Antimicrobial activities of some medicinal essential oils

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S u m m a r y

In this study, the antimicrobial properties of essential oils obtained from *Coriandrum sativum, Foeniculum vulgare* Miller, *Salvia triloba, Laurus nobilis* L., *Citrus limon* and *Origanum smyrnaeum* L. were investigated. A total of eight microbial organisms belonging to six species of bacteria, namely *Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes,* as well as two fungi, *Candida albicans* and *Aspergillus niger*, were studied using a disc-diffusion and agar dilution (minimal inhibition concentration) method. The antimicrobial activity of essential oils obtained from the six plants turned out to be more effective in the case of bacteria than against fungi. The antimicrobial activity against Gram-positive bacteria was more pronounced than against Gram-negative ones. All the investigated plants are known as having healing properties and are used to treat various diseases. The essential oils obtained from *L. nobilis* and *O. smyrnaeum* showed the highest antifungal activity against *C. albicans* and *A. niger*, while the essential oils obtained from *F. vulgare* showed the highest antimicrobial activity against *P. aeruginosa* and *E. coli.* On the other hand, the essential oils obtained from *O. smyrnaeum* showed stronger antibacterial activity in the case of *E. aerogenes* and *S. aureus*, but were not equally effective against *E. coli*. The other crude essential oils showed varied levels of antibacterial and antifungal activity.
The minimal inhibition concentrations (MIC) of the essential oils obtained from *O. smyrnaeum* and of those obtained from *L. nobilis* ranged from 1.17 to 4.71 mg/ml, and 2.4 to 19.2 mg/ml, respectively.

Key words: antimicrobial activity, essential oil

INTRODUCTION

Antibacterial activity is the most widely studied aspect of essential oils. Recently, the essential oils and various plant extracts have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies in treating many infectious diseases and food preservatives, ensuring protection from the toxic effects of oxidants. In particular, the antimicrobial activities of plant oils and extracts have become the basis for many applications, including raw and processed food preservatives, pharmaceuticals, alternative and natural medicines [1].

Essential oils are complex mixtures of numerous compounds obtained from various parts of the plants. Some of the main groups of compounds found in essential oils include alcohols, aldehydes, esters, ethers, ketones, phenols and terpenes [2].

Essential oils have been used for flavouring food and beverages for hundreds of years. Due to their pleasant fragrance, many essential oils have been used to produce cosmetic products and perfumes. Antimicrobial activities of essential oils have been recognised for many years and recently have been studied extensively [3, 4, 5]. Generally, phenolics and terpenoids are the major compounds responsible for antimicrobial properties of essential oils.

Essential oils that contain high amounts of monoterpenes, eugenol, cinnamaldehyde, thymol and carvacrol have been reported to have eleven strong antibacterial properties [1]. Apart from their antimicrobial activity, essential oils and various plant extracts have been widely known as natural antioxidants. About 100 pure components of essential oils were tested for their antioxidant effectiveness and phenols were found to have the highest antioxidant activity [6].

Antifungal infections, a hot topic today, are caused by overuse and abuse of antibiotics by most medical professionals. It is commonly known that antibiotics are first and foremost microscopic fungi [7, 8]. The concentrations and ratios of active compounds in essential oils depend on the plant variety, place of origin, time of harvest, as well as processing and storage conditions [9].

Origanum majorana L. (*Lamiaceae*) grown in the south-western part of the Mediterranean region is characterised by rich oil yield with high carvacrol content, but *O. majorana* grown in the western part of Turkey is poor in oil and contains only trace amounts of carvacrol [10].

In this study, the antimicrobial properties of essential oils obtained from *Coriandrum sativum, Foeniculum vulgare, Salvia triloba, Laurus nobilis, Citrus limon* and *Origanum smyrnaeum* were investigated. A total of eight microbial organisms belonging to six species of bacteria, namely *Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes,* as well as two fungi, *Candida albicans* and *Aspergillus niger*, were studied using a disc-diffusion and agar dilution (minimal inhibition concentration) method.

Some plants that had already been studied by other researchers were also included in this study because different methods were used and different microorganisms or strains were used during the assay.

MATERIALS AND METHODS

Botanical characteristics of plants

Coriandrum sativum L. (*Umbelliferae*) is an annual herbaceous plant, 20-50 cm high, with white or pink flowers. Its fruit can be used as a spice [11]. It is found in the Balkan region and in the Aegean and Mediterranean regions of Turkey [12].

Foeniculum vulgare Miller (*Umbelliferae*) is an herbaceous plant with yellow flowers, reaching the height of 1-2 m. It is reported that the fruit can be used as a gas absorbent and an aroma source [11]. The plant is found growing wild in Germany, France, Italy, and in the Balkan region. It is also found in the Caucasus, South Asia, the Aegean, Mediterranean and Black Sea regions of Turkey [12].

Salvia triloba L. (*Salicaceae*) is a perennial shrub or herbaceous plant. The plant grows wild in central Europe and in the Balkan region, while in Turkey it is grown in gardens. It is also occasionally found near the Mediterranean coast [12].

Laurus nobilis L. (*Lauraceae*) is a shrub or wood, 2-5 metres high, an evergreen with yellow flowers [13]. The flowers can be used to extract an essential oil, and the leaves of the plant are used as a spice. It is grown in Spain, France, Italy, Greece and Turkey [12].

Origanum onites L. (syn. *smyrnaeum* L.) from the *Labiatae* family is a hermaphrodite plant, 40-50 cm high, with white flowers. Its flowers and leaves can be used as a medicine [13]. The plant grows in the Aegean and Mediterranean regions of Turkey [12].

Citrus limon (L) Burm. (*Rutaceae*) is a small tree, 3-5 metres high, with whitish or pink aromatic flowers. It is also an evergreen, originating from China and India, grown in the Mediterranean region of Turkey [13].

Determination of essential oil content of plant material and oleorensis

A laboratory distillation of essential oil from plant material is often necessary to evaluate the raw material that is to be used for large–scale commercial distillations. Determining the essential oil content is also important when assessing the quality of spices and oleoresins.

Such determinations may be conveniently carried out in a special apparatus designed by Clevenger [14]. This apparatus offers following advantages: compactness, cohobation of distillation waters, the actual distillation and separation of the essential oil (so that certain chemical and physical properties may be determined, and so that the odour and flavour of the oil may be studied) and an accurate determination of the essential oil content. The huge advantage of the method is that only small quantities of plant material are used. Furthermore, the apparatus may be used for steam rectification of small amounts of essential oils. The apparatus consist of traps and a small cold-finger type condenser. The trap is supplied for an oil lighter than water.

Test strains and culture media

Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, Maryland). Antimicrobial activities of six crude essential oil samples obtained from the studied plants were assayed against *Enterobacter aerogenes* (ATCC13048), *Salmonella typhimurium* (CCM5445), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Candida albicans* (ATTC 60192) and *Aspergillus niger* (no number). The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Brot (Merck). *C. albicans* and *A. niger* were grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentrations of bacterial suspensions were adjusted to 10^8 cells/ml, while those of fungal suspensions to 10^7 cells/ml.

Antimicrobial assay

Antifungal activity was measured using the method of disc diffusion agar [15]. In order to test antifungal activity, the fractions of the crude essential oil samples were dissolved to 70%. 20 ml of Sabouraud Dextrose Agar (Oxoid) were poured into each 15 cm Petri dish. *C. albicans* and *A. niger* were grown in Sabouraud Dextrose Broth (Difco) at 27°C for 48 hours. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Sabouraud Dextrose Broth (Difco). Mueller Hinton Agar (Merck) and Mueller Hinton Brot (Merck) was used in the case of bacteria. Then 100 μl of each suspension with approximately 10^8 bacteria or 10^7 fungi per millilitre were placed in Petri dishes over agar and dispersed. Then, sterile paper discs (6 mm in diameter) were placed on agar to put a 15 μl sample of each crude essential oil. One hundred units of nystatin were used as a positive control, while in the case of bacteria amoxicillin and cefazolin were used as a positive control and alcohol as a negative control. Inhibition zones were determined after incubation at 27°C for 48 hours. All the tests were done in triplicate.

Microdilution assay

The agar dilution method, described by Vander Berghe and Vietinck [16], was used with slight modifications for the antibacterial screening. Instead of 96-well microtiter plates 24-well Corning plates were used. The samples of crude essential oils were dissolved in 70% ethanol and physiological Tris buffer (Amresco 0826-500G) 1:4 and mixed with an equal amount of 3% agar solution (Sabouraud Dextrose Agar (Oxoid) in the case of fungi, while in the case of bacteria with Mueller Hinton Agar (Merck) at 45°C. Each crude essential oil sample was tested in concentrations of 200, 100, 50, 25, 12.5 and 6.25 μl/mg. From the test solutions 400 μl were transferred into each well of the tissue culture plate. After solidification each well was inoculated with 10 μl of freshly prepared suspension of 10^8 bacteria and 10^7 fungi per 1 ml and incubated at 37°C for 24 hours. In the case of bacteria as

positive control amoxicillin and cefazolin (200, 100, 50, 25, 12.5 and 6.25 μl/mg) and in the case of fungi nystatin were used as positive control, and 70% alcohol as negative control. The bacterial and fungal growth was assessed with a stereomicroscope after the incubation period. All the tests were done in triplicate.

RESULTS AND DISCUSSION

The results of the antibacterial and antifungal screening of samples of essential oils obtained from different plants are reported in Table 1. Antimicrobial activity of essential oils obtained from *C. sativum, F. vulgare, S. triloba, L. nobilis, C. limon* and *O. onites* against various pathogenic fungi and bacteria were investigated. The crude essential oil sample obtained from *C. sativum* showed antibacterial and antifungal activity (7-20 mm/15μl inhibition zone) against the test organisms. It did not show, however, antifungal activity against *A. niger*. On the other hand, the oil obtained from *C. sativum* showed the highest antibacterial activity (18mm/15 μl inhibition zone) against *S. aureus* and against *E. coli* (20mm/15 μl inhibition zone). The crude essential oil sample obtained from *F. vulgare* showed antibacterial and antifungal activity (8-25 mm/15 μl inhibition zone) against the test organisms. On the other hand, this oil showed the highest antibacterial activity against *P. aeruginosa* and *E. coli* (18 and 25 mm/15 μl inhibition zone, respectively). This oil also showed antifungal activity against both fungi (12 mm/15 μl inhibition zone).

Table 1.

Results of antimicrobial screening of the crude essential oils from six plants determined by the agar-well diffusion method (minimum inhibitory concentration, MIC, in mg/ml) and agar diffusion method (inhibition zone in mm).

Plant species and family	Inhibition zone (mm) microorganisms								MIC (mg/ml) microorganisms							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Coriandrum sativum (*Umbelliferae*)	7	18	10	12	20	12	10	-	17.26	1.07	8.63	4.35	2.15	4.315	4.31	-
Foeniculum vulgare (*Umbelliferae*)	8	16	12	18	25	10	12	12	19.56	4.88	9.76	9.76	1.22	4.88	4.88	9.76
Salvia triloba (*Salicaceae*)	7	35	10	11	22	7	15	13	18≥	2.29	18.38	9.91	4.59	11.4	2.29	2.29
Laurus nobilis (*Lauraceae*)	12	7	20	11	9	20	18	15	9.6	19≥	2.4	19.2	19≥	2.4	2.4	2.4
Citrus limon (*Rutaceae*)	12	10	25	13	7	13	20	8	4.25	17≥	1.06	2.12	17.02	4.25	1.06	17.02
Origanum onites (*Labiatae*)	17	25	18	7	-	35	25	13	2.35	1.17	2.35	-	4.71	4.71	1.17	4.71
amoxicillin	32	39	33	34	35	33	ND	ND	-	-	-	-	-	-	-	-
cefazolin	28	36	30	23	30	29	ND	ND	-	-	-	-	-	-	-	-
nystatin	ND	ND	ND	ND	ND	ND	16	15	-	-	-	-	-	-	-	-

-: no inhibition; ND: not detected; 1 - *Salmonella typhimurium*; 2 - *Staphylococcus aureus*; 3 - *Staphylococcus epidermidis*; 4 - *Pseudomonas aeruginosa*; 5 - *Escherichia coli*; 6 - *Enterobacter aerogenes*; 7 - *Candida albicans*; 8 - *Aspergillus niger*.

The crude essential oil sample from *S. triloba* showed antibacterial and antifungal activity (7-35 mm/15 μl inhibition zone) against the tested organisms. This oil showed the highest antibacterial activity (22-35 mm/15 μl inhibition zone) against *E. coli* and *S. aureus*. In the case of this oil the highest antifungal activity (15 mm/15 μl inhibition zone) was observed against *C. albicans*. The crude essential oil from *S. triloba* and *L. nobilis* showed antibacterial and antifungal activity (7-20 mm/15 μl inhibition zone) against the test organisms. This oil showed the highest antibacterial activity (22-35 mm/15 μl inhibition zone) against *E. coli* and *S. aureus*. However, this oil showed weak antibacterial activity (7-9mm/15 μl inhibition zone) against *S. aureus* and *E. coli*. This oil showed almost weak antifungal activity (18-15mm/15 μl inhibition zone) against both tested fungi. The crude essential oil from *Citrus limon* showed antibacterial and antifungal activity (7-25 mm/15 μl inhibition zone) against the tested organisms. This oil showed the highest antibacterial activity (25 mm/15 μl inhibition zone) against *S. epidermidis,* while only weak antifungal activity (8 mm/15 μl inhibition zone) against *A. niger*. This oil showed the highest antifungal activity (20 mm/15 μl inhibition zone) against *C. albicans*.

The crude essential oil sample from *O. onites* showed antibacterial and antifungal activity (7-35 mm/15 μl inhibition zone) against all the tested organisms except for *E. coli*. Still, this oil showed the highest antibacterial activity (25-35 mm/15 μl inhibition zone) against *S. aureus* and *E. aerogenes*.

Evaluation of MICs of the essential oils obtained from some plants by means of agar dilution experiment method is reported in Table 1. The crude essential oil sample from *C. sativum* required an MIC of 1.07mg/ml for *S. aureus*, and of 4.31 mg/ml for *C. albicans*. No concentration of this oil was observed to inhibit the growth of *A. niger*. The crude essential oil sample from *F. vulgare* required an MIC of 4.88 mg/ml for *S. aureus*, *E. aerogenes* and *A. niger*, and a lower MIC value (1.22 mg/ml) against *E coli*. The crude essential oil sample from *S. triloba* required an MIC of 2.96 mg/ml for *A. niger* and *C. albicans*. Lower MIC values (1.14 mg/ml) were required against *E. aerogenes,* and the highest MIC value (1.14 mg/ml) was needed against *S. epidermidis*. The crude essential oil sample from *L. nobilis* required an MIC of 2.4 mg/ml for *C. albicans, A. niger*, *E. aerogenes* and *S. epidermidis*, but no concentration of this essential oil inhibited the growth of *S. aureus* and *E. coli*. The crude essential oil sample from *C. limon* required an MIC of 1.06 mg/ml for *S. epidermidis* and *C. albicans*, but no concentration of this essential oil inhibited the growth of *S. aureus*. The average MIC value obtained for *C. limon* against *A. niger*, *E. aerogenes* and *E. coli* was 4.71 mg/ml.

In recent years, although technology and medicine have developed extensively, due to decrease in natural richness and the drawbacks some countries have made it obligatory to use the natural products for many goals. For these reasons, like other countries in the world, in Turkey too, plants known by people are picked up and used for treating of various diseases.

The oregano essential oil inhibits growth of Gram-positive and Gram-negative bacteria, as well as fungi and moulds [5, 17, 18]. The basil essential oil (distilled

from *Ocimum basilicum* L.) contains methyl chavicol and linalool as the basic compounds. The basil essential oil has been reported to be an antimicrobial against a variety of Gram-positive and Gram-negative bacteria, as well as yeasts and moulds [19]. It was found that 10 μL of pure essential oil of anise, basil, and coriander added in wells of the iso-sensitest agar did not show inhibitory effects against *E. coli* after a 48-hour incubation at 25°C [20-21]. However, most researchers have recognized oregano EO as one of the oils with the strongest bacteriostatic and bactericidal properties towards *E. coli* and *L. monocytogenes* [22-23].

The major compounds of oregano EO are carvacrol and thymol. However, the concentrations and ratios of the active compounds depend on the plant variety, place of origin, time of harvest, as well as on the processing and storage conditions. For example, the oregano variety *Origanum vulgare* harvested from the northern parts of Greece was rich in thymol (30.3% to 42.8%) and had low carvacrol amounts (1.7% to 2.5%), whereas that from the southern parts was rich in carvacrol (57.4% to 69.6%) but had only from 0.2% to 4.1% of thymol [24]. Antimicrobial activity of commercial cardamom, cinnamon, cumin and nutmeg oils against various pathogenic fungi and bacteria were investigated in a number of previous papers [15-17].

The activity of some crude extracts used in the study was similar to that of cefozin and amiktodolin (10mg/ml) against the tested organisms. However, the antifungal activity of some crude plant extracts was stronger than in the case of the standard antifungal (100 units of nystatin) against *C. albicans* and *A. niger*.

In this study, the antimicrobial activity against bacteria and fungi of the crude essential oils obtained from six plants has been determined. The plants are known to have healing properties and are used for treating various diseases affecting people. The antimicrobial activity of essential oils of six plants against bacteria was more effective than against fungi, which is similar to the results of some previous studies [25, 26, 27]. The antimicrobial activity against Gram-positive bacteria was more pronounced than against the Gram-negative ones, which is in accordance with the results reported [28, 29]. The fraction 6 and 7 of crude extract from *Viscum album* were found to be active against *P. aeruginosa, S. aureus, B. subtilis, E. coli* and *C. albicans* [30].

The results indicated that each crude essential oil from six plants exhibited more or less pronounced antibacterial and antifungal potencies in the case of both Gram-positive and Gram-negative bacteria and fungi. Especially, the crude essential oil samples from *S. triloba, O. onites* and *L. nobilis* oils showed antibacterial and antifungal activity against the tested organisms. As a consequence of this study, we will try to isolate compounds responsible for the antimicrobial activity observed in the case of the crude oil showing the largest inhibitory activity against bacteria and fungi.

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DZIAŁANIE PRZECIWDROBNOUSTROJOWE WYBRANYCH OLEJKÓW ROŚLINNYCH

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S t r e s z c z e n i e

Podczas relacjonowanych tu badań analizowano przeciwdrobnoustrojowe własności olejków pozyskiwanych z *Coriandrum sativum, Foeniculum vulgare, Salvia triloba, Laurus nobilis, Citrus limon* oraz *Origanum smyrnaeum*. Badano łącznie osiem mikroorganizmów należących do sześciu gatunków bakterii, mianowicie *Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Escherichia coli* oraz *Enterobacter aerogenes*, a ponadto dwa grzyby, *Candida albicans* i *Aspergillus niger*. Wykorzystano metodę płytkową oceny wartości MIC. Olejki pozyskane z sześciu badanych roślin wykazywały silniejsze działanie w wypadku bakterii niż grzybów. Wyraźniej widać było działanie na bakterie Gram-dodatnie niż na Gram-ujemne. Wszystkie badane rośliny są znane z właściwości zdrowotnych i są wykorzystywane do leczenia różnych chorób. Olejki pozyskane z *L. nobilis* oraz *O. smyrnaeum* wykazywały najsilniejsze działanie na grzyby *C. albicans* oraz *A. niger*, a olejek otrzymany z *F. vulgare* wykazywał najwyższe działanie w wypadku *P. aeruginosa* oraz *E. coli.* Olejki pozyskane z *O. smyrnaeum* działały silniej na *E. aerogenes* i *S. aureus*, nie wykazały natomiast zbliżonego działania w wypadku *E. coli*. W wypadku pozostałych olejków otrzymano różne wartości. Wartości MIC olejków otrzymanych z *O. smyrnaeum* oraz *L. nobilis* wahały się odpowiednio od 1,17 do 4.71 mg/ml oraz od 2,4 to 19,2 mg/ml.

Słowa kluczowe: działanie przeciwdrobnoustrojowe, olejki roślinne