Original paper

The effect of clary sage oil on staphylococci responsible for wound infections

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Abstract

Introduction: The spreading of bacterial antibiotic resistance among clinical strains of pathogenic bacteria has made investigators to search for other active antibacterial agents which could provide a valuable complement to the existing therapies.

Aim: To determine the antibacterial activity of clary sage oil (*Salvia sclarea* L) against *Staphylococcus* clinical strains which were isolated from patients with wound infections.

Material and methods: A comprehensive evaluation of *Staphylococcus* clinical strain resistance to antibiotics was performed. The constituents of clary sage oil were assayed by GC-FID-MS analysis. The minimal inhibitory concentration (MIC) of the tested essential oil against staphylococci by the micro-dilution broth method was determined.

Results: The clary sage oil was active against *Staphylococcus aureus*, *S. epidermidis* and *S. xylosus* with MIC values ranging from 3.75 to 7.00 µl/ml.

Conclusions: The results of the *in vitro* tests encourage to use formulations containing sage oil as the active natural antimicrobial agent. Because of its antimicrobial properties clary sage oil may be applied to treat wounds and skin infections.

Key words: clary sage oil, minimal inhibitory concentration, *Staphylococcus,* wounds.

Introduction

The genus of *Staphylococcus* is a major Gram-positive coccus responsible for severe infections including dermatological ones. The major nosocomial pathogen is methicillin-resistant *Staphylococcus aureus* (MRSA) [1, 2], however coagulase-negative species (CoNS), including *S. epidermidis* and *S. xylosus,* are responsible for difficult to treat infectious diseases. The spread of multidrug resistant bacterial strains in patients, medical staff and hospital environment is largely caused by widespread use of antibiotic therapy [3, 4]. Many classes of antibiotics used before have become therapeutically useless. The risk of local and systemic infections development is high in hospital wards, especially in the elderly, immunosuppressed patients and those debilitated by chronic diseases [5–7].

Literature data report that essential oils are used to treat the respiratory tract, digestive system, skin infections and also may be applied in anticancer therapy and cardiovascular and nervous system disorders to reduce the level of cholesterol, as well as to decrease and regulate the glucose level. Various essential oils are components of the cosmetic cleaning products and food preservatives [8–11].

One of the most important essential oils which has been successfully used in medicine and cosmetology is tea tree oil [12]. The study by Hammer *et al.* [13] showed the susceptibility of transient and commensal skin flora

to the essential oil of *Melaleuca alternifolia*. Their results suggest that tea tree oil may be useful in removing transient skin flora but maintains resident flora. Due to strong antifungal properties of essential oils they can be used in mixed skin infections. According to Adam *et al.* [14], the essential oils of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia*, and *Salvia fruticosa* exhibited antifungal properties against the human pathogens of *Malassezia furfur, Trichophyton rubrum*, and *Trichosporon beigelii.* The analyzed essential oils were tested with the Ames test and did not exhibit any mutagenic activity. The genus of *Salvia* is represented by over 900 species. One of the most important medicinal plants is *Salvia officinalis*. It has been used in treatment of tuberculosis, dysentery, coughing, indigestion, ulcer, psoriasis, as well as in skin and hair care [15]. The antimicrobial activity of plant extracts and essential oils from some species of *Salvia* is well documented in the research [16, 17]. Clary sage extracts and essential oil possess antioxidant and antimicrobial properties [18, 19]. Dźamić *et al.* [20] showed fungicidal activity of the sage oil against *Aspergillus, Penicillium, Fusarium, Trichoderma, Mucor, Candida* and also its fungistatic and fungicidal activity against *Cladosporium, Trichophyton, Alternaria* and *Phoma* in very low levels. Hristova *et al.* [21] presented interesting results connected with high activity of the clary sage oil against many clinical strains of *Candida* species.

Aim

The present study on *Staphylococcus* isolates obtained from difficult-to-heal wound infections provided the information regarding strain sensitivity to the clary sage oil.

Material and methods

Origin and identification of bacterial strains

The tested *Staphylococcus* clinical strains were isolated from the swabs of patients with recurrent, difficult-to-treat wound infections treated at the Department of Dermatology, Pediatric Dermatology and Oncology, Medical University of Lodz, Poland, in 2012. The study included 61 patients; 41 males and 20 females (mean age of 56–63 years) with non-healing wounds arising in the course of diabetes, cardiovascular disorders, burns and surgery. Before admission, these patients were subjected unsuccessfully several times to outpatient treatment. The clinical material was collected before the antibiotic therapy. Staphylococci isolated from wounds were identified according to standard methods of culturing on Columbia Agar (bioMerieux), on Mannitol Salt Agar (bioMerieux), and determining the ability of bacteria to produce catalase and coagulase (bioMerieux). Microorganism species were identified by using API Staph tests (bioMerieux). The bacteria were incubated in 37°C for

24 h. *Staphylococcus aureus* ATCC 29213 strain was used as a control.

Preparation and GC-FID-MS analysis of clary sage oil

Salvia sclarea L. (*Lamiaceae*) oil was obtained from POLLENA-AROMA Poland. It was analyzed by GC-FID-MS in the Institute of General Food Chemistry, Lodz University of Technology, using a Trace GC Ultra apparatus (Thermo Electron Corporation) with FID and MS DSQ II detectors and FID-MS splitter (SGE). Operating conditions: apolar capillary column Rtx-1ms (Restek), 60 m × 0.25 mm i.d., film thickness 0.25 μm; temperature program, 50–300°C at 4°C/min; SSL injector temperature 280°C; FID temperature 300°C; split ratio 1 : 20; carrier gas helium at a regular pressure 200 kPa; FID temperature 260°C; carrier gas, helium; 0.5 ml/min; split ratio 1 : 20. Mass spectra were acquired over the mass range 30–400 Da, ionization voltage 70 eV; ion source temperature 200°C.

Identification of components was based on the comparison of their MS spectra with those in a laboratory-made MS library, commercial libraries (NIST 98.1, Wiley Registry of Mass Spectral Data, 8th Ed. and MassFinder 4) and with literature data [22, 23] along with the retention indices on an apolar column (Rtx-1, MassFinder 4) associated with a series of alkanes with linear interpolation (C_8–C_{26}).

Staphylococcus susceptibility to antibiotic testing

Staphylococcus strains were cultivated on Columbia Agar medium and incubated at 37°C for 24 h. Bacterial suspensions with an optical density of 0.5MF scale were prepared with the bioMerieux densitometer. Susceptibility testing was carried out with the use of disk-diffusion method on Mueller-Hinton II Agar and incubated at 37°C for 18 h. The following antibiotics (Becton Dickinson) were used: *Staphylococcus aureus*, *S. epidermidis* and *S. xylosus*: FOX – cefoxitin (30 μg), P – penicillin (10 IU), E – erythromycin (15 μg), DA – clindamycin (2 μg), TE – tetracycline (30 μg), TGC – tigecycline (15 μg), C – chloramphenicol (30 μg), CIP – ciprofloxacin (5 μg), RA – rifampin (5 μg), GM – gentamicin (10 μg), SXT – trimethoprim-sulfamethoxazole (1.25 μg /23.75 μg), LZD – linezolid (30 μg), FD – fusidic acid (10 μg), QDA – quinupristin-dalfopristin (15 μg), VA – vancomycin (30 μg), and DPC – daptomycin (15 μg).

The results were found according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [24].

Staphylococcus susceptibility to clary sage testing

The clary sage antistaphylococcal activity was tested by the micro-dilution broth method. The essential oil was diluted in ethanol used as a stock solution. This stock solution was mixed with a 100 μl of Mueller-Hinton broth

to obtain concentrations from 3.5 µl/ml to 7.25 µl/ml. An inoculum containing 1–2 × 10⁸ cells/ml (10 µl) per well was added to broth with various oil concentrations, as well as to broth with no oil added (strain growth control) and were transferred to 96-well microtiter plates. The minimal inhibitory concentration (MIC) was determined after 24 h of incubation at 37°C under aerobic conditions [25]. Control media containing only alcohol (at concentrations used in the dilutions of clary sage) did not inhibit the growth of tested bacteria.

Results

Constituents of *Salvia sclarea* oil

Detailed analysis of the clary sage oil showed 56 constituents, out of which linalyl acetate (57.9%) and linalool (12.4%) were determined as the main ones. Moreover, α-pinene (4.5%), α-terpineol (3.5%), sabinene (3.3%), β-pinene (3.0%), geranyl acetate (1.6%), myrcene (1.5%) and neryl acetate (1.0%) were identified in large quantities (Table 1).

The evaluation of *Staphylococcus* clinical strain resistance

Three of *Staphylococcus* genera were isolated from patients with wound infections, including *Staphylococcus aureus* (*n* = 28), and coagulase-negative species: *S. epidermidis* (*n* = 19) and *S. xylosus* (*n* = 14). The number of resistant *Staphylococcus* strains to recommended antibiotics is presented in Figure 1.

The results of susceptibility testing show that the *Staphylococcus* strains isolated from wound infections

Table 1. Constituents of *Salvia sclarea* L. (clary sage) essential oil

Number	Compound	%	RI	Number	Compound	%	RI
1	α-Thujene/Tricyclene	0.3	923	29	Ascaridole	0.1	1221
2	α-Pinene	4.5	931	30	Linalyl acetate	57.9	1249
3	Camphene	tr	941	31	Safrole	0.2	1268
4	Sabinene	3.3	966	32	α-Terpinyl acetate	0.1	1334
5	β-Pinene	3.0	970	33	Neryl acetate	1.0	1344
6	Myrcene	1.5	983	34	α-Cubebene	tr	1349
7	α-Phellandrene	0.1	996	35	Geranyl acetate	1.6	1363
8	Car-3-ene	0.2	1004	36	α-Copaene	tr	1371
9	α-Terpinene	0.4	1009	37	β-Bourbonene	0.2	1377
10	p-Cymene	0.5	1012	38	β-Cubebene	0.1	1385
11	1,8-Cineole	0.8	1020	39	β-Elemene	0.1	1389
12	Limonene	0.9	1021	40	β-Caryophyllene	0.8	1420
13	(Z)-β-Ocimene	0.1	1028	41	β-Copaene	tr	1428
14	(E)-β-Ocimene	0.2	1038	42	trans-α-Bergamotene	tr	1433
15	γ-Terpinene	0.5	1049	43	(E)-β-Farnesene	tr	1447
16	*trans*-Sabinene hydrate	0.1	1054	44	α-Humulene	0.1	1452
17	*trans*-Linalool oxide (f)	tr	1059	45	Germacrene D	0.5	1477
18	*cis*-Linalool oxide (f)	tr	1073	46	β-Selinene	tr	1483
19	Terpinolene	0.3	1079	47	Myristicin	1.6	1492
20	Linalool	12.4	1090	48	δ-Cadinene	0.1	1514
21	*cis*-Sabinene hydrate	tr	1099	49	Elemicin	tr	1520
22	*cis*-p-Menth-2-en-1-ol	tr	1108	50	Spathulenol	tr	1565
23	Borneol	tr	1150	51	Caryophyllene oxide	0.1	1571
24	Terpinen-4-ol	0.9	1163	52	β-Eudesmol	tr	1625
25	α-Terpineol	3.5	1175	53	α-Eudesmol	tr	1637
26	γ-Terpineol	0.2	1181	54	Sclareoloxide	tr	1881
27	Linalyl acetate	0.1	1200	55	Geranyllinalool	tr	1906
28	Nerol	0.6	1216	56	Sclareol	0.1	2206

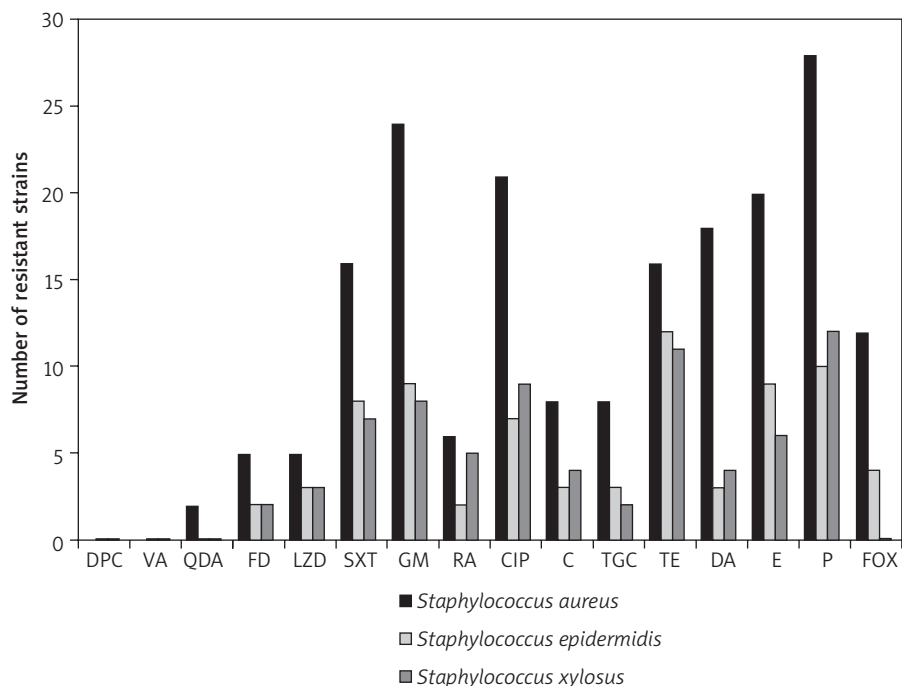*tr < 0.05%, % – percentage of constituents, RI – retention index.*

Figure 1. *Staphylococcus* clinical strains isolated from wounds resistant to recommended antibiotics

in the study were highly resistant to most of the β-lactam (penicillin), macrolide (erythromycin), lincosamides (clindamycin), tetracycline, fluoroquinolone (ciprofloxacin), aminoglycoside (gentamicin) antibiotics and sulfonamide (trimethoprim-sulfamethoxazole). *Staphylococcus aureus* clinical strains were much more resistant to recommended antibiotics than *S. epidermidis* and *S. xylosus* strains. There were 11 MRSA and 16 MSSA strains among the *S. aureus* isolates, but all of them were resistant to penicillin. The highest resistance of the coagulase-nega-

tive species was found for penicillin, erythromycin, tetracycline, ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole.

The susceptibility of *Staphylococcus* clinical strains to clary sage oil

The clary sage oil has the high antibacterial potential against all bacterial genera of *Staphylococcus* with MIC between 3.75–7.00 μl/ml. The approximate MIC values for *S. aureus* and *S. epidermidis* isolated from wounds were obtained. *Staphylococcus aureus* isolates including eleven MRSA and sixteen MSSA were inhibited by the clary sage oil at the concentrations of 3.75–5.25 μl/ml. The concentrations from 4.50 μl/ml to 6.25 μl/ml inhibited the growth of *S. epidermidis* clinical strains. Strains of *S. xylosus* were the least sensitive to *Salvia sclarea* oil, with the MIC values ranging from 6.25 μl/ml to 7.00 μl/ml. The activity of clary sage oil against *S. aureus*, *S. epidermidis* and *S. xylosus* is presented in Figure 2.

Discussion

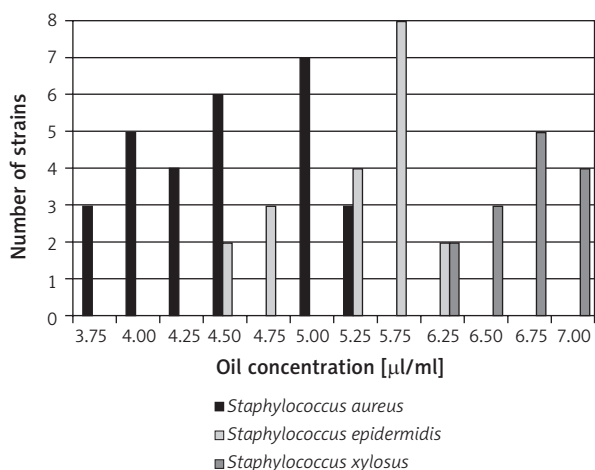The study results show that the *Salvia sclarea* essential oil has the strong antistaphylococcal activity against clinical strains isolated from wound infections. Interestingly, the strains of species *S. aureus* and *S. epidermidis* were more susceptible to the clary sage oil, followed by strains of *S. xylosus*. *Staphylococcus aureus* strains including MRSA were simultaneously the most resistant

Figure 2. The activity of clary sage oil against *Staphylococcus* clinical strains isolated from wounds

to recommended antibiotics. Linalyl acetate (57.9%) and linalool (12.4%) were found to be the main compounds out of 56 constituents of the clary sage oil. It should be emphasized that the oil tested in the present study was consistent with requirements of the European Pharmacopoeia 6 [26] and the Polish Pharmacopoeia VIII [27] which specify precise amounts of linalyl acetate (56–78%) and linalool (6.5–24%). The clary sage oil contains linalyl acetate (52.83%) and linalool (18.18%) which have been studied by Dźamić *et al.* [20] and show the antifungal activity expressed as MIC in the range of 2.5 µl/ml to 25.0 µl/ml. Our previous studies demonstrated that the lavender oil at the concentration from 1.5 µl/ml to 3.0 µl/ml inhibited the growth of resistant *S. aureus* strains isolated from wounds [28]. The composition of the analyzed lavender oil was similar to clary sage oil; linalyl acetate (33%) and linalool (34.1%) were the main constituents of oil from *Lavandula angustifolia Mill.* (*Lamiaceae*). The tested clary sage oil was active against all tested *S. aureus* clinical strains with MIC values ranging from 3.75 µl/ml to 5.25 µl/ml. The strains of *S. epidermidis* and *S. xylosus* were susceptible to the clary sage oil at the concentration from 4.50 µl/ml to 7.00 µl/ml. Peana *et al.* [29] have proven that linalool and linalyl acetate play a major role in the anti-inflammatory activity of essential oils. They recommended all plant species producing these monoterpene compounds as potential anti-inflammatory agents. In recent years, anti-microbial, anti-inflammatory and immune stimulating properties of essential oils have become a subject of interest to clinicians. This is largely due to serious problems in the therapeutic treatment of recurrent infections caused by resistant pathogens. Research shows that many of the oils can be used in the treatment of wounds and skin infections. Riella *et al.* [30] in their studies on animal models showed that essential oil from *Lippia gracilis* Schauer (*Verbenaceae*) with its major component – thymol is a promising compound to be used in treatment of inflammatory processes as well as wound healing. According to Tavares *et al.* [31], the essential oil from *Distichoselinum tenuifolium* (Lag.) Garcia Martin & Silvestre revealed significant antifungal activity against *Cryptococcus neoformans* and dermatophyte strains and significantly inhibited nitric oxide production stimulated by LPS in macrophages, without affecting cell viability at concentrations ranging from 0.64 µl/ml to 1.25 µl/ml. Yoon *et al.* [32] proved that essential oil from *Abies koreana* can be used in the treatment of acne because of their antibacterial potential against *Propionibacterium acnes* and *S. epidermidis*. The tested oil reduced the LPS-induced secretion of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, nitric oxide and prostaglandin E(2) in RAW 264.7 macrophages, indicating that it has anti-inflammatory effects. Süntar *et al.* [33] presented the interesting results connected with the use of a new ointment formulation to provide more efficient wound healing. This ointment containing

essential oils from *Hypericum perforatum* L., *Origanum majorana* L., *Origanum minutiflorum* Schwrd. et Davis and *Salvia triloba* L. not only affected wound healing, but also exerted bactericidal and candicidal activities. Due to the enormous interest in staphylococci, including MRSA and coagulase-negative species that are increasingly being the cause of the difficult infections, the *Staphylococcus* genus was chosen for the investigations with clary sage oil. The studies of the clary sage oil activity against Gram-negative pathogens are also planned. The *Escherichia, Enterobacter, Acinetobacter, Citrobacter, Pseudomonas* and *Proteus* species which were isolated from recurrent, difficult-to-treat wound infections will be investigated. Our results may provide the basis for further research on the local use of the clary sage oil as a complement of existing anti-infective therapies in clinical conditions. Data on the antimicrobial activity of numerous plants have been scientifically confirmed inter alia against pathogenic microorganisms resistant to antimicrobials [34, 35].

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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