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Composition and Antifungal Activity on Soil-Borne Pathogens of the Essential Oil of *Salvia sclarea* from Greece

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The hydrodistilled essential oils of the aerial parts of wild-growing *Salvia sclarea* originated from two localities in Greece were analyzed by GC-MS. Sixty-six compounds, representing 93.26−98.19% of the oils, were identified. Linalyl acetate (19.75−31.05%), linalool (18.46−30.43%), geranyl acetate (4.45−12.1%), and α-terpineol (5.08−7.56%) were the main components. The antifungal activity of the oil of one locality and of the main components, linalyl acetate and linalool, was evaluated in vitro against three soil-borne pathogens.

KEYWORDS: ***Salvia sclarea*; GC-MS analysis; essential oil; linalool; linalyl acetate; antifungal activity; soil-borne pathogens**

INTRODUCTION

Lamiaceae is a plant family within which there can be found several species, with potential therapeutic activity especially due to their essential oils. Pharmacology, pharmaceutical botany, medical and clinical microbiology, phytopathology, and food preservation are some fields in which essential oils can be applied. The antimicrobial activity of the essential oils or their main components on important human pathogenic organisms and microorganisms that cause food spoilage has been reported by several researchers (*1*−*5*). Recently, interest in the research of the effectiveness of essential oils to control plant pathogens has increased (*6*−*8*).

Salvia sclarea L. (clary sage) belongs to section *Aethiopis* Bentham. It is a biennial or perennial shrub up to 100 cm high native to southern Europe (*9*). Clary sage oil has been reported to show anticonvulsive activity in animals. Except being moderately irritating to rabbit skin, relevant data indicate clary sage oil to be generally nontoxic (*10*). The herb has been used as a stomachic in digestive disorders and in kidney diseases. Moreover, clary sage oil is extensively used in food and in cosmetic products (*11*). Due to its uses *S. sclarea* is cultivated in France, Russia, and the United States.

In Greece *S. sclarea* is known by the common name "agiannitis" (*12*). Locally it is used for coughs, colds, and blood cleaning, on wounds and sore eyes, and as a diuretic (*13*).

Researchers have studied the chemical composition of *S. sclarea* essential oil of different origin (*14*−*18*). Recently Foray et al. (*17*) have reported a strong cytotoxic activity of *S. sclarea* essential oil, equivalent to that of doxorubicin. *S. sclarea* oil showed significant antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albicans*. To our knowledge the antifungal activity on soil-borne pathogens of *S. sclarea* oil has never been studied before.

The purpose of the present work was to determine the chemical composition of the essential oil of *S. sclarea* from two different localities (central and northwestern Greece) by GC-MS analysis and to evaluate the efficacy of the oil (sample B) and its pure major components on the radial growth of three phytopathogenic fungi, *Sclerotinia sclerotiorum*, *Sclerotium cepivorum*, and *Fusarium oxysporum* f. sp. *dianthi*, in view of a use, if possible, as natural fungicides. The oil of sample B was tested as it was available in a sufficient amount, enough for all of the assays.

MATERIALS AND METHODS

Materials. Aerial parts of wild-growing *S. sclarea* L. were collected during the flowering stage from Mt. Katara [sample A; 1700 m, in Prefecture Trikala (central Greece)] in July 1995 and from Monodendri [sample B; 1060 m, Prefecture Ioannina (northwestern Greece)] in July 1999. Voucher specimens are kept at the Laboratory of the Pharmacognosy Department, University of Athens. Pure commercial oil components were purchased from the Sigma-Aldrich Co.

Isolation of the Essential Oils. Semicrushed air-dried leaves were subjected to hydrodistillation for 2 h, using a modified Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and kept at −4 °C until it was analyzed.

Gas Chromatography—Mass Spectrometry (GC-MS). Analysis of the essential oils was performed using a Hewlett-Packard 5973-6890 GC-MS system operating in EI mode at 70 eV, equipped with an HP-5 MS capillary column (30 m × 0.25 mm; film thickness = 0.25 μm). The initial temperature of the column was 60 °C and was raised to 280 °C at a 3 °C/min rate. Carrier gas was He, flow rate = 1 mL/

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Table 1. Chemical Composition of *S. sclarea* Essential Oils from Two Localities

compound	RI[a]	sample[b] A	sample[b] B
cis-3-hexenol		tr[c]	
α-pinene	907	tr	tr
benzaldehyde	931		tr
camphene	939	tr	
β-pinene	949	tr	tr
myrcene	963	1.76	1.40
α-terpinene	993		tr
p-cymene	1002	tr	tr
limonene	1005	0.62	0.54
1,8-cineole	1008		tr
(*Z*)-β-ocimene	1013	0.61	0.77
benzeneacetaldehyde	1019		tr
(*E*)-β-ocimene	1023	0.75	1.31
γ-terpinene	1033		tr
cis-linalool oxide	1047		tr
terpinolene	1063	tr	0.59
trans-linalool oxide	1065		tr
linalool	1084	30.43	18.46
cis-β-terpineol	1132	tr	
α-terpineol	1169	5.08	7.56
δ-3-carene	1190		0.42
nerol	1207	tr	1.24
geraniol	1230	4.21	
linalyl acetate	1238	19.75	31.05
geranial	1282	0.96	
geranyl formate	1300	0.48	
α-cubebene	1322		0.31
neryl acetate	1339	7.78	2.01
α-ylangene	1342		tr
α-copaene	1347		1.67
geranyl acetate	1359	12.1	4.45
β-cubebene	1361		0.52
β-elemene	1363		tr
(*E*)-caryophyllene	1390	1.95	2.28
β-gurjunene	1398		tr
α-*trans*-bergamotene	1405		tr
aromadendrene	1407		tr
α-humulene	1422	tr	tr
cis-muurola-4(14),5-diene	1432	tr	tr
germacrene D	1451	2.57	
valencene	1456	0.39	0.49
epicubebol	1462	tr	tr
bicyclogermacrene	1464		tr
α-muurolene	1469		tr
germacrene A	1489	tr	tr
(*E*,*E*)-farnesene	1492	tr	tr
δ-cadinene	1506	tr	0.53
α-calacorene	1524	tr	tr
1,5-epoxysalvial-4(14)-diene	1548	tr	0.44
(*Z*)-3-hexenyl benzoate	1554	tr	
germacrene-D-4-ol	1558	tr	
spathulenol	1560		0.66
caryophyllene oxide	1566	0.72	2.34
salvial-4(14)-en-1-one	1568		0.44
β-copaen-4-α-ol	1570	tr	tr
β-oplopenone	1593	tr	tr
β-eudesmol	1636	1.27	1.05
α-eudesmol	1638	0.40	0.83
7-epi-α-eudesmol	1641	tr	
3-hydroxydodecanoic acid methyl ester	1650	0.27	
benzyl benzoate	1749	tr	
8,13-epoxy-15,16-dinorlab-12-ene	1873	1.02	3.03
farnesyl acetate[d]	1911	0.50	0.47
manoyl oxide[d]	1986	0.35	1.07
epi-13-manoyl oxide	2007	tr	0.63
manool	2033	0.69	1.15
sclareol	2200	3.53	5.55
total (%)		98.19	93.26

[a] RI = retention indices relative to C_9–C_{23} *n*-alkanes on the HP-5MS column (*22*). [b] Relative percentage obtained from peak area. [c] tr = trace (<0.05%). [d] Correct isomer not identified.

min. Split ratio was 1:10. Identification of the chemical constituents was based on comparisons of their relative retention times and mass spectra with those obtained from authentic samples and/or the NIST/NBS and Wiley libraries and the literature (*19*).

Fungal Strains and Media. Strains of the phytopathogenic fungi *F. oxysporum* f. sp. *dianthi*, *Sclerotinia sclerotiorum*, and *Sclerotium cepivorum* were supplied by the Benaki Phytopathological Institute. Cultures of the phytopathogenic organisms were maintained on potato dextrose agar (PDA) medium.

Antifungal Assays. The antifungal assays were carried out in vitro, in Petri dishes, 9 cm in diameter, containing PDA. The essential oil was dispersed as an emulsion in water using ethanol and Tween 20 and added to PDA immediately before it was emptied into the Petri dishes at a temperature of 45−50 °C. The concentrations tested were 500, 1000, 1500, and 2000 μL/L. The controls received the same quantity of ethanol and Tween 20 mixed with PDA. The phytopathogenic fungi were inoculated immediately after preparation of the Petri dishes by placing in the center of each plate a 6 mm diameter disk of the test species, cut with a sterile cork borer from the periphery of actively growing cultures on PDA plates. The Petri dishes were kept wrapped in aluminum foil at a temperature of 23 ± 1 °C. After a 4 day incubation period, the minimum inhibitory concentration (MIC) and the EC_{50} values (concentration causing 50% inhibition of mycelial growth on control media) were determined. Fungitoxicity was expressed in terms of percentage of mycelial growth inhibition. EC_{50} values were calculated from the data subjected to probit analysis (statistical software SPSS 7.0 Inc., Chicago, IL). To ascertain if the essential oil and its main components showed a fungicidal or fungistatic activity, parts of media from plates without mycotic growth were transferred into new PDA plates; no fungal growth after an incubation of 10 days was indicative of fungicidal activity. The main components of the oil of *S. sclarea*, linalyl acetate and linalool, were also screened for their antifungal activity using the method described above. The concentrations tested were 50, 100, 250, 500, 750, 1000, 1250, and 1500 μL/L.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oils. The oils obtained in 0.68% (sample A) and 0.42% (sample B) yields from the wild clary sage were yellowish and possessed a strong characteristic aroma. The physical properties of the essential oils of *S. sclarea* from the two localities were $[\alpha]_D^{20}$ (in $CHCl_3$) +2.15 and −3.73, respectively. The volatile constituents identified in the oils of *S. sclarea* are listed in **Table 1** in the order of elution from the HP-5 MS column. A total of 67 compounds, representing 93.26−98.19% of the oils, were identified. The qualitative oil compositions did not present any differences; however, there was considerable quantitative variation. The most abundant constituents of *S. sclarea* oil (sample A) were linalool (30.43%), linalyl acetate (19.75%), geranyl acetate (12.1%), α-terpineol (5.08%), and neryl acetate (7.78%), whereas in the oil of sample B, linalyl acetate was the dominating component (31.05%) followed by linalool (18.46%), α-terpineol (7.56%), and sclareol (5.55%). In sample B geranyl acetate and neryl acetate were found in much lower amounts (<5%).

Both oils (samples A and B) were characterized by a high content of oxygenated monoterpenes (80.79 and 64.77%, respectively). In sample A sesquiterpene hydrocarbons amounted to 4.91%, diterpenes to 4.22%, monoterpene hydrocarbons to 3.74% and oxygenated sesquiterpenes to 2.89%, whereas sample B contained 11.43% diterpenes, 6.23% oxygenated sesquiterpenes, 5.80% sesquiterpenes, and 5.03% monoterpene hydrocarbons.

Souleles and Argyriadou (*14*) have already reported the chemical composition of wild-growing *S. sclarea*, originated from northern Greece (Thessaloniki). According to their study the main constituents of the oil were linalool (17.2%), linalyl acetate (14.3%), geraniol (6.5%), geranyl acetate (7.5%), α-terpineol (15.1%), nerol (5.5%), neryl acetate (5.2%), and

Table 2. EC_{50} Values of the Oil of Sample B and Its Main Components on Fungal Species Tested

	Sclerotinia sclerotiorum	*Sclerotium cepivorum*	*Fusarium oxysporum* f. sp. *dianthi*
sample B	492.55 (419.71, 554.86)[a]	544.17 (511.06, 580.70)	584.36 (460.43, 690.93)
linalyl acetate	549.62 (458.67, 696.54)	>1500	>1500
linalool	146.15 (128.13, 166.08)	563.94 (513.64, 613.57)	661.76 (552.52, 816.63)

[a] Numbers in parentheses indicate 95% confidence limits determined by probit analysis.

sclareol (5.2%). From the comparison of the results of the two studies we noted significant quantitative differences, especially in the contents of α-terpineol [5.08% (sample A), 7.56% (sample B), and 15.1% (Souleles and Argyriadou)] and nerol [traces (sample A), 1.24% (sample B), and 5.5% (Souleles and Argyriadou)].

Torres et al. (*15*) reported that *S. sclarea* essential oil of Spanish origin was characterized by the presence of linalool (32.97%), α-terpineol (5.63%), linalyl acetate (16.85%), and germacrene D (7.57%), whereas in the essential oil of *S. sclarea* of French origin linalyl acetate (81.1%) was the dominating component followed by linalool (10.7%) (*17*).

The essential oil of *S. sclarea* growing wild on the Italian island of Sardinia presented noticeably different qualitative and quantitative results. Moretti et al. (*16*) detected as main components methylchavicol (49.02%), linalyl acetate (19.20%), and linalool (9.93%), whereas Peana et al. (*18*) found α-terpinyl acetate (22.1%), α-terpineol (47.4%), and linalyl acetate (12.7%).

According to Lawrence (*20*) the major difference between commercial oils of *S. sclarea* is in the linalool/linalyl acetate contents. In the present study in sample A the linalyl acetate content was lower than linalool, whereas in sample B the percentages were reversed.

The presence of sclareol, an important bioactive diterpene (*21*), was noticeable, especially in sample B (5.55%).

Antifungal Activity. Clary sage caused a dose-dependent inhibition of mycelial growth of the three fungi tested. The radial growth of *Sclerotinia sclerotiorum* was totally inhibited by *S. sclarea* essential oil at 1000 μL/L, whereas the growth of *S. cepivorum* and *F. oxysporum* f. sp. *dianthi* were inhibited 94.44 and 72.04% at 2000 μL/L. Moreover, the oil exhibited a fungicidal effect on *S. sclerotiorum* at the concentration of 2000 μ/L.

The EC_{50} values (**Table 2**) of the essential oil of *S. sclarea* (sample B) obtained for each fungus were *Sclerotinia sclerotiorum* (EC_{50} = 492.55 μL/L), *Sclerotium cepivorum* (EC_{50} = 544.17 μL/L), and *F. oxysporum* f. sp. *dianthi* (EC_{50} = 584.36 μL/L).

Pure commercial linalool and linalyl acetate were tested independently for their inhibitory action on the radial growth of *Sclerotinia sclerotiorum*, *Sclerotium cepivorum*, and *F. oxysporum* f. sp. *dianthi* at concentrations up to 1500 μL/L. Both linalool and linalyl acetate exhibited various degrees of inhibition depending on the fungi tested. The EC_{50} values of linalool obtained for *S. sclerotiorum*, *S. cepivorum*, and *F. oxysporum* f. sp. *dianthi* were 146.15, 563.94, and 661.76 μL/L, respectively. Linalyl acetate presented inhibitory effects on *S. sclerotiorum* (EC_{50} = 549.62 μL/L), whereas with the other fungi tested the EC_{50} values exceeded 1500 μL/L.

Linalool, which comprised 18.46% of the total oil, exhibited an effect comparable to that of the total oil on *S. cepivorum* and *F. oxysporum* f. sp. *dianthi*, whereas on *S. sclerotiorum* linalool was more effective. On the contrary, linalyl acetate (31.05%) was slightly effective on *S. cepivorum* and *F. oxysporum* f. sp. *dianthi,* whereas on *S. sclerotiorum* it presented an activity similar to that of the oil.

From the above results we concluded that the activity of the oil was attributable to the presence of not only linalool but also other components that could exert if not a direct activity, at least a synergic effect on linalool's action. Linalyl acetate might offer a certain degree of inhibition on *S. sclerotiorum*, but on the other two fungi tested it seemed to be quite inactive.

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