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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF *SALVIA SCLAREA* (LAMIACEAE) ESSENTIAL OIL

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Abstract — Clary sage (*Salvia sclarea* L.) is native to Southern Europe and is cultivated worldwide. The essential oil of clary sage was analyzed as a potential antifungal agent. The main compounds in the oil were linalyl acetate (52.83%) and linalool (18.18%). Food poisoning agents, spoilage fungi, and plant and animal pathogens were among the tested fungal species. The microdilution method was used to establish minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC). The commercial antimycotic bifonazole was used as a control. A concentration of 25 µl/ml showed fungicidal activity against *Aspergillus*, *Penicillium*, and *Fusarium* species and *Trichoderma viride*. For the species *Mucor mucedo* and *Aspergillus viride*, the MFC was 15µl/ml; for *Candida albicans*, it was 10 µl/ml, as in the case of bifonazole. Fungistatic and fungicidal activities of the oil against *Cladosporium cladosporioides* and *Trichophyton menthagrophytes* were recorded at concentrations of 2.5 µl/ml and 5 µl/ml. The most sensitive micromycetes were *Cladosporium fulvum*, *Alternaria alternata*, *Phomopsis helianthi*, and *Phoma macdonaldii*, where a concentration of 2.5 µl/ml was lethal.

Key words: *Salvia sclarea,* essential oil, antifungal activity, micromycetes

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INTRODUCTION

Salvia sclarea L. is a stout biennial or perennial herb up to one meter high with large hairy leaves that are green with a hint of purple. Its small flowers are blue, white, or pink. Clary sage is native to Southern Europe and is cultivated worldwide, especially in the Mediterranean region and Central Europe. In aromatherapy, it is a good relaxant for stress, asthma, and digestive and menstrual problems. Essential oil from *S. sclarea* is used as an antidepressant, antiseptic, antispasmodic, carminative, and aphrodisiac. Clary sage oil is also extensively used in processed food of all types, as well as in alcoholic and soft drinks (Lavabre, 1998; Lawless, 2002). This oil showed antimicrobial (Pitarokili, 2005) and larvacidal activity against the house mosquito *Culex pipiens* (Cetin et al., 2006) and *Spodoptera littoralis* (Pavela, 2005).

The most important components in the oils are alcohols (linalool, terpineol) and esters (linalyl acetate, α-terpinyl acetate, geranyl acetate) (Peana et al., 1999; Pitarokili et al., 2002; Fraternale et al., 2005; Farkaš et al., 2005).

The aim of this work was to examine the chemical composition of *S. sclarea* oil and evaluate its effect on growth of 18 micromycetes and the yeast *Candida albicans*.

MATERIAL AND METHODS

The essential oil used in the experiment was a commercial sample obtained from the Dr. Josif Pančić Institute for Medicinal Plant Research (Belgrade, Serbia).

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

Qualitative and quantitative analyses of *S. sclarea* oil was performed using GC and GC/MS. The GC analysis was carried out on a GC HP-5890 II apparatus equipped with a split-splitless injector attached to an HP-5 column (25 m x 0.32 mm, 0.52 μm film thickness) and fitted to an FID. The carrier gas (H_2) flow rate was 1 mL/min at a split ratio of 1:30. The injector temperature was 250°C, the detector temperature 300°C. The column temperature was linearly programmed from 40° to 240°C (at a rate of 4°/min). The same analytical conditions were employed for GC/MS analysis, where an HP G 1800C Series II GCD system equipped with an HP-5MS column (30 m x 0.25 mm, 0.25 μm film thickness) was used. The transfer line was heated to 260°C. Mass spectra were acquired in the EI mode (70 eV) in an m/z range of 40-400. Identification of individual oil components was accomplished by comparison of retention times with standard substances and by matching mass spectral data with MS libraries (NIST/NBS and Wiley 275.l) using computer search and literature sources (A d a m s , 2001). For quantitative analysis, area percent data obtained by FID were used as the base.

Antifungal assay

Antifungal activity was tested using the following micromycetes: *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *A. versicolor* (ATCC 11730), *A. flavus* (ATCC 9170), *A. terreus* (ATCC 16792), *Alternaria alternata* (ATCC 13963), *Aureobasidium pullulans* (ATCC 9348), *Cladosporium cladosporioides* (ATCC 13276), *C. fulvum* (TK 5318), *Fusarium tricinctum* (CBS 514478), *F. sporotrichoides* (ITM 496), *Mucor mucedo* (ATCC 52568), *Penicillium ochrochloron* (ATCC 9112), *P. funiculosum* (ATCC 10509), *Phoma macdonaldii* (CBS 38167), *Phomopsis helianthi* (ATCC 201540), *Trichoderma viride* (IAM 5061), *Trichophyton mentagrophytes* and yeast *Candida albicans*.

Microdilution method

In order to investigate the antifungal activity of essential oil, a modified version of the microdilution technique was used (H a n e l and R a e t h e r , 1988; D a o u k et al., 1995). Fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 μL per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum. Determination of MIC values was performed by a serial dilution technique using 96-well microtiter plates. The investigated essential oils were dissolved in MA or SDA broth containing fungal inoculum. The microplates were incubated for 72 h at 28°C. The lowest concentrations without visible growth (under a binocular microscope) were defined as the minimal concentrations which completely inhibited fungal growth (MIC). The minimal fungicidal concentrations (MFC) were determined by serial subcultivation of a 2-μL volume on microtiter plates containing 100 μL of broth per well and further incubation for 72 h at 28°C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum compared to bifonazole.

Micromycetes were cultivated on malt agar (MA) medium at room temperature for 24 h (B o o t h , 1971). Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) were determined. The lowest concentrations without visible growth were defined as the MIC, while MFC values were determined as the lowest concentrations with no visible growth after reinoculation of the original inoculum. The commercial drug bifonazole was used as a positive control.

RESULTS AND DISCUSSION

The results of chemical analysis of *S. sclarea* essential oil are presented in Table 1. The 34 identified components represent 98.94% of the total oil. The main components were linalyl acetate (52.83%), linalool (18.18%), α-terpineol (5%), α-pinene (4.57%), 1.8-cineole (2.29%), limonene (1.55%), β-caryophyllene (1.83%) and β-terpineol (1.19%).

Table 1. Chemical composition (expressed as %) of *Salvia sclarea* L. essential oil.

Component	%	KI*
α-pinene	4.57	939
β-pinene	0.90	979
β-myrcene	1.01	991
p-cymene	0.18	1026
limonene	1.55	1029
1.8-cineole	2.29	1031
cis-β-ocymene	0.32	1037
trans-β-ocymene	0.66	1050
isoterpinolene	0.41	1089
linalool	**18.18**	1097
camphor	0.30	1147
β-terpineol	1.19	1163
borneol	0.64	1169
α-terpineol	5.00	1189
γ-terpineol	0.90	1199
linalyl formate	0.14	1216
nerol	0.26	1230
linalyl acetate	**52.83**	1257
bornyl acetate	0.83	1289
α-terpinyl acetate	0.18	1350
neryl acetate	0.52	1362
α-copaene	0.55	1377
β-bourbonene	0.94	1388
β-cubebene	0.67	1390
β-elemene	0.35	1391
β-caryophyllene	1.83	1418
α-hummulene	0.08	1454
germacrene D	0.84	1485
bicyclogermacrene	0.17	1500
δ-cadinene	0.13	1523
spathulenol	0.13	1576
caryophyllene oxide	0.27	1581
sclareole oxide	0.08	2220
sclareol	0.06	2223
total	**98.94**	

* In elution order on HP-5 column

A previous study reported that *S. sclarea* oil contains linalyl acetate, linalool, geranyl acetate, and terpineol as the main components (Pitarokili et al., 2002). Oil of clary sage from Italy possessed linalool, linalyl acetate, geranyl acetate, trans-β-ocimene, and caryophyllene oxide as the dominant components (Fraternale, 2005). Sokvić (2001) reported the chemical composition of wild *S. sclarea* from Southern Serbia. According to her study the main constituent of the oil was the diterpene sclareol (28.29%). Farkaš et al. (2005) found that clary sage oil from flowers was characterized by high content of linalool, sclareol, and linalyl acetate, whereas gemacrene D, bicyclogermacrene, β-caryophyllene and spathulenol were found as major components in the leaf oil.

The minimal inhibitory and fungicidal concentrations (MIC and MFC) of *S. sclarea* oil are presented in Table 2.

In tests based on the microdilution method, the essential oil exhibited fungicidal characteristics with MIC and MFC values of 2.5-25 μl/ml. A concentration of 25 μl/ml showed fungicidal activity against *Aspergillus*, *Penicillium*, and *Fusarium* species and *Trichoderma viride*. For the species *Mucor mucedo* and *Aspergillus viride*, the MFC was 15 μl/ml; and for *C. albicans*, it was 10 μl/ml, as in the case of bifonazole. Fungistatic and fungicidal activities of the oil against *Cladosporium cladosporioides* and *Trichophyton menthagrophytes* were recorded at concentrations of 2.5 μl/ml and 5 μl/ml. The most sensitive micromycetes were *C. fulvum*, *A. pullulans*, *A. alternata, P. helianthi*, and *P. macdonaldii*, where a concentration of 2.5 μl/ml was lethal.

According to Yousefzadi et al. (2007) *S. sclarea* oil showed moderate to high antimicrobial activity against bacteria, but weak activity against the yeasts *Candida albicans* and *Saccharomyces cerevisiae*. Clary sage oil caused total inhibition of mycelia growth in three soil-borne pathogens (Pitarokili, 2003), as well as in the phytopathogenic fungi *Fusarium oxysporum*, *Alernaria solani*, *Botritys cinerea*, and *Rhizoctonia solani* (Fraternale, 2005).

Previous results indicate that the chemical composition of essential oils can affect their antimicrobial activity. Some chemical configurations had greater potency, while others were less potent. A previous study revealed that linalyl acetate was slightly effective in suppressing mycelial growth (Sokvić, 2001). This suggests that a relationship exists between the high presence of linalyl acetate

Table 2. Minimal inhibitory (MIC) and fungicidal (MFC) concentrations of *Salvia sclarea* essential oil.

Fungi	*S. sclarea*		bifonazole	
	MIC(µl/ml)	MFC(µl/ml)	MIC(µl/ml)	MFC(µl/ml)
Alternaria alternata	2.5	2.5	10	10
Aspergillus niger	25	25	10	10
Aspergillus ochraceus	10	25	10	15
Aspergillus flavus	25	25	10	15
Aspergillus terreus	15	25	10	15
Aspergillus versicolor	10	15	10	10
Aureobasidium pullulans	2.5	2.5	5	10
Cladosporium cladosporioides	2.5	5	10	10
Cladosporium fulvum	2.5	2.5	5	10
Fusarium tricinctum	15	20	15	20
Fusarium sporotrichioides	20	25	15	20
Mucor mucedo	10	15	15	15
Penicillium funiculosum	10	20	15	20
Penicillium ochrochloron	25	25	15	20
Phomopsis helianthi	2.5	2.5	10	10
Phoma macdonaldii	2.5	2.5	10	15
Trichoderma viride	25	25	15	20
Trichophyton menthagrophytes	2.5	5	10	15
Candida albicans	5	10	10	15

and linalool in *S. sclarea* oil and the moderate antifungal activity of this oil.

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ХЕМИЈСКИ САСТАВ И АНТИФУНГАЛНА АКТИВНОСТ ЕТАРСКОГ УЉА *SALVIA SCLAREA* (LAMIACEAE)

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Шарлахна жалфија (*Salvia sclarea* L.) је као самоникла врста распрострањена у Јужној Европи, а култивисана широм света. У раду је анализирано етарско уље ове врсте и утврђивана његова антифунгална активност. Главне компоненте етарског уља су линалил ацетат (52.83%) и линалол (18.18%). Као тест организми коришћене су гљивице које изазивају кварење хране, као и патогени биљака и животиња. Коришћењем микродилуционе методе одређиване су минималне инхибиторне (MIC) и минималне фунгицидне концентрације (MFC). Комерцијални антимикотик бифоназол је коришћен као контрола. У концентрацији од 25 μl/ml уље је деловало фунгицидно на врсте родова *Aspergillus*, *Penicillium* и *Fusarium* и врсту *Trichoderma viride*. За врсте *Mucor mucedo* и *Aspergillus viride* MFC је била 15 μl/ml, и 10 μl/ml за *C. albicans*, слично као за бифоназол. Потпуно заустављање раста мицелија *Cladosporium cladosporioides* и *Trichophyton menthagrophytes* је постигнуто при концентрацијама 2.5 μl/ml и 5 μl/ml. Најосетљивије микромицете биле су *Cladosporium fulvum*, *Alternaria alternata, Phomopsis helianthi* и *Phoma macdonaldii*, за које је летална концентрација била 2.5 μl/ml.