# The influence of essential oils on Staphylococcus spp. isolated from skin microbiota

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### Introduction

Essential oils, being mixtures of various natural compounds, constitute an attractive source of novel antimicrobial agents. They are often used in cosmetology because of their anti-inflammatory, antibacterial and antiviral properties.

## Aim of the study

The aim of the study was to determine the antistaphylococcal activity of six essential oils and to evaluate their influence on staphylococci isolated from healthy skin microbiota.

## Materials & methods

#### STUDIED ESSENTIAL OILS

The study tested the activity of six commercially available essential oils derived from *Origanum vulgare* L, *Thymus vulgaris* L, *Melissa officinalis* L, *Melaleuca alternifolia, Mentha piperita* L and *Salvia officinalis* L

#### MICROBROTH DILUTION METHOD

The antistaphylococcal activity of essential oils was determined with the microbroth dilution method. Briefly, essential oil stocks (100 ma/ml in DMSO) were two-fold diluted in Mueller-Hinton Broth (MHB) to obtain a concentration 16 - 0.0156 mg/ml. Then, a microbial suspension (0.5 MacFarland) was 100-fold diluted in MHB and 2 ul was added to each well. After incubation at 35°C for 20 h, MIC (minimum inhibitory concentration) was read spectrophotometrically ( $\lambda$ =600 nm). The MBC (minimum bactericidal concentration) was determined by plating 5 µl from each well on Mueller-Hinton Agar followed by incubation at 35°C for 16-20 h. The assay included positive control (growth control) and negative control (MHB) as well as control of tested essential oil. All experiments were conducted in triplicate.

#### STUDIED STRAINS

The activity of essential oils was tested against two reference staphylococci: *Staphylococcus aureus* ATCC 29213 and *S. epidermidis* ATCC 12228 as well as 21 strains of staphylococci isolated from healthy human skin, three strains of each of the following species: *S. saprophyticus, S. haemolyticus, S. lentus, S. warneri, S. aureus, S. epidermidis* and *S. hominis*.

## RESULTS

Table 1 & 2. Antistaphylococcal activity of essential oils.											
	Me	entha pip	erita L.	Mel	aleuca al	ternifolia	Origanum vulgare L.				
Strains	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC		
	mg/ml			mg/ml		11120/11110	mg/ml				
S. saprophyticus	8-16	16	1-2	4-8	16	2-4	1	1-2	1-2		
S. haemolyticus	8-16	16	1-2	8	16	2	0.5-1	1-2	2		
S. lentus	8-16	16	1-2	4-8	16	2-4	0.5-1	1-2	2		
S. warneri	16	16	1	4-8	16	2-4	2	2	1		
S. aureus	16	16	1	8	16	2	1-2	2	1-2		
S. epidermidis	8-16	16	1-2	4	8-16	2	1	2	2		
S. hominis	4-16	16	1-4	4-8	16	2-4	1-2	2-4	1-2		
S. aureus ATCC 29213	8	16	2	2	2	1	1	1	1		
S. epidermidis ATCC 12228	8	16	2	1	1	1	1	1	1		

	Thy	mus vulg	garis L.	Ме	lissa offi	cinalis L.	Salvia officinalis L.			
Strains	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	
	mg/ml			mg	ı/ml		mg/ml			
S. saprophyticus	0.5-1	1-2	2	2-8	8-16	2-4	16	16	1	
S. haemolyticus	1-2	2	1-2	4	8-16	2-4	16	16	1	
S. lentus	1-2	2	1-2	4	4-8	1-2	16	16	1	
S. warneri	2	2-4	1-2	8	16	2	16	16	1	
S. aureus	1-2	2	1-2	8	8	1	16	16	1	
S. epidermidis	1	2-4	2-4	2-8	8-16	2-4	8	16	2	
S. hominis	1-2	2-4	1-2	2—4	8-16	2-4	8-16	16	1-2	
S. aureus ATCC 29213	1	2	2	4	4	1	4	8	2	
S. epidermidis ATCC 12228	0.5	1	2	4	8	2	2	4	2	

In the case of staphylococci isolated from skin microbiota, MIC, MBC and MBC/MIC values were presented as range. MBC to MIC ratio equal or lower than 4 – bactericidal mode of action, higher than 4 – bacteriostatic.

	v	Origa ulgare		v	Thym ulgaris		off	Melis icinali:			elaleu ternifo		pi	Ment perita		off	Salvia icinali:	
	1	0,5	1	1	1	1	8	4	8	16	8	8	8	16	16	16	16	16
S. saprophyticus	1	0,5	1	0,5	1	0,5	4	2	8	8	8	8	8	16	8	16	16	16
	1	1	1	1	1	1	4	2	2	8	4	4	8	8	8	16	16	16
	2	1	1	2	2	2	8	4	4	8	8	8	16	16	16	16	16	16
S. haemolyticus	2	1	1	2	2	2	4	4	4	8	8	8	16	16	16	16	16	16
	1	0,5	0,5	2	1	1	4	4	4	8	8	8	8	8	8	16	16	16
	2	1	1	1	2	1	4	4	8	8	4	4	8	16	8	16	16	8
S. lentus	1	1	1	2	2	2	4	4	4	8	8	8	16	16	16	16	16	16
	0,5	1	0,5	1	1	1	4	4	4	16	8	8	16	16	16	16	16	16
	2	2	2	2	2	2	8	8	8	8	4	4	16	16	16	16	16	16
S. warneri	2	2	2	2	2	2	4	8	8	16	8	8	16	16	16	16	16	16
	2	2	1	2	2	2	8	8	8	16	8	8	16	16	16	16	16	16
	2	2	2	2	2	2	8	8	16	16	8	8	16	16	16	16	16	16
S. aureus	1	1	1	1	1	1	8	4	8	16	8	8	16	16	16	16	16	16
	2	2	2	2	2	2	8	8	8	16	8	8	8	16	16	16	16	16
	2	1	1	2	1	1	8	2	2	8	4	4	8	16	16	8	16	8
S. epidermidis	2	1	1	2	1	1	16	4	8	8	4	4	16	8	8	8	16	8
	2	1	1	2	1	1	8	4	4	8	4	4	8	8	8	8	16	8
	2	2	2	2	2	2	8	4	4	16	8	8	16	16	16	16	16	16
S. hominis	0,5	1	1	1	0,25	1	8	2	2	8	4	4	4	4	4	8	16	8
	2	2	2	2	1	1	8	4	4	8	4	4	8	8	8	8	16	8

Fig. 1. Heatmap presenting the influence of essential oils on staphylococci isolated from healthy human skin. Red indicates high influence (low MIC), green low influence (high MIC) on skin microbiota.

CONCLUSIONS	
<ol> <li>Tested essential oils exhibited a wide range of antistaphylococcal activity (MIC from 0.5 to 16 mg/ml).</li> <li>The highest activity against skin staphylococci was noted for oregano and thyme essential oils (MIC from 0.5 to 2 mg/ml) and the lowest activity for mint and salvia oils (MIC from 4 to 16 mg/ml).</li> <li>All tested essential oils exhibited bactericidal properties.</li> <li>None of the tested essential oils acted selectively on skin staphylococci.</li> <li>Certain essential oils (thyme, oregano) should be used carefully in topical formulations because they can disrupt the natural homeostasis of skin microbiota.</li> </ol>	