacea BC 3213	-	-	-	-	24(KF 30)	7.8
Escherichia coli BC 1402	12.9±0.3	250±0.0	-	-	22(OFX10)	15.62
Escherichia coli BC 1818	10.9±0.8	500±0.0	7.3±0.5	500±0.0	12(AZM15)	31.25
Flavobacterium indologenes BC 1520	11.8±0.2	500±0.0	-	-	27(AZM15)	7.8
Klebsiella pneumoniae BC 1749	9.4±0.3	500±0.0	-	-	30(OFX10)	62.25
Klebsiella pneumoniae BC 3126	-	-	-	-	25(OFX10)	31.25
Listeria monocytogenes BC 8353	15.2±0.5	15.6±0.0	12.4±0.9	125±0.0	22(SAM20)	7.8
Proteus mirabilis BC 2644	18.6±5.5	250±0.0	-	-	28(OFX10)	125
Proteus vulgaris KÜKEM 1329	18.2±0.4	7.8±0.0	13.1±0.1	31.25±0.0	18(AMC30)	7.8
Providencia alkalifaciens BC 0236	14.5±0.9	62.5±0.0	-	-	33(OFX10)	62.5
Pseudomonas aeruginosa ATCC 9027	14.3±0.3	15.6±0.0	7.4±0.4	500±0.0	36(TE30)	7.8
Pseudomonas aeruginosa ATCC 27859	16.2±0.5	7.8±0.0	9.6±0.6	125±0.0	35(TE30)	7.8
Pseudomonas pseudoalkaligenes BC3445	17.1±0.6	7.8±0.0	9.2±0.4	500±0.0	32(OFX10)	125
Pseudomonas putida BC 1617	20.4±1.2	125±0.0	-	-	16(TE30)	125
Salmonella typhimurium RSSK 95091	12.6±0.6	500±0.0	10.7±0.4	500±0.0	12(TE30)	7.8
Staphylococcus aureus ATCC 29213	14.9±0.9	7.8±0.0	7.2±0.5	500±0.0	34(TE30)	7.8
Staphylococcus aureus BC 7231	13.6±0.5	31.2±0.0	7.9±0.7	500±0.0	22(KF30)	7.8
Staphlococcus hominis BC 2288	12.5±0.5	250±0.0	-	-	24(KF30)	15.62
Streptococcus pyogenes ATCC 176	28.3±0.8	7.8±0.0	15.5±0.7	7.8±0.0	25(OFX10)	7.8
Yersinia enterocolitica BC 0184	-	-	-	-	26(OFX10)	31.25
Mean values for Gram positives	**16.8**	**81.5**	**8.5**	**162.9**		
Mean values for Gram negatives	**12.6**	**161.3**	**3.4**	**126.8**		

Inhibition zone data are mean ± SD values of three parallel experiments.
-: Not active
[a] DD, inhibition zone (diameter in mm) around the disks (6 mm) impregnated with 10 µL of essential oil and 300 µg per disk of hexane extract (Disk diffusion value).
[b] In µg/mL.
[c] DD, inhibition zone (diameter in mm) around the standard antibiotic disks. Positive reference standards antibiotic disks (Oxoid) were as follows: OFX10, ofloxacin (10 µg per disk); SAM20, 10 µg of sulbactam+10 µg of ampicillin per disk; AMC30, 20 µg of amoxicillin + 10 µg of clavulanic asit per disk; KF 30, 30 µg of cephalothin per disk; AZM15, 15 µg of azithromycin per disk; TE30, 30 µg of tetracycline per disk;
[d] Clarithromycin (µg/mL) was used as the reference antibiotic in the microwell dilution assay (Sigma).

Table 3. Antifungal activities of the oil and *n*-hexane extract of *C. erythraeum* (DC.) Boiss against fungi and yeast.

Test microorganisms	The oil		Hexane extract		Antibiotics	
	DD[a]	MIC[b]	DD[a]	MIC[b]	DD[c]	MIC[d]
Yeast						
Candida albicans ATCC 1223	13.6±2.1	7.8±0.0	20.5±0.8	7.8±0.0	15	250
Sacharomyces boulardii BC 6128	18.3±1.3	125±0.0	11.0±0.5	500±0.0	9	62.5
Sacharomyces cerevisiae BC 6541	17.6±0.6	125±0.0	9.9±0.7	500±0.0	8	62.5
Mean values	**16.5**	**85.9**	**13.8**	**335.9±0.0**	**10.7**	**125.0**
Fungi						
Aspergillus niger BC 102	41.8±0.3	7.8±0.0	33.0±0.4	7.8±0.0	21	62.5
Geotrichum candidum BC 107	35.6±0.7	7.8±0.0	31.0±0.7	15.6±0.0	29	250
Penicillium jensenii BC 110	28.1±0.9	31.2±0.0	22.2±0.4	31.2±0.0	11	250
Mean values	**35.2**	**15.6**	**28.7**	**18.2**	**20.3**	**520.8**

Inhibition zone data are mean ± SD values of three parallel experiments.
[a] DD, inhibition zone (diameter in mm) around the disks (6 mm) impregnated with 10 µL of essential oil and 300 µg per disk of hexane extract (Disk Diffusion value).
[b] Minimal inhibitory concentration as µg/mL.
[c] DD, inhibition zone in diameter (mm) around the standard. Amphotericin B (20 µg per disk)-impregnated disks were used as the positive reference standards antibiotic disk (Sigma).
[d] Amphotericin B (µg/mL) was used as the reference antibiotic in the microwell dilution assay (Sigma).

3.2. Chemical composition and antimicrobial activities of the essential oil and hexane extract of E. tenuifolia

In previous works, the characteristic compounds of *E. tenuifolia* oil were methyl eugenol, α-phellandrene, δ-3-carene and *p*-cymene [12,16-18,27-31]. Our results (Table 4) are in agreement with these previous work. In the present study, the essential oil isolated from Turkish *E. tenuifolia* contains

mainly methyl eugenol (53.0%), *p*-cymene (17.0%), α-phellandrene (13.2%) and carvacrol (3.6%). On the other hand, as can be seen from Table 4, *δ*-3-carene is not detected in this oil. Likewise, it has been found that the main components of the essential oil of Turkish *E. tenuifolia* were *δ*-3-carene (17.93%), *p*-cymene (8.99%), methyl eugenol (16.41%), and α-phellandrene (9.33%) [12]. The chemical composition of the hexane extract of *E. tenuifolia* was firstly analyzed by GC and GC-MS in the present study. The hexane extract displayed a different chemical composition and aliphatic compounds, *n*-tricosane (75.0%) and *n*-pentacosane (7.6%) were the predominant compounds. Monoterpenes, *p*-cymene (6.5%), α-Phellandrene (3.6%) and methyl eugenol (3.2%), major components of the oil are found in the low amounts in the extract.

The antimicrobial activities of the essential oil of *E. tenufolia* assayed against the microorganisms in the present study were assessed by evaluating the presence of inhibition zones and MIC values. The results are given in Table 5. The results showed that the essential oil of *E. tenufolia* had antimicrobial activity against all tested microorganisms except for *Enterobacter cloacea*. The maximal and minimum inhibition zones and MIC values for the strains were in the range of 29.93-6.56 mm, and 62.5-500 μL/mL, respectively (Table 5).

Table 4. Chemical composition of the essential oil and *n*-hexane extract of *E. tenuifolia* L.

RI[a]	Components	Oil (%)	Extract (%)	Identification methods
994	Myrcene	t	t	GC, MS, RI
1012	α-Phellandrene	13.2	3.6	GC, MS, RI
1034	*p*-Cymene	17.0	6.5	GC, MS, RI
1035	*o*-Cymene	t	-	MS, RI
1091	Terpinolene	t	-	GC, MS, RI
1095	*p*-Cymenene	t	-	GC, MS, RI
1167	*cis*-Dihydro-α-terpineol	0.7	-	GC, MS, RI
1172	Borneol	2.3	t	GC, MS, RI
1185	*p*-Cymen-8-ol	1.5	t	GC, MS, RI
1190	α-Terpineol	3.2	t	GC, MS, RI
1289	Thymol	t	t	GC, MS, RI
1296	Carvacrol	3.6	t	GC, MS, RI
1357	Eugenol	1.6	t	GC, MS, RI
1387	Isolongifolene	0.6	-	GC, MS, RI
1409	Methyl eugenol	53.0	3.2	GC, MS, RI
1526	Myristicin	2.2	-	GC, MS, RI
2011	Squalene	-	0.9	MS
2300	*n*-Tricosane	-	75.0	GC, MS, RI
2500	*n*-Pentacosane	-	7.6	GC, MS, RI
Grouped components (%)				
Monoterpene hydrocarbons			30.2	10.1
Oxygenated monoterpenes			68.1	3.2
Sesquiterpene hydrocarbons			0.6	-
Oxygenated sesquiterpenes			-	-
Others			-	83.5
Total			**98.9**	**96.8**

[a] Retention index relative to *n*-alkanes (C_8-C_{28}) on SGE-BPX5 capillary column.
GC: co-injection with standards; MS; tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data [17]; RI: identification based on comparison of retention index with those of published data [17]; t: trace (less than 0.1%).

When compared strains it was shown that *Enterobacter cloacea* was the most resistant strain and *Staphylococcus aureus* was the most sensitive bacteria (29.93 mm). Another remarkable result from this study was that gram-positive bacteria were more sensitive to the oil. DD average of Gram positive bacteria (26.13 mm) was significantly greater than gram negatives (13.19 mm) (p<0.01). Similarly, *Enterobacteriacea* (6.11 mm) was more resistant than another bacteria (20.45 mm) used in the study (p<0.01). As known *Enterobacteriacea* has a gram negative cell property. Enterobacteriacea family contains important foodborne bacterial strains as *Salmonella, E. coli* and *Klebsiella*. Furthermore, important foodborne pathogen bacteria (*S. aures, S. typhimurium, E. coli*) were inhibited by essential oil of the *E. tenufolia*. The antimicrobial activity of the essential oil isolated from *E. tenuifolia* growing in different regions of the world was previously reported and these reports showed that the essential oils have antimicrobial activities against various microorganisms [12,32-36]. Our

results are in accordance with these findings. Previously, the antimicrobial activity of the essential oil of *E. tenuifolia* L. subsp. *sibthorpiana* was tested against 13 bacterial and 2 fungal microorganisms. The lowest minimum inhibitory concentration of the oil was found to be 62.5 g/mL against *Bacillus cereus* whereas its antifungal activity was greater than 1000 g/mL for both *Candida albicans* and *Saccharomyces cerevisiae* [12]. The essential oil contain *δ*-3-carene (60.86%), α-phellandrene (7.12%), *p*-cymene (6.22 %) and myrcene (4.82%) as the predominant components was the most prominent against *E. coli* and *P. aeruginosa* and the most resistant bacterial species against the *E. spinosa* oil was the *S.aureus* [35]. However, in the current study, the essential oil was sensitive against *S. aureus*, it can be attributed to the major component, methyl eugenol (amounting to 53.0%) of the oil.

4. Conclusions

The present results showed that the essential oils of two spice plants, *C. erythraeum* and *E. tenuifolia* have antimicrobial activity at a broad spectrum of the tested microorganisms and therefore they can be used in food preservation. In particular, Psychrotrophilic bacteria are important for food preservation and the most important factor affecting the shelf life of foods stored in the cold. When Tables 3 and 5 were examined, it was seen that Psychrotrophilic bacteria such as *Pseudomonas* and *Acinetobacter* were inhibited by the essential oils of *C. erythraeum* and *E. tenuifolia*.

Table 5. Antimicrobial activities of the essential oil of *E. tenuifolia* L.

Test Bacteria	Essential oil		Antibiotics	
	DD[a]	MIC[b]	DD[c]	MIC[d]
Acinetobacter lwoffi BC 2819	25.90±0.36[c]	125	27	7.8
Enterobacter cloacea BC 3213	-	-	26	7.8
Escherichia coli BC 1818	8.10±0.15	500	14	31.25
Flavobacterium indologenes BC 1520	6.56±0.20	500	23	7.8
Klebsiella pneumoniae BC 1749	9.25±0.05	500	30	62.25
Proteus mirabilis BC 2644	16.07±0.31	250	24	125
Pseudomonas aeruginosa ATCC 9027	27.94±0.25	62.5	42	7.8
Pseudomonas fluorescens BC 7324	19.78±0.65	125	31	125
Pseudomonas putida BC 1617	11.17±0.20	250	27	125
Salmonella typhimurium RSSK 95091	7.10±0.17	500	33	7.8
Staphylococcus aureus ATCC 29213	29.93±0.63	62.5	38	7.8
Staphylococcus aureus BC 7231	27.96±0.20	62.5	13	7.8
Streptococcus pyogenes ATCC 176	20.49±0.67	250	35	7.8
Candida albicans 58	8.93 ±0.21	500	15	250

[a] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 10 µL of essential oil and 300 µg per disk of hexane extract (Disk Diffusion value).
[b] Minimal inhibitory concentration as µg/mL.
[c] DD, inhibition zone in diameter (mm) around the standard antibiotic disks; OFX5 (Ofloxacin 5 μg/disk), was used as positive reference standards antibiotic disks (Oxoid).
[d] Clarithromycin (µg/mL) was used as reference antibiotic in micro well dilution assay (Oxoid).

Author Disclosure Statement

No competing financial interests exist.

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