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Antimicrobial Activity and Chemical Composition of Some Essential Oils

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In this study the composition and antimicrobial properties of essential oils obtained from *Origanum onites, Mentha piperita, Juniperus exalsa, Chrysanthemum indicum, Lavandula hybrida, Rosa damascena, Echinophora tenuifolia, Foeniculum vulgare* were examined. To evaluate the *in vitro* antibacterial activities of these eight aromatic extracts; their *in vitro* antimicrobial activities were determined by disk diffusion testing, according to the NCCLS criteria. *Escherichia coli* (ATTC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATTC 27853 were used as standard test bacterial strains. *Origanum onites recorded* antimicrobial activity against all test bacteria, and was strongest against *Staphylococcus aureus*. For *Rosa damascena, Mentha piperita* and *Lavandula hybrida* antimicrobial activity was recorded only to *Staphylococcus aureus*. *Juniperus exalsa, and Chrysanthemum indicum* exhibited antibacterial activities against both *Staphylococcus aureus* and *Escherichia coli*. We also examined the *in vitro* antimicrobial activities of some components of the essential oils and found some components with antimicrobial activity.

Key words: Antimicrobial activity, Essential oils, Chemical composition, Disk diffusion, Capillary Gas Chromatography, Plant extracts

INTRODUCTION

Botanical extracts have long been used to treat disease, and plant sourced materials play a major role in primary health care in many developing countries. Moreover, the screening of such plant extracts for antimicrobial activity has always been of great interest to scientists looking for new sources for drugs for the treatment of various diseases (Oka *et al.*, 2000; Sökmen *et al.*, 2000).

Essential oils are generally used in the cosmetic, medical and food industries. Moreover volatile compounds obtained from plants, have known antimicrobial, antifungal and insecticidal activities (Janssen *et al.*, 1987; Kurita *et al.*, 1981; Oka *et al.*, 2000).

These essential oils are also called volatile oils, and are generally aromatic oils obtained by the steam or hydro distillation of plants. Essential oils are the odorous principles found in the various parts of plant, and when exposed to the air at ordinary temperatures they substantially evaporate, which also explains the use of terms like volatile oils or ethereal oils Essential was chosen to describe these oils because they represent the "essence" or characteristic odour of a plant (Tanker *et al.*, 1990). Different parts of plants have been used to obtain essential oils. These include the flowers, leaves, seeds, roots, steams, bark, and wood though secretionary parts, such as, the internal multicellular cavities and secretionary ducts are of particular value.

They are chemically complex mixtures, and are generally composed of more than a hundred or so components. However, the number of components that are actually responsible for the characteristic odors and flavours are limited.

Essential oils are composed mainly of *terpenes* and *phenilpropens,* but the majority of essential oils contain predominantly (90%) *terpenes*. These *terpenes* contain mono *terpenes* with ten carbons, *sesquiterpenes* with fifteen carbons and *diterpenes* with twenty carbons (Hay *et al.*, 1993; Zakaria 1991).

Essential oils have many therapeutic effects, which

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include vasodilatation, irritation, hypersecretion (in saliva and sweat secretions), hyperperistaltism, the stimulation of heart muscle, and they aid the distribution of drugs and antiseptics (Palevitch *et al.*, 1991). Their most important characterstics are their anti- infection, antibacterial, antifungal, allelopatic, and antioxidative effects (Altanlar *et al.*, 1999; Larssen *et al.*, 1987; Kurita *et al.*, 1981). Today, most of the volatile oils are also used as insecticides, fungicides, and herbicides, and for their nematocidal activity.

We undertook to investigate the antimicrobial activities of volatile oils extracted from seven different plants and *Chrysanthemum indicum*. In the hope that we would identify an ibacterial or antiseptic effects, or chemical species of pharmacological interest

MATERIALS AND METHODS

General

The composition and antimicrobial properties of essential oils obtained from different plants were investigated

Plant materials

Origanum onites, Mentha piperita, Juniperus exalsa, Chrysanthemum indicum, Lavandula hybrida, Rosa damascena Echinophora tenuifolia, Foeniculum vulgare ssp were collected in the Isparta region. Specimens were deposited at the Department of Field Crops, Faculty of Agriculture, and Süleyman Demirel University-Isparta-Turkey.

Extraction and isolation

The essential oils were freshly extracted from the appropriate plant organs by Clevengers steam distillation and the amount extracted is expressed a percentage on a v/w basis. In the case of *Chrysanthemum indicum* only the substrate was examined.

Natural volatile oils were extracted from various parts of plants. For *Origanum onites, Mentha piperita, Echinophora tenuifolia* the leaves, for *Lavandula hybrida, Rosa damascena, and Chrysanthemum indicum* the flowers, and for *Juniperus exalsa* and *Foeniculum vulgare* the fruit and semens were used, respectively.

Capillary gas chromatography conditions

The chemical compositions of the aromatic extracts obtained were determined by capillary gas chromatography (GC) (Başer 1993; Hethelyi *et al.*, 1990; Warner *et al.*, 1997) using a Fractovap Carlo Erba 4300 with a FID detector, fitted with an MN-DB-23 column (60 m× 0.25 m).

Investigations were carried out using the following QC parameters.

Detector temperature : 240°C
Injector temperature : 250°C
Over programme : 120°C/3 min//3°C/min//
Column programme temperature : 200°C/6 min//3°C/ min, 120°C/3 min
Gas flows : air 400 ml/min, H_2 40 ml/min
Carrier gas : He
Split flow : *1/50 ml/min*
Syringe capacity (sample injected) : 1 µl
Integrator : Shimatzu C R3A

Individual components of the essential oils were tentatively identified by matching retention times with those of authentic compounds. The relative percentages of components of essential oils on were calculated by a computer program (Turbo Crom. Navigator, USA).

Determination of antimicrobial activity

The antimicrobial activities of aromatic extracts and volatile oils were determined using the disk diffusion method, according to NCCLS criteria (Isenberg 1995; NCCLS 1990; NCCLS 1991).

Test microorganisms

Escherichia coli (E. Coli) (ATTC 25922), *Staphylococcus aureus (S. aureus)* (ATCC 25923) and *Pseudomonas aeruginosa (P. aeruginosa)* (ATTC 27853) were used as standard test bacterial strains.

Preparation of a bacterial suspension

For the inoculum, colonies were selected from 18-24 h old plates. Turbidity was visually adjusted to that of a 0.5 Mc Farland turbidity standard (1.5×10^8 CFU/ml) (Murray *et al.*, 1999) using sterile Mueller-Hinton broth and after vortexing.

Inoculation of agar plates

The inoculums were swabbed onto the surface of Mueller-Hinton agar plates three times, by rotating the plates approximately 60° with a sterile cotton swab. Inoculated plates were allowed to stand for at least 3 minutes before applying antimicrobial disks.

Laboratory preparation of antimicrobial disks

Sterile filter paper disks were prepared to a diameter of 6.35 mm and sterilized in a Pasteur – oven, (at 170°C for 2 hour). Volatile oils and extracts were sterilized by passing through 0.22 mm pore-size membrane filters and then 20 µl (0.02 ml) (Isenberg 1995) of the solution of volatile oils was pipetted into the center of each disk to achieve the desired potency. Disks were air-dried in a contamination free environment.

Application of disks

The antimicrobial disks were applied to a Mueller-Hinton

agar plate surface by using a sterile forceps, which was also used to apply gentle pressure to ensure complete contact of disk with the agar. Disks were not placed closer to each other than 24 mm, measured from center to center. Plates were incubated at 37°C for 18-24 h. After overnight incubation, the diameter of the zone of inhibition around each disk was measured in mm. Disk diffusion tests were performed in duplicate.

RESULTS AND DISCUSSION

The compositions of the essential oils were determined by gas chromatography (GC). On the basis of the GC retention times components of the seven essential oils their compositions were determined and are summarized in Table I.

The antimicrobial activities of the extracts of *Chrysanthemum indicum* and of the essential oils of the seven other different plants against *E. coli, P. aeruginosa* and *S. aureus* were investigated and the results are presented in Table II.

According to the GC investigation of the essential oils, it was found that the sample obtained from the *Origanum onites* contained *carvacrol* principal component, which amounted to 78.2%. *Origanum onites* was found to have potent antimicrobial activity against all of the reference strains (*E. coli, P. aeruginosa S. aureus*).

Biondi *et al.* (1993) demonstrated that the volatile oils of *Origanum onites* and of *Thymus capitatus* have both antibacterial and antifungal activity. They also showed that the volatile oils of *Origanum onites,* contain 61.7% carvacrol, and have antibacterial activity against *E. coli, P. aeruginosa* and *S. aureus*. On the other hand, Deans *et al.* (1990) found that volatile oil of *Origanum majorana* had no antibacterial activity against reference strains of *S. aureus*. We propose that this discrepancy is due to species differences. We also found that the volatile oils extracted from *Rosa damascena* (second oil), *Mentha piperita* and *Lavandula hybrida* have antimicrobial activity only against *S. aureus*, whereas *Juniperus exalsa* and *Chrysanthemum indicum* showed the same level of activity against both *E.coli* and *S. aureus*. The substrate (not the essential oil extract) of *Chrysanthemum indicum* showed potent antibacterial effects against *E.coli* and *S. aureus*.

In the present study, the main components of some essential oils with antibacterial activity were tentatively identified by capillary gas chromatography. The names of

Table I. On the basis of GC data, the essential oils contents and compositions of the specimens

Botanical name of plants	% (v/w)	Compositions (%)
Origanum onites	2.5	Carvacrol (%78.2), γ terpinene (%4.3), borneol (%8.1), cineole (%1.5), β pinene (%1.1), linalyl acetate (%1.0), myrcene (%0.5)
Rosa damascena	0.02	First oil: Citronellol (%10.3), geraniol (%2.8), nerol (%1.3), linalool (%0.6) Second oil:Citronellol (%46.7), geraniol (%23.3), nerol (%11.9), linalool (%0.8)
Mentha piperita	2.5	Menthon (%44.1), menthol (%29.5), menthylacetate (%3.8), menthofuron (% 0.9)
Echinophora tenuifolia	1.6	α phelladrene (45.9), m-eugenol (%29.5), p-cymene (%24.4)
Lavandula hybrida	6.1	Linalool (%32.8), linalyacetate (%29.9), citronellol(%6.7), camhor (%5.3), α-terpineol(%2.8), ocimene (%2.5), bisabolone (%1.9), 1.8-cineole (%1.8), borneol (%1.6), geraniol (%1.4), α-humulene (%1.0)
Foeniculum vulgare	4.5	Anethol (76.4), limonene (%7.7), fenchone (%3.3), estragole (%3.2) 1.8-cineole (%1.6), anisketone(%1.2), γ-terpinene (%0.9), α-pinene (%0.3)
Juniperus exalsa	1.9	Karen (%29.1), α-pinene (%26.8), limonene (%25), sabinene (%9), β pinene (%3.3)

Table II. Antimicrobial activity of essential oils and the extracts of plants (Zone of inhibition in mm)

Botanical name of the plants	Standard test bacteria		
	E. coli	*S. aureus*	*P. aeruginosa*
Origanum onites (essential oil)	25 mm	35 mm	15 mm
Rosa damascane (essential oil)	–	8 mm	–
Mentha piperita (essential oil)	–	12 mm	–
Echinophora tenuifolia (essential oil)	–	–	–
Lavandula hybrida (essential oil)	–	8 mm	–
Juniperus exalsa (essential oil)	18 mm	32 mm	–
Chrysanthemum indicum	26 mm	32 mm	–

Table III. Main components of some essential oils with antibacterial activity

Botanical name of the plants		Main component (%)
Origanum onites		Carvacrol (78.2%)
Rosa damascena	First oil	Citronellol (10.3%)
		Geraniol (2.8%)
	Second oil	Citronellol (46.7%)
		Geraniol (23.3%)
Mentha piperita		Menthon (44.1%)
		Menthol (29.5%)
Lavandula hybrida		Linalool (32.8%)
		Linalyl acetate (29.9%)
Juniperus exalsa		Karen (29.1%)

the components and their percentages are presented in Table III, and the antimicrobial activities of some of these components were examined independently. The results were presented in Table IV.

According to the GC analysis, the volatile oils of *Origanum onites* contained *carvacrol* as a principal component at 78.2%. *Cineole* and *γ-terpinene* showed no antimicrobial activity and their percentage compositions were 4.3% and 1.5% respectively (Table III). Their inhibition zones are presented in Table IV. We suggest that *carvacrol*, as the most prominent volatile component in *Origanum onites*, also contributes most to its antibacterial activity (Deans *et al.*, 1990; Oka *et al.*, 2000). *Rosa damascena is* found only in the Isparta region in Turkey. The important components of *Rosa damascena* were *citronellol, geraniol* and *nerol* and their corresponding percentages were 10.3%, 28%, and 1,3% in first oil (direct oil obtained from fresh flowers) and 46,7%, 23,3%, 11,9% in second oil (redistillated oil of the first oil), respectively.

We found that *citronellol, geraniol* and *nerol* have more potent antimicrobial activity individually than in the mixture form. Because in the compound form of *Rosa damascena* antimicrobial activity was determined only against *Staphylococcus aureus* strains and the inhibition zone was 8 mm, whereas in *geraniol* and *nerol* antimicrobial activity was determined against both *Staphylococcus aureus* and *Escherichia coli strains. Their* inhibition zone diameters were 21 mm, 19 mm, 12 mm and 14 mm, respectively (Table IV). The essential oils of *linalool* (32.8%) and *linalyl acetate* (29.9%) in *Lavandula hybrida* proved to be very effective and to have potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli strains*. We found that *menthon* and *menthol* in *Mentha piperita* were ineffective antimicrobials. Therefore, we suggest that *menthon* and *menthol* are effective in the mixture form.

Table II. Antimicrobial activity of some components of essential oils (Zone of inhibition in mm)

Components	Standard test bacteria		
	E. coli	*S. aureus*	*P.aeruginosa*
Rosa damascena			
Citronellol	–	20	–
Geraniol	12	21	–
Nerol	14	19	–
Lavandula hybrida			
Linalyl acetate	–	12	–
Linaloo	19	20	–
Origanum onites	–	–	–
Cineole	–	–	–
γ terpinene	–	–	–
Mentha piperita	–	–	–
Menthon	–	–	–
Menthol	–	–	–

Our results suggest that essential oils have potential use as antimicrobials. Essential oils and main components of some of these oils, such as *carvacrol, citronellol, geraniol, and nerol* have been previously reported to have antibacterial activity (Janssen *et al.*, 1987).

The intensive use of antibiotics has often resulted in the development of resistant strains. Because of this drug resistance, the search for new antibiotics continues unabated. In this context plants continue to be a rich source of therapeutic drugs, as the active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution made by plants to the drug industries has been made possible by the large number of biological studies undertaken (Sidney *et al.*, 1980; Srinivasan *et al.*, 2001).

Therefore, further experiments are needed to more fully evaluate the antibacterial and antifungal activity of the components of essential oils.

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