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RESEARCH ARTICLE

Evaluation of the chemical composition and biological activities of *Salvia officinalis* subsp. *lavandulifolia* (Vahl) Gams essential oil

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Abstract

The main aim of the present study was to investigate the antibacterial effect of the commercial Pharma Grade *S. officinalis* subsp. *lavandulifolia* (Lamiaceae) essential oil against the skin pathogen *Staphylococcus aureus*. The chemical composition of the essential oil was confirmed by GC and GC/MS, simultaneously. Camphor (30.5%), 1,8-cineole (24.8%), α-pinene (6.5%), linalool (4.0%) and linalyl acetate (3.5%) were found as major components. The bioactivity of the essential oil and its main compounds were tested using the *in vitro* microdilution technique. Minimum inhibitory concentration (MIC) values of α-pinene, essential oil, camphor, linalool and linalyl acetate were in the range of 2.5-10 mg/mL, respectively. The results showed that the tested pathogen was only moderately susceptible against the essential oil and its main compounds when compared with standard antibiotics. In addition, the *in vitro* antioxidant activity was evaluated using the radical scavenging activity mediated by 1,1-diphenyl-2-picrylhydrazyl (DPPH). The activity range was found more than 30 mg/mL for all tested oil samples, compared with the standard. The results suggested that the oil and its major constituents represent antimicrobial activity supporting its antiseptic use in folk medicinal use.

Keywords: *Salvia lavandulifolia* Vahl., essential oil, antibacterial activity, antioxidant activity

Introduction

The *Salvia* genus, with more than 900 species throughout the world, is one of the most widespread members of the Lamiaceae family, with many species classified as culinary or medicinal. *S. officinalis* subsp. *lavandulifolia* (Lamiaceae) commonly known as "Spanish sage" is an aromatic plant. *S. officinalis* subsp. *lavandulifolia* has commonly been used in folk medicine as spasmolytic, antiseptic, analgesic, sedative and antioxidant, among others. Furthermore, it is used in dementia therapy attributed to its sedative, antioxidant, anti-inflammatory, estrogenic and anticholinesterase activities (Perry et al., 2003). It is a small woody herbaceous perennial shrub up to 17–100 cm with mauve-blue flowers that grow preferably on the sandy–calcareous soils of mountainous areas (at 350–2000 m) of Spain, southeast of France and northwest of Africa (Kintzios, 2000; Porres-Martinez et al., 2013).

Staphylococcus aureus is a Gram-positive, ubiquitous organism that is found in both hospital and common areas. *S. aureus* is found on the skin, mucous membranes, and humans are the major reservoir for this organism. It is estimated that up to half of all adults are colonized (Fanelli et al., 2011). While *S. aureus* colonizes the skin, it can also be responsible for localized cutaneous infection such as acne (Deleo et al., 2010; Chambers & Deleo, 2009). Antibiotics are commonly used in treatment of acnes caused by *S. aureus* (Deleo et al., 2010).

In this present study, it was aimed to investigate the antimicrobial and antioxidant activity of the pharma grade *S. officinalis* subsp. *lavandulifolia* essential oil and its major components against the skin pathogenic bacterium *S. aureus.*

Materials and Methods

Materials

Pharma grade *S. lavandulifolia* essential oil was obtained from Aromapharm Company, Germany, the standard antibiotic ciprofloxacin, Gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH)• and major components were also acquired from commercial sources like Sigma-Aldrich (St. Louis, USA) if not otherwise stated.

Methods

Gas chromatography and gas chromatography-mass spectrometry (GC and GC-MS) analysis

The essential oils were analysed by GC using a Hewlett Packard 6890 system and an HP Innowax fused silica capillary column (FSC) (60 m x 0.25 mm Ø, with 0.25 µm film thickness) was used with nitrogen as carrier gas at 1 mL/min. Initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220°C, then kept constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage compositions of the individual components were obtained from electronic integration using flame ionization detection (FID, 250 °C) (Demirci et al., 2008). Relative percentages of the separated compounds were calculated from FID chromatograms as cited in Table 1. GC-MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd, Istanbul, Turkey) and Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425 as previously reported (Demirci et al., 2008). Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC-MS Library, MassFinder Software 4.0) (McLafferty & Stauffer, 1989, Hochmuth, 2008), and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & König, 1998) was used for the identification as also previously reported in detail (Demirci et al., 2008).

Antibacterial activity

The microbial strain used was obtained from the American Type Culture Collection (ATCC) in lyophilized form. The microorganism was stored at -85 °C in glycerol until inoculation and purity testing.

The potential antibacterial activity of both the essential oil and the main compounds, α-pinene, camphor, linalool, linalyl acetate and 1,8-cineole were evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute (CLSI) method (Anonymous, 2006; Demirci et al., 2015). *Staphylococcus aureus* ATCC 11632 was used as test microorganism. The samples (20-0.019 mg/mL) were dissolved in sterile dimethyl sulfoxide (DMSO) for the initial stock solution. 100 µL of samples were applied to 96-well microplates and 2 fold serial dilutions were performed. After the dilutions, 50 µL aliquots of tubidometrically adjusted microorganism was inoculated (10^5-10^6 CFU /mL) on to the plates. After incubation at 37 °C for 24 h the first well was treated with 20 µL of resazurin, which insured on all microplates the Minimum Inhibitory Concentrations (MIC), where the lowest concentration of the samples prevented visible growth. The standard antibiotic ciprofloxacin (128-0.25 µg/mL) were used as standard control. Solvent and microbial controls were also added to the assay plate. Antibacterial assays were repeated three times for all the test samples.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

Serial dilutions were carried out with stock solutions (10 mg/mL) of the essential oils to obtain the concentrations of 10-3125×10^{-4} mg/mL. Diluted solutions were mixed with DPPH and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm at room temperature using a microplate spectrophotometer. The experiment was performed three times and average absorption was noted for each concentration. Gallic acid was used as a positive control. The percentage of inhibition was calculated using equation. The EC_{50} value, which is the concentration of the test materials that inhibits 50% of the free radical concentration, was calculated as mg/mL (Kumarasamy et al., 2007).

$$\text{Percentage Inhibition} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] x100$$

Results and Discussion

Gas chromatography and gas chromatography-mass spectrometry (GC and GC-MS) analysis

The chemical composition of the essential oil *S. officinalis* subsp. *lavandulifolia* was characterized by GC and GC-MS analysis. The results are shown in Table 1. The major components were camphor (30.5%), 1,8-cineole (24.8%), *α*-pinene (6.5%), linalool (4.0%) and linalyl acetate (3.5%). Thirty-two components were identified representing 99.1% of the sample, confirming the Pharmacopoeia (PHEUR 9.0) standard (Anonymous, 2016).

Table 1. Volatile components of *S. officinalis* subsp. *lavandulifolia* essential oil

RRI[a]	Component	%[b]
1014	Tricyclene	0.3
1032	*α*-Pinene	6.5
1035	*α*-Thujone	0.1
1076	Camphene	5.2
1118	*β*-Pinene	4.6
1132	Sabinene	1.0
1174	Myrcene	2.0
1203	Limonene	4.1
1213	1,8-Cineole	24.8
1246	(*Z*)-*β*-Ocimene	0.1
1255	*γ*-Terpinene	0.2
1266	(*E*)-*β*-Ocimene	tr
1280	*p*-Cymene	0.6
1290	Terpinolene	0.2
1474	*trans*-Sabinene hydrate	0.1
1532	Camphor	30.5
1553	Linalool	4.0
1565	Linalyl acetate	3.5
1590	Bornyl acetate	0.9
1611	Terpinen-4-ol	0.4
1612	*β*-Caryophyllene	1.5
1658	Sabinyl acetate	1.9

Table 1. cont.

RRI[a]	Component	%[b]
1687	α-Humulene	0.3
1706	α -Terpineol	1.0
1709	α-Terpinyl acetate	0.9
1719	Borneol	3.4
1755	Bicyclogermacrene	0.1
1765	Geranyl acetate	0.1
1819	Geranyl isobutyrate	0.3
1857	Geraniol	0.2
1864	*p*-Cymene-8-ol	0.1
	Total	**99.1**

[a]RRI: Relative retention indices calculated against n-alkanes; [b]%: calculated from FID data; tr, identification based on the retention times (tR) of genuine standard compounds on the HP Innowax column

In a previous study, the main constituent of *S. officinalis* subsp. *lavandulifolia* essential oil was reported to contain 1,8-cineole (21.4-33.8 %), followed by α-pinene (10.5-17.5 %), β-pinene (6.0- 17.3 %), limonene (5.6-10.4 %) camphor (6.1- 9.4 %) and β-caryophyllene (4.0-8.5 %), respectively (Usano-Alemany et al., 2012). According to Pierozan (2009), the main components of *S. officinalis* subsp. *lavandulifolia* essential oil were β-thujone (19.9%), camphor (18.9%), α-thujone (18.9%), 1,8-cineole (8.1%), and β-pinene (3.9%) (Pierozan et al., 2009). In other work, among 27 identified constituents, camphor was the most abundant one (29.1%), along with 1,8-cineole (20.3%) and α-pinene (8.2%) (Nikolic et al., 2014).

Antibacterial activity

The antibacterial activity of *S. officinalis* subsp. *lavandulifolia* essential oil and major components were examined against human pathogen *Staphylococcus aureus* ATCC 11632. *S. aureus* was inhibited at a concentration of 2.5 mg/mL by α-pinene with the best performance among the tested samples. The other samples tested, except for 1,8-cineole showed rather moderate inhibitory effects. 1,8-Cineole showed the lowest inhibition against *S. aureus*. The comparative results are given in Table 2.

Table 2. Antibacterial evaluation of the oil and its major components (MIC, mg/mL)

Test samples	*Staphylococcus aureus* ATCC 11632
S. officinalis subsp. *lavandulifolia* essential oil	5
Camphor	5
α-Pinene	2.5
Linalool	10
Linalyl acetate	10
1,8-Cineole	>20
Ciprofloxacin	0.03

As a result of this present work, the *S. officinalis* subsp*. lavandulifolia* essential oil was evaluated for its *in vitro* biological activities such as antibacterial and antioxidant activities. The chemical analysis, confirmed with camphor (30.5%), 1,8-cineole (24.8%), *α*-pinene (6.5%), linalool (4.0%), linalyl acetate (3.5%), respectively, the quality. One of the major skin pathogens *S. aureus* presented higher sensitivity for the essential oil rather its major components, except for *α*-pinene. The lowest MIC was observed when *S. aureus* was exposed to 2.5 mg/mL *α*-pinene, while the lowest sensitivity with MIC of >20 mg/mL value was obtained when *S. aureus* was exposed to 1,8-cineole. It is also difficult to say that the main components act synergistically.

According to the literature, MIC= 2.3 mg/mL was observed for the pathogenic *S. aureus* strain against when interacted with *S. officinalis* subsp. *lavandulifolia* essential oil (Pierozan et al., 2009). Nikolic et al. (2014) reported that, MIC was 6**.**2 mg/mL against *S. aureus* (Nikolic et al., 2014). In addition, Tzakou et al. (2001) were determined MIC of main components, *α*-pinene and 1,8-cineole, 7.5 and 9.5 mg/mL, respectively (Tzakou et al., 2001).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was used to determine the antioxidant capacity of *S. officinalis* subsp. *lavandulifolia* essential oil. However, EC_{50} values of essential oils were found more than 30 mg/mL. EC_{50} value of gallic acid which used as a positive control, was found 0.004 mg/mL. When the compared with gallic acid, the samples were showed weak DPPH radical scavenging activities.

Recently, Cutillas et al. (2017) studied the antioxidant activity of *S. officinalis* subsp*. lavandulifolia* essential oil and its main components by DPPH method and found activity lower than 0.05 units at a maximum assay concentration of 100 mM.

As an overall result, our antibacterial and antioxidant results are comparable with literature findings. The results obtained show correlation with previous published data.

Conclusion

More in detail evaluations on biological activity both on *in vitro* and *in vivo* levels are needed to exhaust the potential of essential oils from *Salvia* sp. Further work is ongoing.

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