Antimicrobial Activity of Some Essential Oils and Major Constituents of Essential Oils

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The screening of the antimicrobial activity of sage, rosemary, eucalyptus, melissa, lavender and thyme essential oils and active compounds 1.8-cineole, citral, linalyl acetate and thymol was conducted by a diffusion test against Gram-positive and Gram-negative bacteria. The most active essential oils, eucalyptus and rosemary oils were tested for the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The activity was more pronounced against Gram-positive bacteria than against Gram-negative bacteria with MIC ranging from 0.097 mg/ml-0.390 mg/ml, and MBC ranging from 0.390 mg/ml-12.5 mg/ml.

Key words: Plants, Medicinal; Anti-Infective Agents; Anti-Bacterial Agents; Oils, Volatile; Essential Oils.

Introduction

The antimicrobial properties of essential oils have been recognized for many years. Several compounds of essential oils are considered to possess biological activites. The antimicrobial activity of essential oils has been the subject of numerous investigations (1). The mechanism of reaction of essential oils and their components is unclear. A number of factors hamper the evaluation of the antimicrobial activity of essential oils, their volatility at room temperature, their water insolubility and their complexity (2). There are a number of different testing methods with different testing set-ups (2, 3, and 4). The aim of this study was to carry out a comparative analysis of the antimicrobial activi-

ties of the essential oils - sage, rosemary, eucalyptus, melissa, lavender, thyme and their active components (1.8-cineole, citral, linalyl acetate, and thymol).

Materials and methods

Essential oils were purchased from commercial samples in local stores.

Essential oils

1. Salviae aetheroleum, Sage essential oil, "Aromatica"
2. Rosmarini aetheroleum, Rosemary essential oil, "Aromatica"
3. Eucalypti aetheroleum, Eucalyptus essential oil, "Aromatica"
4. Melissae aetheroleum, Melissa essential oil, "Atea"
5. Lavandulae aetheroleum, Lavender essential oil, "Aromatica"
6. Thymi aetheroleum, Thyme essential oil, "Aromatica"

Aromatica, Atea, Croatia, producers of essential oils

Active compounds

Pure 1.8-cineole (Sigma-Aldrich), citral (Aldrich), linalyl acetate (Fluka), thymol (Fluka) and a 50% solution in dimethyl sulfoxide DMSO (Merck).

Organisms and media

Test organisms used in this study: *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027). The strains were maintained and tested on medium E (*Bacillus subtilis)*, medium A (*Staphylococcus aureus*) and Mueller-Hinton agar (*Escherichia coli, Pseudomonas aeruginosa*). Media were made up according to the European pharmacopeia directions (5).

Agar diffusion hole assay

Microorganisms were suspended in a sterile broth with turbidity corresponding to 0.5 McF units (approximately 10^{8} CFU mL^{-1}). Suspensions of microorganisms were incorporated in the appropriate medium (1 ml/100 ml media). Holes (0.5 mm diameter) were punched in the agar plate. Pure DMSO was used as a negative control while erythromycin discs (15 μg), gentamicin discs (30 μg) and penicillin discs (6 μg) were used as positive controls. The plates were observed after 18^{h} at 37°C. The antibacterial activity was expressed as the mean of inhibition diameters (mm). Tests were performed in triplicate. The doze diameters were measured with the Readbiotic apparatus.

Broth dilution assay (Minimal inhibitory and minimal bactericidal concentration)

Essential oils were serially diluted twofold in Tryptone Soya broth. The final concentration of oils in the medium ranged from 50 %-0,012 % (v/v). A 2 ml of essential oils in the medium was seeded with the broth culture overnight (0.5 McF units). The samples were incubated 18^{h} at 37°C. After incubation the last tube without any visible growth of the bacteria was taken to represent the minimum inhibitory concentration (MIC). All samples showing no turbidity were sub-cultured but the lowest concentration, from which the microorganisms did not recover, was the minimal bactericidal concentration (MBC).

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of oil or active compound inhibiting the visible growth of bacteria. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of oil or active compound in the test tube showing no growth in sub-culture. Control samples (positive and negative) were incubated under the same conditions.

Results

The results of the diffusion test are listed in Table 1.

Table 1. Antimicrobial activity of essential oils

Essential oils	Diameter of inhibition zone (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Sage	13.2	14.2	0.7	-
Rosemary	15.9	**20.0**	**13.0**	9.0
Eucalyptus	12.5	18.0	**13.0**	10.5
Melissa	15.0	18.0	-	-
Lavender	11.5	14.5	9.0	-
Thyme	**20.1**	19.0	12.0	**11.5**
Positive control	31.8 erythromycin	25.0 erythromycin	16.0 gentamicin	- penicillin
Negative control (DMSO)	-	-	-	-

Table 2. Antimicrobial activity of compounds of essential oils

Compounds	Diameter of inhibition zone (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
1.8-Cineole	10.4	14.5	-	-
1.8-Cineole 50 %	-	9.0	-	-
Citral	20.0	23.0	9.0	-
Citral 50%	17.1	23.0	-	-
Linalyl acetate	7.0	8.5	-	-
Linalyl acetate 50 %	7.0	8.3	-	-
Thymol	**22.0**	**23.0**	**14.5**	**11.5**
Thymol 50 %	**21.9**	**23.0**	**14.0**	**9.0**
Positive control	31.8 erythromycin	25.0 erythromycin	16.0 gentamicin	penicillin
Negative control (DMSO)	-	-	-	-

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of eucalyptus and rosemary essential oils

Essential oils	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Eucalyptus	0.390	3.215	**0.097**	12.5	0.390	0.390	0.390	0.390
Rosemary	0.195	0.781	**0.097**	6.25	0.390	0.390	0.390	0.781

The results of the antimicrobial activity assays indicated that essential oil of thyme exhibited higher activity against the *S. aureus* (20.1 mm), essential oil of rosemary against the *B. subtilis* (20.0 mm), essential oils of rosemary and eucalyptus against *E. coli* (13.0 mm) and essential oil of thyme against *P. aeruginosa* (11.5 mm).

Table 2. shows antimicrobial activity of active compounds. Thymol, a phenolic constituent of thyme oil, showed the highest activity.

Table 3. summarizes the MIC and MBC of tested essential oils. Rosemary and eucalyptus oils exhibited higher activity against *B. subtilis*.

Discussion

Among the six essential oils tested, thyme, eucalyptus and rosemary oils showed the highest activity. Gram-positive bacteria are known to be more susceptible to essential oils than Gram-negative bacteria (6). *P. aeruginosa* was least susceptible to the essential oils. The weak antibacterial activity against Gram-negative bacteria was ascribed to the presence of their cell wall, lip polysaccharide (7). *B. subtilis* was the most susceptible micro-organism to the rosemary essential oil. Concerning the activity of pure active compounds, the most susceptible bacteria to thymol was *B. subtilis* (23.0 mm) and the most resistant was *P. aeruginosa* (11.5 mm). Eucalyptus essential oil contained about 80 v/v % 1.8-cineole, but the antimicrobial activity of eucalyptus oil was greater than the antimicrobial activity of 1.8-cineole. Other components contributed significantly to the antibacterial activity of eucalyptus essential oil. A similar situation occurred for lavender essential oil. Linalyl acetate (16-30 v/v %) was a major component to the lavender essential oil, but it was not found to be a major contributor to the antimicrobial activity.

Conclusion

P. aeruginosa appeared to be the most resistant to the essential oils and active compounds. The active compound with the widest spectrum of activity was thymol. Gram-positive bacteria S. *aureus* and *B. subtilis* were more sensitive to essential oils than the Gram-negative bacteria. The antimicrobial activity of essential oils results from the combined effect of compounds.

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