J.Serb.Chem.Soc. 68(1)17–24(2003)
JSCS – 3016

UDC 615.281:635.74+547.262
Original scientific paper

Chemical constituents and antimicrobial activity of the ethanol extracts obtained from the flower, leaf and stem of *Salvia officinalis* L.

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(Received 19 August 2002)

Abstract: In this paper a comparison of the chemical composition and antimicrobial action of the ethanol extracts from the flower, leaf and stem of the herbal species *Salvia officinalis* L. (Lamiaceae), originating from the southeast region of Serbia was carried out. The chemical composition of the extracts was determined by GC-FID and GC-MS analyses. Manool has the highest level of all the components (9.0–11.1 %). Antimicrobial activity was determined by the diffusion and dilution method, whereby the latter one was modified by use of cellulose discs, and it was applied for the determination of the minimal inhibitory (MIC) and minimal lethal concentrations (MLC). The leaf extract has a stronger antimicrobial activity than those of the flower and stem.

Keywords: *Salvia officinalis* L., Lamiaceae, extracts composition, manool, antimicrobial activity.

INTRODUCTION

Salvia is a large and polymorphous genus of the family Lamiaceae, comprising about 900 species with almost cosmopolitan dissemination.[1] The Flora of Serbia comprises 14 species of this genus.[2] A special position among them has the herbal species *S. officinalis* L. The content of some components varies depending on the locality, extraction procedures and extracting agents. The prevailing components in the extract obtained by ultrasound extraction[3] were alpha-thujone (48.4 %) and camphor (14.2 %), in the methylene chloride extract[4] were alpha-thujone (15.7–59.3 %), 1,8-cineole (10.9–43.1 %) and beta-thujone (4.9–25.8 %), whereas 1,8-cineole was the dominant component in the SF extract[5] (54.4 %).

Some components of the extracts and the essential oils of *S. officinalis* have antimicrobial activity. Linalyl acetate and terpineol have the greatest power of bacterial inhibi-

tion.[6] Antifungal action of alpha-bisabolol, farnesol, anethole, carvacrol has been proved.[7] Salvin from acetone extract of the dried flowers is effective against *Staphylococcus aureus*.[8]

In this work a comparison of the chemical composition and antimicrobial action of the extracts from the flower, leaf and stem of *Salvia officinalis* L. was carried out.

EXPERIMENTAL

Plant material. The sage *Salvia officinalis* L (Lamiaceae), originating from Sićevačka Klisura gorge (Gradište village, the southeast part of Serbia), was identified by Prof. Dr. Novica Randjelović (Faculty of Technology, University of Niš). A voucher specimen has been deposited in the General Herbarium of the Balkan Peninsula (BEO), the Natural History Museum in Belgrade, Yugoslavia (BEO 32147). The plants were collected during the flowering phase at the end of May 1998.

Extraction. Extracts from the dried, ground plant material (flower, leaf, stem) were obtained by maceration (hydromodule 1:5) with ethanol (96 % vol).[9]

Identification procedure. The extracts were analyzed by analytical GC-FID and GC-MS and most of the constituents were identified by comparison of their mass spectra with those from the Wiley MS library. The obtained results were correlated with retention indices.[10,11]

GC-FID: A Hewlett Packard 5890 II Gas Chromatograph, equipped with a 25 m × 0.32 mm fused silica capillary column, with a 0.53 μm film thickness of HP-5, and FID was used. The operating conditions were: column temperature program 40 º – 280 ºC at 4 ºC/min with an injector temperature of 250 ºC and a detector temperature of 280 ºC; carrier gas: H_2 (1 mL/min).

GC-MS: The analyses were performed on a Hewlett Packard, model G 1800 C, equipped with a fused silica 30 m × 0.25 mm, HP-5 capillary column, with a film thickness 0.25 μm; the carrier gas was H_2 (1 mL/min) with the same temperature program as for the analytical GC. Electrons at 70 eV performed the ionization. 1 μL of the extract is injected (splitless mode).

Antimicrobial activity. The antimicrobial action of the extracts was investigated by the diffusion and dilution methods. The dilution method was modified by use of cellulose discs to avoid addition of emulsifiers for better dissolution of the samples, and it was applied for the determination of minimal inhibitory (MIC) and minimal lethal concentration (MLC). Cultures of the following microorganisms were used: *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Sarcina lutea* ATCC 9341, *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763, obtained from Oxoid, as well as *Aspergillus niger* from the collection of micro-organisms of the Biological laboratory of "Zdravlje" Pharmaceutical and Chemical industry, Leskovac.

The following nutritive media were used for the diffusion method: Antibiotica-Agar No. 1 (Merck, Darmstadt, Germany) for bacteria, Tripton soya agar - TSA (Torlak Institute, Belgrade, Yugoslavia) for *C. albicans* and *A. niger*, Sabouraud dextrose agar - SDA (Torlak) for *S. cerevisiae* and Medium for total bacterial count (Torlak), for the determination of the total number of micro-organisms.

The following nutritive media were used for the dilution method: Medium 3 (Difco Laboratories Detroit Michigan USA) for bacterial growth, Tripton soya broth – TSB (Torlak) for growth of *C. albicans* and *A. niger*, Sabouraud liquid medium - SDB (Torlak) for growth of *S. cerevisiae*.

Medium for the total bacterial count (Torlak) for the determination of the total number of micro-organisms, selective media for the identification of micro-organisms: Endo agar (Torlak) for *E. coli*, SS agar (Torlak) for *S. enteritidis*, Cetrimid agar for *P. aeruginosa*, Brilliant Green agar (Torlak) for *B. subtilis*, Chapman medium (Torlak) for *S. aureus* and *S. lutea*, Tripton soya agar (Torlak) for *C. albicans* and *A. niger* and Sabouraud dextrose agar (Torlak) for *S. cerevisiae*. Diffusion method: 0.1 mL of micro-organism suspension, formed during 24 h culture on oblique agar with 10 mL 0.9 %NaCl, was introduced into 10 mL of the nutritive medium. A Petri dish was filled with this system. 5 μL of the pure extracts were applied by micropipette onto sterile cellulose discs, diameter 6 mm (Biolife Italiana SRL - Milano, Italy), and the disc

was placed into the center of an 86 mm internal diameter Petri dish. Following 2 h prediffusion at +4 ºC, the incubation was carried out for 24 h at 37 ºC for bacteria and 48 h at 26 ºC for fungi. The initial number of micro-organisms in the suspension was detemined after thermostating the medium for the total bacterial count during 24 h at 37 ºC mixed with 1 mL of 10^4 – fold diluted suspension.

Dilution method: From the suspension of micro-organism, formed with 10 mL 0.9 % NaCl and 24 h culture on oblique agar, the inoculum was made by introducing 0.1 mL of the suspension into 9.9 mL Medium 3 (for bacteria), Tripton soya broth (for *C. albicans* and *A. niger*) and Sabouraud liquid medium (for *S. cerevisiae*). Onto 12.7 mm diameter sterile cellulose discs (Schleicher & Shuell, Dassel, Germany), the following quantities were applied by micropipette: 2, 4, 6, 8, 10, 12, 14, 18, 20, 25, 30, 40, 50, 60 μL of the extracts for *B. subtilis*, *S. aureus*, *S. lutea*, *C. albicans*, *A. niger*; 40, 50, 60, 80, 100, 120, 140, 160, 180, 200 μL of the extracts for *E. coli*, *S. enteritidis*, *P. aeruginosa*, *S. cerevisiae*, which were submerged into sterile test tubes with 0.5 mL of the suitable medium, followed by the addition of 1 mL of the inoculum. The incubation was carried out at 37 ºC for bacteria and at 26 ºC for fungi. 1 mL of inoculum dilution 10^{-4} was overspread with the medium for the total bacterial count, which was used for the determination of the initial number of micro-organisms in the inoculum after thermostating for 24 h at 37 ºC. The changes in the clarity of the inoculum in the test tubes were monitored during 3 days. Re-inoculation was carried out from individual test tubes onto the medium for the total bacterial count as well as for the introduction of inoculum by loopfool onto selective media (determination of MIC and MLC).

RESULTS AND DISCUSSION

The obtained yields of the dry extracts were 3.5 % (flower), 3.1 % (leaf) and 1.2 % (stem). The results of GC-FID and GC-MS analyses of the ethanol extracts of *S. officinalis* from the flower, leaf and stem are presented in Table I. The number of identified components in the examined extracts are 60, 61 and 44, respectively. Manool was the major component (9.0–11.1 %). The examined extracts contain all the specific components defining the chromatograph profile[12] of the essential oil of *S. officinalis*: alpha-pinene, camphene, limonene, 1,8-cineole, linalool, *cis*- and *trans*-thujone, camphor, bornyl acetate and alpha-humulene. Comparing the contents of each part of the plant separately, the flower has the highest level of alpha-pinene and 1,8-cineole. Camphene, limonene, *cis*-thujone, *trans*-thujone, camphor, bornyl acetate and alpha-humulene are most present in the leaf extract. Linalool is most present in the stem extract. The other identified components present in higher percentage are: *n*-pentacosane (8.3 % in the stem extract), (*E*)-caryophyllene (5.3 % in the leaf extract and 4.7 % in the flower extract), *trans*-ferruginol (4.8 % in the flower extract and 1.1 % in the stem extract), *cis*-ferruginol (4.4 % in the flower extract) and viridiflorol (3.7 % in the leaf extract, 3.6 % in the flower extract and 2.7 % in the stem extract).

The similarity of the chemical composition between the sage from the Sićevačka Klisura gorge and from other localities can be discerned.[3–5,13] Components like menthone, neomenthol, menthol, spathulenol and others are present in the extract of sage originating from all the localities except the Sićevačka Klisura gorge. Also, there is a similarity in the composition of the extracts and the essential oils from the same localities.[14,15]

The results of the examined antimicrobical activities of the extract obtained from the flower, leaf and stem of *S. officinalis* by the diffusion method are shown in Table II. The leaf extract showed significantly higher antimicrobial activity compared to the other extracts. The antimicrobial activity of the extract against *E. coli*, *S. enteritidis*, *P. aeruginosa, C. albicans* and *S. cerevisiae* is not discerned. The MIC and MLC values (μL of extract/mL

TABLE I. Percentage compositions of the flower, leaf and stem extracts of *Salvia officinalis* L.

Constituents	KI	RIexp	Flower	Leaf	Stem
Furfural	836	893			0.3
Tricyclene[m]	927	923	0.2	0.2	
α-Pinene[m]	939	938	2.1	1.6	0.2
Camphene[m]	954	950	0.5	0.7	0.2
Sabinene[m]	975	974	tr.	tr.	0.3
β-Pinene[m]	979	976	1.8	0.9	0.8
3-Octanone	984	980	tr.	0.6	
Myrcene[m]	991	988	0.5	0.1	tr.
3-Octanol	991	995	0.1	tr.	
α-Phellandrene[m]	1003	989	0.1	0.2	tr.
α-Terpinene[m]	1017	1022	tr.	tr.	tr
p-Cymene[m]	1025	1029	tr.	tr.	tr.
Limonene[m]	1029	1033	tr.	0.1	tr.
1,8-Cineole[m]	1031	1036	7.3	3.1	1.7
(*Z*)-β-Ocimene[m]	1037	1041	0.1	0.2	tr.
(*E*)-β-Ocimene[m]	1050	1050	0.1	0.1	tr.
γ-Terpinene[m]	1060	1060	0.1	0.1	tr.
trans-Linalool oxide[m]	1073	1071	0.1	tr.	
Terpinolene[m]	1089	1088	0.3	0.3	
Linalool[m]	1097	1098	0.4	tr.	0.7
cis-Thujone[m]	1102	1105	0.6	4.5	2.1
trans-Thujone[m]	1114	1115	0.1	0.6	0.2
Camphor[m]	1146	1141	0.5	1.3	0.6
Borneol[m]	1169	1163	0.6	0.8	0.3
Terpinen-4-ol[m]	1177	1177	0.1	0.1	tr.
3-Decanone	1188	1187			0.3
α-Terpineol[m]	1189	1189	0.1	0.1	
n-Dodecane	1200	1221	0.3	0.4	
n-Decanol	1272	1270			0.3
Isobornyl acetate[m]	1286	1280	0.1	0.1	1.5
Bornyl actate[m]	1289	1285	tr.	0.1	
Carvacrol[m]	1299	1303	tr.	tr.	0.3
n-Tridecane	1300	1304	tr.	1.1	
α-Cubebene[s]	1351	1348	0.1	0.2	
Eugenol[m]	1359	1356	tr.	0.2	

TABLE I. Continued

Constituents	KI	RIexp	Flower	Leaf	Stem
α-Ylangene[s]	1375	1370	tr.	tr.	
α-Copaene[s]	1377	1371	0.3	0.6	tr.
β-Bourbonene[s]	1388	1379	0.1	0.1	0.4
β-Cubebene[s]	1388	1386	0.1	tr.	
(*E*)-Caryophyllene[s]	1419	1414	4.7	5.3	1.9
Aromadendrene[s]	1441	1435	tr.	0.1	0.4
α-Humulene[s]	1455	1449	4.0	5.6	1.9
allo-Aromadendrene[s]	1460	1455	0.1	0.1	
γ-Muurolene[s]	1480	1472	0.6	1.0	tr.
α-Muurolene[s]	1500	1496	0.5	0.8	
γ-Cadinene[s]	1514	1509		0.5	
δ-Cadinene[s]	1523	1519	0.2	1.3	0.3
trans-Calamenene[s]	1529	1528	0.7	0.1	
(*E*)-γ-Bisabolene[s]	1531	1532	0.1	0.1	
α-Calacorene[s]	1546	1537	0.1	0.1	
β-Calacorene[s]	1566	1557		0.1	
Caryophyllene oxide[s]	1583	1579	0.1	tr.	0.3
Viridiflorol[s]	1593	1591	3.6	3.7	2.7
n-Hexadecane	1600	1592	0.3	0.1	0.5
Humulene epoxide II[s]	1608	1602		0.8	
Epi-α-muurolol[s]	1642	1635	0.1	0.2	0.4
n-Hexadecanol	1876	1879	0.1		
Dibutyl phthalate	1990	1959	0.2	0.2	0.8
Ethyl hexadecanoate	1993	1996	0.3	0.1	tr.
n-Eicosane	2000	2010	0.1	0.1	tr.
Manool[d]	2057	2050	11.1	9.0	9.9
n-Octadecanol	2078	2110	tr.		0.2
Methyl octadecanoate	2125	2154		2.8	0.6
trans-Totarol[d]	2314	2299	0.6	0.4	4.0
trans-Ferruginol[d]	2332	2317	4.8	0.1	1.1
cis-Ferruginol[d]	2371	2340	4.4		
n-Pentacosane	2500	2477	0.1	tr.	8.3

KI – retention index by Kovats
RI – retention index experimental determined (medium value)
tr. – traces
m – monoterpenoids, s – sesquiterpeoids, d – diterpenes

TABLE II. Diameters of the inhibition zones (mm) caused by the action of the extracts of *Salvia officinalis* L.

Micro-organisms	CFU/0.1 mL of suspension	Flower	Leaf	Stem
Escherichia coli ATCC 25922	30×10^7	0	0	0
Salmonella enteritidis ATCC 13076	14×10^7	0	0	0
Pseudomonas aeruginosa ATCC 9027	19×10^7	0	0	0
Bacillus subtilis ATCC 6633	75×10^5	15.1	17.0	8.5
Staphylococcus aureus ATCC 6538	15×10^7	10.1	11.6	6.6
Sarcina lutea ATCC 9341	80×10^6	7.0	10.2	0
Candida albicans ATCC 10231	50×10^5	0	0	0
Saccharomyces cerevisiae ATCC 9763	85×10^5	0	0	0
Aspergillus niger	20×10^6	9.9	11.6	0

CFU – Number of Colony Forming Units

TABLE III. Values of MIC and MLC (μL/mL) of the extracts of *Salvia officinalis* L.

Micro-organisms	CFU/mL of inoculum	Flower		Leaf		Stem	
		MIC	MLC	MIC	MLC	MIC	MLC
Escherichia coli ATCC 25922	56×10^6	80	100	60	80	120	120
Salmonella enteritidis ATCC 13076	65×10^6	50	60	50	60	80	100
Pseudomonas aeruginosa ATCC 9027	55×10^6	60	100	60	80	100	120
Bacillus subtilis ATCC 6633	60×10^6	6	8	6	8	10	12
Staphylococcus aureus ATCC 6538	45×10^6	12	14	10	14	16	18

TABLE III. Continued

Micro-organisms	CFU/mL of inoculum	Flower		Leaf		Stem	
		MIC	MLC	MIC	MLC	MIC	MLC
Sarcina lutea ATCC 9341	70×10^6	20	25	20	25	40	50
Candida albicans ATCC 10231	80×10^5	40	60	40	60	40	50
Saccharomyces cerevisiae ATCC 9763	11×10^6	160	–	180	–	180	–
Aspergillus niger	24×10^6	25	50	30	60	30	70

CFU – Number of Colony Forming Units
MIC – minimal inhibitory concentration, MLC – minimal lethal concentration
– there was no lethal activity (200 μL/mL)

of inoculum) of the *S. officinalis* extracts are shown in Table III. The stem extract shows the highest activity to *C. albicans*, followed by the flower and then the leaf extract. Regarding *E. coli*, *S. enteritidis*, *P. aeruginosa* and *S. cerevisiae*, a larger quantity of the extract was needed to achieve antimicrobial effects.

The extracts show stronger antibacterial activity than the essential oils of sage from the same locality. However, more extract is required for antifungal activity to be realized.[14]

CONCLUSIONS

The examined extracts contain all the specific components defining the chromatograph profile of the essential oil. There is a similarity of the chemical composition with that of essential oils.

The leaf extract showed significantly higher antimicrobial activity compared to the other extracts as determined by the diffusion and dilution methods. The stem extract showed the highest activity to *C. albicans*, followed by the flower and then the leaf extracts (the dilution method is more precise). Regarding *E. coli*, *S. enteritidis*, *P. aeruginosa* and *S. cerevisiae*, a larger quantity of the extract is needed to achieve antimicrobial effects.

ИЗВОД

ХЕМИЈСКИ САСТАВ И АНТИМИКРОБНО ДЕЛОВАЊЕ ЕТАНОЛНИХ ЕКСТРАКАТА ДОБИЈЕНИХ ИЗ ЦВЕТА, ЛИСТА И СТАБЉИКЕ *Salvia officinalis* L.

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Испитан је хемијски састав и антимикробно дејство етанолних екстраката цвета, листа и стабљике биљне врсте *Salvia officina*lis L., пореклом из југоисточне Србије. Испитивани екстракти садрже све карактеристичне компоненте које одређују хроматографски профил етарског уља *S. officinalis* према Нацрту међународног стандарда ИСО/ДИС 11024, док је компонента са највећим уделом у свим екстрактима маноол (дитерпен) (9,0–11,1 %). Антимикробна активност је одређена дифузионом и дилуционом методом, при чему је друга модификована употребом целулозних дискова. Екстракт листа има нешто већу антимикробну активност од екстраката цвета и стабљике.

(Примљено 19. августа 2002)

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