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EXPERIMENTAL PAPER

Activity of essential oils against *Staphylococcus aureus* strains isolated from skin lesions in the course of staphylococcal skin infections

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Summary

Introduction: *Staphylococcus aureus* is an important etiological agent of skin and soft tissue infections. Due to the increasing resistance of this bacterium to antimicrobial agents, treatment of staphylococcal infections remains a great challenge for clinicians and requires an alternative treatment options. **Objective:** The aim of the study was to determine the antimicrobial activity of essential oils: caraway (CEO), patchouli (PEO) and geranium (GEO) against *S. aureus* strains isolated from skin lesions in the course of staphylococcal skin infections. **Methods:** The antibacterial activity of essential oils was tested using the dilution method in Mueller-Hinton broth. **Results:** The antimicrobial effect of CEO, PEO and GEO was observed. The highest antimicrobial activity showed PEO (MIC = 1.7±0.8 μl/ml), the lower was observed for GEO (MIC = 5.4±2.0 μl/ml) and CEO (MIC = 18.8±10.3 μl/ml). **Conclusion:** All tested essential oils showed antibacterial activity against *S. aureus* strains isolated from skin lesions of patients with staphylococcal skin infections. Application of the CEO, PEO and GEO can become an alternative method of treatment of staphylococcal infections, but further microbiological tests and clinical trials should be assessed.

Key words: *Staphylococcus aureus, caraway oil, patchouli oil, geranium oil, staphylococcal skin infections*

INTRODUCTION

Staphylococcus aureus is a Gram-positive, facultatively anaerobic, catalase-positive coccus, with single cells arranged mostly in irregular clusters. This one of the major human bacterial pathogens is responsible for a great number of invasive infections also with severe and life-threatening course. *S. aureus* is the most common etiological agent of purulent skin infections (impetigo, furunculosis, folliculitis, hidradenitis suppurativa, mastitis etc.), as well as infections of the subcutaneous tissue [1].

This bacterium colonizes the mucous membranes of anterior nares and nasopharynx, but is also found e.g. on skin of armpits or anal area of humans [2]. It is proved that about 20–30% of human population is persistent and 50–60% are transient *S. aureus* carriers [3]. The carrier state is undoubtedly a major risk factor for staphylococcal infections and has an impact on prevalence, course and duration of an infection. It has been shown that recurrent folliculitis and furunculosis are associated with *S. aureus* nasal carrier state [4]. This pathogen is transmitted mostly *via* hands of colonized patients or medical staff.

S. aureus carries multiple factors and multiple mechanisms of antibiotics resistance thereby is able to cause health and life-threatening infections. The major virulence factors include enzymes, cell wall components and toxins. Surface proteins (protein A, *clumping factor*) play an important role in skin infections promoting adhesion of bacterium to damaged tissue. Hemolysins as well as Panton-Valentine leukocidin possess cytocidal activity, whereas hyaluronidase, lipases, nucleases, coagulase and fibrinolysin facilitate spreading the toxins within the human body [5-7].

Staphylococcal skin infection is a result of skin breakdown and tissue destruction, may also initiate infections of deeper parts of skin as well as soft tissues, thereby requiring supportive antibiotic treatment. The following antibiotics: mupirocin, first and second generation of cephalosporins, macrolides or clindamycin are highly efficient drugs, currently used in staphylococcal skin infections treatment. Co-trimoxazole, tetracyclines or linezolid are also therapeutic option. The latter are used in the therapy of *community-acquired* methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections, when MRSA or macrolide-lincosamide-streptogramin B (MLS_B) resistance mechanisms severely limit the number of efficient drugs [8, 9]. It should be kept in mind that despite an appropriate antibiotic treatment is implemented, the clinical effect is not always reached. Staphylococcal skin infections still remain a challenge for clinicians - therefore it is reasonable to seek a novel treatment alternatives.

Essential oils are mixtures of natural compounds, obtained from plants mainly by hydrodistillation, steam distillation or extrusion. Caraway *Carum carvi* L. (CEO), patchouli *Pogostemon cablin* (Blanco) Benth (PEO) and geranium *Pelargonium graveolens* L'Hér. (GEO) oils show a wide range of properties including antimicrobial, antioxidant, antiproliferative, hypoglycemic, diuretic, insecticidal and anti-cancer activities [10-12]. These oils are widely used in food, cosmetic and pharmaceutical industries worldwide.

The biological activity is determined by the composition of essential oils. Essential oil content depends on the weather conditions, geographical origin, time of harvest, parts of the plant from which an essential oil is obtained; as well as

on essential oil isolation methods and technology of the process [13, 14]. It is proved that the CEO is rich in carvacrol, carvone, α-pinene, limonene, γ-terpinene, linalool, *p*-cymene and carvenone. It has white or yellowish colour and possesses a characteristic smell of caraway seeds conditioned by the enantiomer (*S*)-(+)-carvone presence [10, 15]. Current data show that the main ingredient of PEO is patchouli alcohol, accompanied by small amounts of germacrene A, germacrene D, α-patchoulene, β-patchoulene, γ-patchoulene, α-guaiene and globulol. Due to the content of all those compounds, PEO can take brown to dark yellow color [16]. In contrast, GEO contains citronellol, geraniol, caryophyllene oxide, menthone, linalool, β-bourbonene, *iso*-menthone and geranyl formate [17]. GEO usually takes pale yellow color and has a characteristic smell of roses and mint [18].

The aim of the study was to determine the antimicrobial activity of CEO, PEO and GEO against *S. aureus* strains isolated from skin lesions in the course of staphylococcal skin infections.

MATERIAL AND METHODS

Bacterial strain identification

Thirteen S. *aureus* strains were isolated from skin lesions of patients with staphylococcal skin infections: non-surgical wound (7), surgical wound (2), abscess (2), acne lesion (1) and ulcer (1). Specimen samples were cultured onto blood agar and mannitol salt agar (bioMérieux), incubated overnight at 37°C. After bacterial growth, isolates were identified on the basis of their ability to ferment mannitol and to form fibrin clot in rabbit plasma (Biomed). A positive reaction of Slidex Staph-Kit (bioMérieux) was observed. *S. aureus* (ATCC 29213) was used as a control strain in the study.

Essential oils analysis

Commercial essential oils from Pollena-Aroma: caraway (*C. carvi*; CEO), patchouli (*P. cablin*; PEO) and geranium (*P. graveolens*; GEO) were used in the study. Essential oils were stored in 4°C in dark glass bottles.

The chemical composition of essential oils was determined by gas chromatography with mass selective detector (GC-MS) method. Analyses were performed using an Agilent 6890N gas chromatograph with a 5973N mass selective detector and a 7683 automatic liquid injector. The resolution of analytes was achieved using a HP-5MSI column (30 m length, 0.25 mm I.D., 0.25 μm film thickness). The column temperature was programmed: initial temperature 50°C, ramp rate 4°C/min, final temperature 290°C (total time of analysis: 60 min). Other parameters of applied method: flow rate of carrier gas (helium): 1.2 ml/min, injector temperature:

250°C, MS quad temperature: 150°C; MS source temperature: 230°C. Mass spectra were obtained using electron impact ionization at 70 eV in a full scan mode (range: 20–500 m/z).

Samples for GC-MS were prepared by dissolving of tested essential oil (30 μl) in 1 ml of acetone (p.a.). Collection and processing of chromatographic data were performed using ChemStation program. The identification of the analytes was based on the comparison of their mass spectra with the reference spectra from NIST 02 library. The relative contents of the particular compounds in essential oils were obtained as the peak area percentages in a total ion chromatogram.

Activity of essential oils against tested *S. aureus* strains

The minimum inhibitory concentration (MIC) of essential oils against *S. aureus* strains was determined with dilution method [19]. A stock solution of the tested oil was prepared in Mueller-Hinton broth (MHB) (Sigma-Aldrich) (supplemented with 1% Tween 80) in a concentration ranging from 50 μl/ml to 0.39 μl/ml. A 100 μl of increasing concentration of an essential oil tested was added to each well of 96-well microplate. Next, to each well with an essential oil the bacterial suspension (10 μl) at a concentration of 1.5 x 10^8 cfu/ml was added.

The MIC was estimated after 24 hours of incubation at 37°C. A 20 μl of 0.02% resazurin (responsible for dark blue color of the solution) was added to each plate well [20]. MIC was determined on the basis of the dark blue color appearance in the first tested well after 3 hours of incubation with resazurin. The colour change from blue to pink after a 3-hours of incubation with resazurin at 37°C indicated the presence of bacteria. Each oil was tested in three repetitions. In order to exclude an inhibitory effect of 1% Tween 80 on the *S. aureus* growth, the control assays with MHB and MHB supplemented with 1% Tween 80 were performed.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

In recent years a growing interest in antibacterial properties of plant origin substances is observed. Among them a particular essential oils play an important role.

Using GC-MS method, 4 natural compounds in CEO were identified, with the highest concentration of carvone (52.3%) and limonene (46.8%). A total of 8 and 12 compounds were found in PEO and GEO, respectively. The predominant component of PEO was patchouli alcohol with the 45.7% of relative content, whereas the major components of GEO were citronellol and geraniol with 42.4% and 15.0% of relative content, respectively. The composition of oils examined in this study was similar when compared with other studies [21, 22, 17], thereby indicating

natural origin of CEO, PEO and GEO. The results of chemical analysis are shown in table 1.

Table 1.

The chemical composition of tested commercial essential oils

Essential oil	Compound	Relative content [%]
CEO	Limonene	46.8
	cis-Limonene oxide	0.2
	trans-Dihydrocarvone	0.5
	Carvone	52.3
	Total	99.8
PEO	*β*-Patchoulene	2.1
	Caryophyllene	2.5
	α-Guaiene	20.4
	α-Patchoulene	4.9
	Seychellene	2.6
	β-Guaiene	0.6
	δ-Guaiene	17.1
	Patchouli alcohol	45.7
	Total	95.9
GEO	Linalool	2.9
	cis-Rose oxide	1.5
	iso-Menthone	6.6
	Citronellol	42.4
	Geraniol	15.0
	Citronellyl formate	12.6
	Geranyl formate	3.3
	β-Bourbonene	1.1
	Caryophyllene	2.3
	Germacrene D	2.2
	δ-Cadinene	1.4
	10-epi-*γ*-Eudesmol	6.8
	Total	98.1

CEO – caraway essential oil; PEO – patchouli essential oil; GEO – geranium essential oil

It was observed in the study that addition of 1% Tween 80 has no impact on inhibition of *S. aureus* growth and this has been also noted previously by Honório *et al.* [23]. The highest antimicrobial activity was reported for PEO (MIC = 1.7±0.8 *μ*l/ml). These data were consistent with those of Yang *et al.* [24] which confirmed the antimicrobial properties of the Chinese, Indian and Indonesian

PEO. PEO-suppressive effect against strains of *S. aureus* was also demonstrated in other studies [25, 26].

In the study, the lower activity of GEO (MIC = 5.4±2.0 μl/ml) against isolates of *S. aureus* was observed when compared with PEO. The antimicrobial activity of GEO against clinical isolates of *S. aureus* was considered in details by Bigos *et al.* [27].

CEO demonstrated the lowest antimicrobial activity among all essential oils tested in the study (MIC = 18.8±10.3 μl/ml). Recent studies of Seidler-Łożykowska *et al.* [28] focused on *S. aureus* revealed that MIC of CEO was dependent on the origin of the oil and the carvone/limonene ratio. The same authors mentioned that the highest antimicrobial activity of CEO was reported when carvone relative content was higher than limonene. In our study the relative content of carvone in CEO was higher than limonene, but antibacterial activity of CEO against tested *S. aureus* was lower when compared with PEO and GEO.

Consecutively CEO (MIC = 2.1±0.9 μl/ml), PEO (MIC = 1.0±0.5 μl/ml) and GEO (MIC = 4.2±1.8 μl/ml) showed their antibacterial activity against *S. aureus* ATCC 29213 reference strain. It is noteworthy that CEO showed higher antibacterial activity against the control strain when compared with clinical isolates of *S. aureus*. Higher activity of CEO against reference strain than against clinical isolates of *S. aureus* may be due to its low virulence, but this hypothesis requires further studies.

The results of the antimicrobial activity of essential oils tested against *S. aureus* strains were summarized in table 2 and shown in figure 1.

Table 2.

Antimicrobial activity of the essential oils against *S. aureus* strains

No.	*Staphylococcus aureus*/clinical sample	MIC of essential oils [μl/ml]		
		CEO	PEO	GEO
1.	ATCC 29213	2.1±0.9	1.0±0.5	4.2±1.8
2.	Swab/non-surgical wound	20.8±7.2	1.8±1.2	5.2±1.8
3.	Swab/non-surgical wound	10.4±3.6	2.6±0.9	6.3±0.0
4.	Swab/non-surgical wound	20.8±7.2	1.3±0.5	5.2±1.8
5.	Swab/acne lesion	16.7±7.2	1.3±0.5	5.2±1.8
6.	Swab/surgical wound	20.8±7.2	2.6±0.9	8.3±3.6
7.	Swab/non-surgical wound	12.5±0.0	2.1±0.9	4.2±1.8
8.	Swab/surgical wound	20.8±7.2	2.6±0.9	5.2±1.8
9.	Swab/non-surgical wound	4.2±1.8	0.5±0.2	2.1±0.9
10.	Swab/abscess	16.7±7.2	1.6±0.0	6.3±0.0
11.	Swab/abscess	33.3±14.4	1.6±0.0	6.3±0.0
12.	Swab/non-surgical wound	33.3±14.4	0.8±0.0	4.2±1.8
13.	Swab/non-surgical wound	16.7±7.2	1.6±0.0	6.3±0.0
14.	Swab/ulcer	16.7±7.2	1.6±0.0	6.3±0.0

MIC – minimal inhibitory concentration; CEO – caraway essential oil, PEO – patchouli essential oil; GEO – geranium essential oil. Values are expressed as means±standard deviation

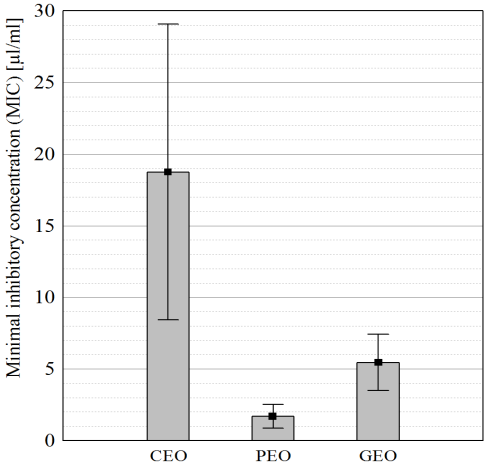

Figure 1

Minimal inhibitory concentration (MIC) of caraway essential oil (CEO), patchouli essential oil (PEO) and geranium essential oil (GEO) against *S. aureus* strains isolated from skin lesions of patients with staphylococcal skin infections

Nowadays, searching for new substances with antibacterial properties is a result of an antibiotic resistance threats. This study supports the idea that essential oils may be a potential antibacterial agent limiting the antibiotic overuse. A direct aromatherapy with the application of diluted essential oil on the skin surface may become an alternative option of treatment in the course of staphylococcal skin infections. This unconventional therapy needs to be preceded by skin tests and by selection of essential oil, its appropriate concentration and the skin exposure time [29].

CONCLUSION

CEO, PEO and GEO demonstrated the antibacterial activity against *S. aureus* isolated from skin lesions of patients with staphylococcal skin infections. The work provides a basis for further studies on potential use of essential oils as antibacterial agents. However, application of essential oils as an alternative treatment of skin infections with *S. aureus* etiology requires association of microbiological data with clinical trials.

Conflict of interest: Authors declare no conflict of interest.

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AKTYWNOŚĆ OLEJKÓW ETERYCZNYCH WOBEC SZCZEPÓW *STAPHYLOCOCCUS AUREUS* IZOLOWANYCH ZE ZMIAN SKÓRNYCH W PRZEBIEGU GRONKOWCOWYCH ZAKAŻEŃ SKÓRY

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Streszczenie

Wstęp: *Staphylococcus aureus* jest kluczowym czynnikiem etiologicznym zakażeń skóry i tkanki podskórnej. Ze względu na narastającą oporność bakterii na antybiotyki lecze-

nie zakażeń o etiologii gronkowcowej pozostaje nadal wyzwaniem dla lekarzy klinicystów, zatem uzasadnione jest poszukiwanie alternatywnych metod leczenia. **Cel:** Celem pracy było określenie aktywności przeciwbakteryjnej olejków eterycznych: kminkowego (CEO), paczulowego (PEO) oraz geraniowego (GEO) wobec szczepów *S. aureus* izolowanych ze zmian skórnych w przebiegu gronkowcowych zakażeń skóry. **Metodyka:** Aktywność przeciwbakteryjną olejków eterycznych badano z wykorzystaniem metody rozcieńczeń w bulionie Mueller-Hinton (MHB). **Wyniki:** Wykazano, iż CEO, PEO i GEO dodane do MHB hamują wzrost *S. aureus*. Najwyższą aktywność przeciwbakteryjną wykazywał PEO (MIC = 1,7±0,8 μl/ml), niższą uzyskano dla GEO (MIC = 5,4±2,0 μl/ml) oraz CEO (MIC = 18,8±10,3 μl/ml). **Wnioski:** Badane olejki eteryczne wykazały aktywność przeciwbakteryjną wobec szczepów *S. aureus* izolowanych od pacjentów ze zmian skórnych w przebiegu gronkowcowych zakażeń skóry. Zastosowanie CEO, PEO i GEO może stać się alternatywną metodą leczenia infekcji gronkowcowych, ale wciąż wymaga powiązania badań mikrobiologicznych i klinicznych.

Słowa kluczowe: *Staphylococcus aureus, olejek kminkowy, olejek paczulowy, olejek geraniowy, gronkowcowe zakażenia skóry*