SCREENING OF THE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF EIGHT VOLATILE ESSENTIAL OILS

ELENA S. ŞERBAN[1*], MIHAELA IONESCU[2], DOINA MATINCA[2], CODRUŢA S. MAIER[1], MARIUS T. BOJIŢĂ[1]

University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca,
[1]Department of Drugs Control, [2]Department of Microbiology,
Louis Pasteur 6, 400349, Cluj-Napoca, Romania
[*]corresponding author: sseptimia@yahoo.com

Abstract

The aim of this study was to investigate the antibacterial and antifungal activity of eight essential oils. The methods used were agar diffusion (well and disc diffusion techniques) and microatmosphere methods. All oils showed antibacterial and particularly high antifungal activity against tested strains.

Rezumat

Scopul acestui studiu a fost de a investiga acţiunea antibacteriană şi antifungică a opt uleiuri esenţiale. Metodele utilizate au fost metoda difuziei pe agar (tehnicile cu godeuri şi discuri) precum şi metoda microatmosferei. Toate uleiurile testate au demonstrat o bună activitate antibacteriană şi mai ales antifungică faţă de tulpinile testate.

Keywords: antibacterial activity, antifungal activity, essential oils

Introduction

The antimicrobial activity of different essential oils is known for many centuries. In the last years, a large number of essential oils and their constituents were investigated for their antimicrobial properties against different bacteria and fungi strains [1, 5]. The assessment of both antibacterial and antifungal activities is achieved with the agar diffusion and dilution methods [2, 3, 4, 5]. Based on the high volatility of essential oils, a so-called microatmosphere method was developed for the assessment of essential oil activity in vapour phase [5, 6].

Materials and methods

Essential oils

The essential oils used in this study were commercial samples of the following essential oils: coriander oil (*Coriandrum sativum* L.), fennel oil (*Foeniculum vulgare* Mill.), dill oil (*Anethum graveolens* L.), peppermint oil (*Mentha piperita* L.), fir oil (*Abies alba* Mill.), juniper oil (*Juniperus communis* L.), rosemary oil (*Rosmarinus officinalis* L.), and lavender oil (*Lavandula hybrida*).

Strains and culture media

The essential oils were tested on the following strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 10231. Bacterial strains were cultured on Muller Hinton agar and *C.albicans* was cultured on Sabouraud agar.

Experimental method

Standardisation of inoculum

Sterilized nutrient broth was inoculated with the test bacteria and incubated at 37°C overnight. The obtained inoculum was diluted as follows: *E. coli* (1:1000), *S. aureus* (1:200) and *C. albicans* (1:10).

Antibacterial and antifungal assay

The antibacterial and antifungal activity was evaluated as in traditional antibiotic susceptibility testing using the disc diffusion method [2]. The *aromatogram method* [4] is an analytical method similar with the disc diffusion assay. The sterile paper discs of 6 mm in diameter were impregnated with 5 µL essential oil and deposited on the agar surface (Figure 1). Penicillin, erythromycin, chloramphenicol, co-trimoxazole (Biseptol®), ciprofloxacin, ampicillin, amphotericin B and 5-fluro-cytosine were used as controls.

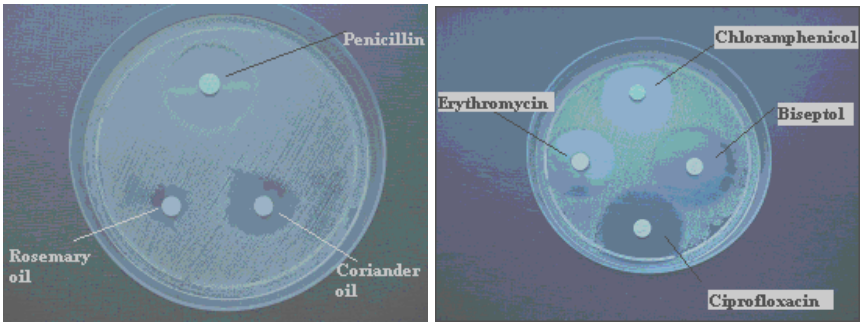

Figure 1

Inhibition zones for testing *Staphylococcus aureus* by the aromatogram technique: (left) - Penicillin, coriander oil, rosemary oil; (right) Chloramphenicol, Trimethoprim+Sulfamethoxazole (co-trimoxazole - Biseptol®), Ciprofloxacin, Erythromycin.

For the agar diffusion method [5], wells were made in the agar medium and filled with 200 µL essential oil (Figure 2). The essential oils were used undiluted and diluted 1:10 with *n*-hexane as dispersing solvent.

The microatmosphere method consists of the qualitative observation of antibacterial/antifungal activity [5, 6]. A sterile filter paper disc moistened with essential oil or its solution is attached to the lid of a Petri dish containing the culture medium and seeded uniformly with bacterial or

fungal strains. The dish was reversed and placed on the upper lid containing the filter paper and then sealed. The Petri dish prepared as described was incubated at 37°C overnight. Control plates without essential oil or moistened only with *n*-hexane as solvent were used as references (Figure 2).

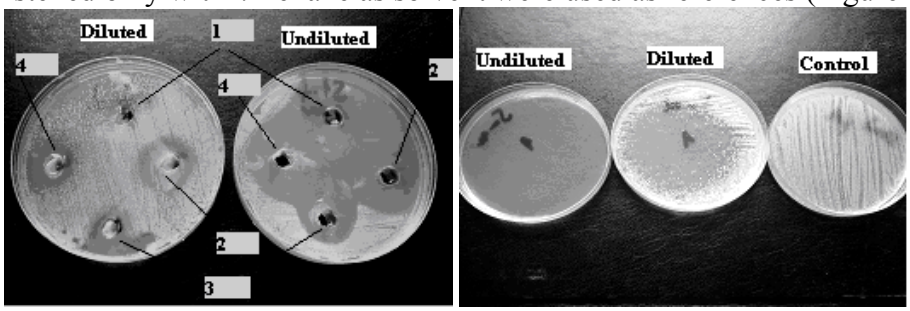

Figure 2

Inhibition zones for testing *Staphylococcus aureus*: (left) - agar diffusion method (well) for peppermint (1), lavender (2), fennel (3), dill (4) oils - comparison diluted – undiluted; (right) - microatmosphere method for peppermint oil – comparison undiluted – diluted – control.

Results and discussion

The chemical composition of the essential oils was determined using GC-MS (gas cromatography – mass spectrometry) technique [7, 8]. The main compounds identified are presented in Table I. The area of each constituent was calculated by normalisation.

All tested essential oils exhibited antibacterial and antifungal activity (Table II, III) with the screened methods.

The solvent *n*-hexane was not suitable for testing the antimicrobial activity against *E.coli*. The results showed an inhibition activity against *E.coli* while no inhibition was observed against *S. aureus* when only *n*-hexane was used for testing. (Table III, entry 9) For this reason the results obtained for diluted oil against *E. coli* (see Table III, column 4, dilution 1:10) were not taken into consideration for conclusions.

Table I

Chemical composition of essential oils

Essential oil	Main components	% Area
Lavender	β-Linalool Linalyl acetate	32.39% 31.03%
Coriander	β-Linalool P-Cymene α-Pinene	50.35% 28.81% 11.99%
Fennel	L-Fenchone Anethole α-Pinene	48.53% 26.76% 10.3%

Table I (continued)
Chemical composition of essential oils

Rosemary	Eucalyptol α-Pinene Camphor β-Pinene	36.3% 15.34% 12.52% 10.96%
Fir	β-Pinene α-Pinene Camphene Limonene	30.94% 26.96% 13.54% 12.36%
Peppermint	Menthone Menthol Eucalyptol	31.01% 19.56% 9.62%
Dill	α-Phellandrene Carvone Limonene	41.5% 22.99% 22.24%
Juniper	α-Pinene o/m-Cymene	60.98% 16.18%

All tested essential oils exhibited antibacterial and antifungal activity (Table II, III) with the screened methods.

The solvent *n*-hexane was not suitable for testing the antimicrobial activity against *E.coli*. The results showed an inhibition activity against *E.coli* while no inhibition was observed against *S. aureus* when only *n*-hexane was used for testing. (Table III, entry 9) For this reason the results obtained for diluted oil against *E. coli* (see Table III, column 4, dilution 1:10) were not taken into consideration for conclusions.

A high activity against *E.coli* was found for coriander (Table III, entry 1), peppermint (Table III, entry 4), and juniper essential oils (Table III, entry 6) based on agar well diffusion test. When disc diffusion test was used, the most active oils against *E.coli* were coriander (Table II, entry 1), rosemary (Table II, entry 7), and juniper (Table II, entry 6). The most active essential oil at a dose of 5 µL was coriander oil (Table II, entry 1). The inhibition zone for coriander oil was smaller than those of the antibiotics tested (Table II, entries 9-12). The microatmosphere test indicated coriander, peppermint, and lavender oils as highly active (results not shown here). The results revealed that lavender oil had a major contribution to the vapour activity, while juniper oil had significant diffusion activity.

An inhibition effect on *S. aureus* was found for coriander (Table III, entry 1), fir (Table III, entry 5), juniper (Table III, entry 6), and rosemary oils (Table III, entry 7) using the agar diffusion method – well technique. Only five oils were still active against *S. aureus* at the 5 µL dose. The highest activity was observed for coriander oil (Table II, entry 1) although

the efficiency was lower than for control antibiotics, at the specified doses (Table II, entries 9, 10, 12, 13, 14). The relationship dose – activity followed a similar trend, except peppermint and rosemary oils at dilution 1:10 (Table III, entries 4, 7). The differences observed may be explained by the diffusion suitability of tested oils in well or disc techniques used for the assay. Inhibition zones were not observed for fennel oil neither for 200 µL doses with dilution 1:10 (Table III, entry 2) nor for the 5 µL doses of undiluted oil (Table II, entry 2). The microatmosphere method showed an inhibition effect on *S. aureus* for peppermint, coriander, and lavender oils. (results not shown here).

All tested essential oils showed high antifungal activity against *C. albicans* by both agar diffusion and microatmosphere methods. Fir oil had the highest activity for the 5 µL dose (Table II, entry 5), better than the activity of the controls (Table II, entries 15, 16).

The essential oils with inhibitory activities against all tested strains were coriander, peppermint, juniper, rosemary, and lavender oils.

Table II

Antibacterial and antifungal activity of essential oils (5µL doses) and traditional antibiotics in agar diffusion method (disc), inhibition zones (in mm.)

Entry	Essential oil / Antibiotic	*E. coli* inhibition area (mm)	*S. aureus* inhibition area (mm)	*C. albicans* inhibition area (mm)
1	Coriander	20	18	15
2	Fennel	0	0	12
3	Dill	13	0	10
4	Peppermint	13	12	11
5	Fir	0	0	26
6	Juniper	14	12	17
7	Rosemary	15	12	15
8	Lavender	10	8	13
9	Chloramphenicol 30µg/disc	30	30	NA
10	Trimethoprim+Sulfamethoxazole (Co-trimoxazole) 1.25µg + 23.75µg/disc	26	30	NA
11	Ampicillin 10µg/disc	25	NA	NA
12	Ciprofloxacin 5µg/disc	25	30	NA
13	Penicillin 6µg/disc	NA	25	NA
14	Erythromycin 15µg/disc	NA	26	NA
15	Amphotericin B 100 µg/disc	NA	NA	20
16	5-Fluro-Cytosine 1 µg/disc	NA	NA	18

NA – not analysed; 0 – no inbition zone.

Table III

Antibacterial activity of essential oils by the agar diffusion method (well), inhibition zones in mm

Entry	Essential oil	Dose	*E. coli* inhibition area (mm)	*S. aureus* inhibition area (mm)
1	Coriander	200µL 200µL dilution 1:10	40 50	50 36
2	Fennel	200µL 200µL dilution 1:10	20 50	25 0
3	Dill	200µL	30 50	40 20
4	Peppermint	200µL dilution 1:10	50 50	44 0
5	Fir	200µL	15 15	40 15
6	Juniper	200µL dilution 1:10	30 36	45 30
7	Rosemary	200µL	20 50	50 0
8	Lavender	200µL dilution 1:10	30 20	36 24
9	*n*-Hexane	200µL	50	0

0 – no inbition zone.

Conclusions

The antibacterial activity of the assessed essential oils against Gram positive and Gram negative microorganisms is dependent on the used dose. At 5 µL of undiluted oil, the effect against *E. coli* was smaller comparing with the antibiotics used as controls. Not all the tested undiluted oils were active against *S. aureus*.

At high doses, undiluted, all tested oils were active against both *E. coli* and *S. aureus*. Diluted ten times, the oils proved to be less active against *S. aureus*, some of them being even inactive: fennel, peppermint, and rosemary. Because the solvent *n*-hexane showed an inhibition activity against *E. coli*, the results obtained for diluted oils against *E. coli* were not taken into consideration for conclusions.

All tested essential oils showed antifungal activity against *Candida albicans* strain. The highest antifungal activity was observed for fir essential oil.

Practical applications of the results obtained herein are predicted.

References

1. Bakkali F., Averbeck S., Averbeck D., Idaomar M. – Biological effects of essential oils – a review, *Food Chem Toxicol*, 2008, 46, 446-475.
2. Bauer A.W., Kirby W.M., Sherris J.C., Turck M. - Antibiotic susceptibility testing by a standardized single disk method, *Am J Clin Pathol.* 1966, 45(4), 493-6.
3. Bădiceanu C.D., Larion C. - Antimicrobial activity of some new thioureides from 2-thiopheneacetic acid, *Farmacia*, 2009, 57(4), 473-478.
4. De Billerbeck VG – Huiles essentielles et bactéries résistantes aux antibiotiques, *Phytothérapie*, 2007, 5, 249-253.
5. Kalemba D., Kunicka A. – Antibacterial and antifungal properties of essential oils, *Curr Med Chem*, 2003, 10(10).
6. Inouye S., Uchida K., Maruyama N., Yamaguchi H., Abe S. – A novel method to estimate the contribution of the vapour activity of essential oils in agar diffusion assay, *Jpn J Med Mycol*, 2006, 47(2), 91-98.
7. Pavel M., Rădulescu V., Ilieş D. C. - GC-MS Analysis of essential oil obtained from the species *Thymus comosus* Heuff. ex Griseb.(*Lamiaceae*), *Farmacia*, 2009, 57(4), 479-484.
8. Rădulescu V., Pavel M., Teodor A., Tănase A., Ilieş D.C. - Analysis of volatile compounds from infusion and hydrodistillate obtained from the species *Thymus pulegioides* L. (*Lamiaceae*), *Farmacia*, 2009, 57(3), 282-289.

Manuscript received: January 7th 2010