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Short communication

SHORT COMMUNICATION

Chemical composition and antimicrobial action of the ethanol extracts of *Salvia pratensis* L., *Salvia glutinosa* L. and *Salvia aethiopis* L.

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Abstract: In this paper the chemical composition and antimicrobial action of the extracts from the flower, leaf and stem of *Salvia pratensis* L., *Salvia glutinosa* L. and *Salvia aethiopis* L. were investigated. The investigated extracts mostly contain monoterpenes to a high degree, except the flower extract of *S. pratensis* and the leaf extract of *S. glutinosa* where sesquiterpenes were found in great amounts. Diterpenes were found only in the extracts of *S. aethiopis*. All these extracts contained 1,8-cineole (to the highest degree (19.1 %) in the stem extract of *S. glutinosa*) and beta-caryophyllene (to the highest degree (7.0 %) in the flower extract of *S. pratensis*). The antimicrobial activities of the extracts were determined by the diffusion and dilution method.

Keywords: *Salvia pratensis*, *Salvia glutinosa*, *Salvia aethiopis*, Lamiaceae, extracts, chemical composition, antimicrobial activity.

INTRODUCTION

The genus *Salvia* L. includes about 900 species[1] and is the most numerous within the family Lamiaceae. The flora of Serbia comprises 14 species of this genus.[2] The chemical composition of the extracts of *S. officinalis* L. has been the subject of several publications.[3–7] There is little information about the chemical composition of the extract of *S. pratensis* L., *S. glutinosa* L. and *S. aethiopis* L. The extraction of *S. pratensis* with hexane gives an extract which contains beta-amyrin, germanicol, loranthol and lupeol.[8] From the acetone extract of dried roots of *S. glutinosa* isotanshinone II has been isolated.[9] In the petrol ether and chloroform extracts of *S. aethiopis*: *n*-eicosane, beta-sitosterol, salvigenin, methoxyluteolin and sitosteryl-3-beta-D-glucoside were found.[10] Aethiopinone was ob-

tained from the root of this species.[11] There is information about the pharmacological properties of the extracts of *S. pratensis*[12,13] and *S. aethiopis*[13] and about the antimicrobial action of the plant extracts of *S. officinalis*.[14,15]

In this study, the chemical composition and antimicrobial action of the extracts from the flower, leaf and stem of *S. pratensis*, *S. glutinosa* and *S. aethiopis* were investigated.

EXPERIMENTAL

Plant material

The herbal species were collected during the flowering phase in 1998 at three localities in southeast Serbia. The *S. pratensis* was collected on the Rtanj Mt. (surrounding of Sokobanja, Rtanj village), at the end of May. The *S. glutinosa* originating from the Streser Mt. (surrounding of Surdulica, Vucedelce village) was picked at the beginning of August. Finally, the *S. aethiopis* was collected from the surroundings of Pirot (Blato village) in the middle of June. Herbaria samples are kept in the General Herbarium of the Balkan Peninsula (BEO) Natural History Museum in Belgrade (Yugoslavia), under the following numbers: BEO 32148 (*S. pratensis*), BEO 32149 (*S. glutinosa*) and BEO 32150 (*S. aethiopis*).

Extraction

The extractions of the dried flower, leaf and stem were carried out by maceration with 96 % ethanol (the plant material to solvent ratio was 1:5).[16]

Identification procedure

The extracts were analyzed on an analytical GC-FID and GC-MS and most of the constituents were identified by comparison of their mass spectra to those from the Wiley MS library. The obtained results were correlated with retention indices.[17,18]

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 a FID was used. The operating conditions were: temperature program 40º – 280 ºC at 4 ºC/min, injector temperature 250 ºC, detector temperature 280 ºC; carrier gas: H_2 (1 mL/min).

GC-MS: 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; 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 was injected (splitless mode).

Antimicrobial activity

The antimicrobial activities were determined by the diffusion and dilution methods. The dilution method was modified by the use of cellulose discs. It was applied for the determination of minimal inhibitory (MIC) and minimal lethal concentraiton (MLC). The 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.

For the diffusion method, the following nutritive media were used: 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 bacteria count (Torlak), for determination of the total number of micro-organisms.

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

bacterial count (Torlak) for the determination of the total number of micro-organisms, selective media for the identification of individual 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 on oblique agar with 10 mL 0.9 % NaCl during 24 h culture, was introduced into 10 mL of the nutritive medium. A Petri dish was filled with this system. 5 μL of a pure extract was applied from a 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 determined 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 a suspension of micro-organisms, 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. cerevisae*). Quantities of 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* and 40, 50, 60, 80, 100, 120, 140, 160, 180, 200 μL of the extracts for *E. coli*, *S. enteritidis*, *P. aeruginosa*, *S. cerevisiae* were applied onto sterile cellulose discs of the diameter 12.7 mm (Schleicher & Schuell, Dassel, Germany), which were submerged into sterile test tubes with 0.5 mL of the suitable medium, followed by addition of 1 mL of the inoculum. The incubation was carried out at 37 ºC for bacteria and at 26 ºC for fungi. The changes in the inoculum clarity 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 the introduction of inoculum by loop onto selective media (determination of MIC and MLC).

RESULTS AND DISCUSSION

Results of the GC-characterization of the examined extracts are presented in Table I. The investigated extracts (all extracts of *S. aethiopis*, the *S. glutinosa* stem extract, and the *S. pratensis* leaf and stem extract), contain monoterpenes mostly in abundance except the *S. pratensis* and *S. glutinosa* flower extracts where sesquiterpenes predominate. Diterpenes are only found in the extracts of *S. aethiopis*. In all extracts, 1,8-cineole was present (19.1 % in stem extract of *S. glutinosa*, 17.0 % and 15.7 % in the flower and leaf extract of *S. aethiopis*, 8.1 % in the leaf extract of *S. pratensis*), as well as beta-caryophyllene (7.0 % in the flower extract of *S. pratensis*, 6.5 % and 4.7 % in the leaf and stem extracts of *S. aethiopis*, 2.3 % in the stem extract of *S. glutinosa*. In addition, the extracts of *S. pratensis* contain significant amounts of 3-*p*-menthene (7.7 % in stem), *n*-tetradecanol (3.5 % in flower), *n*-pentadecanol (1.9 % in flower), *n*-hexadecanol (2.3 % in flower), *n*-octadecanol (4.3 % in leaf), *n*-heneicosane (4.2 % in flower), *n*-pentacosane (7.9 % in leaf). The extracts of *S. glutinosa* are rich in the following components: beta-pinene, (7.5 % in flower), *n*-undecane (1.7 % in stem), (*Z,E*)-farnesyl acetate (4.0 % in flower), *n*-hexadecanol (3.3 % in flower), *n*-octadecanol (6.0 % in leaf), *n*-tetracosane (2.6 % in florer), *n*-pentacosane (3.1 % in flower). The extracts of *S. aethiopis* are rich in the following components: alpha-copaene (2.1 % in leaf), gamma-muurolene (1.3 % in stem), delta-cadinene (1.8 % in leaf), viridiflorol (8.3 % in stem), beta-bisabolol (3.2 % in flower), manool (5.7 % in stem).

The characteristic components of the essential oil of sage (*S. officinalis*; alpha-pinene, camphene, limonene, 1,8-cineole, alpha- and beta-thujone, camphor, linalool, linalyl acetate, bornyl acetate and alpha-humulene)[19] were also present in the extracts of *S. aethiopis*

(with the exception of linalool and linalyl acetate) and *S. pratensis* (with the exception of bornyl acetate, linalool and linalyl acetate). However, the extracts of *S. glutinosa* had a quite different chemical composition (besides 1,8-cineole). There is a similarility in the chemical composition of the essential oils from the herbs[20] and the flowers, leaves and stems extracts of *S. pratensis*, *S. glutinosa* and *S. aethiopis*.

TABLE I. Percentage composition of the flower, leaf and stem extracts of *Salvia pratensis*, *Salvia glutinosa* and *Salvia aethiopis*

Constituents	RI	*Salvia pratensis*			*Salvia glutinosa*			*Salvia aethiopis*		
		Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
n-Nonane	899									0.5
α-Pinene[m]	939		0.9	tr.			1.5	2.5	3.0	0.5
Camphene[m]	953		0.7				0.7	1.5	1.0	0.3
β-Pinene[m]	980		3.9	2.7	7.5	2.9	3.8	2.8	4.0	1.0
3-*p*-Menthene[m]	986	4.1		7.7						
β-Myrcene[m]	991						3.8	1.2	0.6	0.6
p-Cymene[m]	1026		tr.	tr.						
Limonene[m]	1031		tr.	tr.					0.1	
1,8-Cineole[m]	1033	8.0	8.1	3.9	0.6	0.2	19.1	17.0	15.7	6.7
(*Z*)-β-Ocimene[m]	1040							0.2	0.1	
γ-Terpinene[m]	1062								0.2	0.2
Terpinolene[m]	1088								0.2	
n-Undecane	1099	0.6		1.9	1.2	0.3	1.7			
α-Thujone[m]	1102		5.7	10.8			9.1	21.8	12.4	33.4
β-Thujone[m]	1114		0.9	2.0			2.9	2.9	2.2	4.3
Camphor[m]	1143		1.0	1.1			1.7	5.6	1.9	5.6
Pentyl benzene	1158			0.4						
3-Thujanol[m]	1166		0.6	0.9						
n-Nonanol	1171						1.2			
Terpinen-4-ol[m]	1177							0.3	0.2	0.5
3-Decanol	1188				0.5	0.2	0.6			
α-Terpineol[m]	1189							0.4	0.2	
1-Dodecene	1192	2.1	0.9	0.4		0.2	0.4			
Myrtenol	1194								0.2	0.3
n-Dodecane	1199	0.3	0.5	0.6	0.3		0.9	0.2		
α-Fenchyl acetate[m]	1220							0.1		
n-Hexyl-2-methylbutyrate	1234							0.1		
n-Decanol	1272			0.9						

TABLE I. Continued

Constituents	RI	*Salvia pratensis*			*Salvia glutinosa*			*Salvia aethiopis*		
		Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
Bornyl acetate[m]	1285							0.3	0.2	0.8
n-Tridecane	1299	0.8	0.6	0.6						
α-Cubebene[s]	1351			0.2				0.2	0.2	0.3
α-Copaene[s]	1376			1.0		0.2	0.5	1.7	2.1	1.2
β-Bourbonene[s]	1384				0.9	0.4		0.5	0.7	0.4
β-Caryophyllene[s]	1418	7.0	0.2	1.6	1.1	0.8	2.3	2.7	6.5	4.7
β-Gurjunene[s]	1432								0.2	
Aromadendrene[s]	1439		0.4							
α-Humulene[s]	1454	0.4		1.5	0.6		0.7	4.0	7.7	9.8
γ-Muurolene[s]	1477	0.8		0.4				1.3	1.1	1.3
Germacrene D[s]	1480								1.1	
α-Muurolene[s]	1499							0.9	1.5	0.8
δ-Cadinene[s]	1524							0.9	1.8	1.3
α-Calacorene[s]	1542								0.3	
β-Calacorene[s]	1563							0.4	0.1	
Longipinanol	1566		0.4	0.2						
Caryophyllene oxide[s]	1581	7.4			3.0	2.5	0.5	3.4		
Viridiflorol[s]	1590	tr.						2.0	6.2	8.3
Tetradecanal	1611	1.2								
α-Muurolol[s]	1645	0.3	0.4							
β-Bisabolol[s]	1671							3.2	1.0	2.3
Cadalene[s]	1674		0.3	0.3						
n-Tetradecanol	1676	3.5	0.1							
(*Z,E*)-Farnesol[s]	1697				0.5	0.8				
n-Heptadecane	1700			0.2						
Iso-longifolol[s]	1726								0.6	
n-Pentadecanol	1778	1.9	1.6		0.9	1.7				
1-Octadecene	1793								0.5	
n-Octadecane	1800	1.1	0.4							
(*Z,E*)-Farnesyl acetate[s]	1818				4.0	1.0				
n-Hexadecanol	1879	2.3	0.5	1.1	3.3	1.3	0.9			
n-Nonadecane	1900				0.4					
n-Eicosane	2000	1.7								
Manool[d]	2056							0.6	2.1	5,7

TABLE I. Continued

Constituents	RI	*Salvia pratensis*			*Salvia glutinosa*			*Salvia aethiopis*		
		Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
n-Octadecanol	2082	4.1	4.3	0.5	3.8	6.0	0.4			
n-Heneicosane	2100	4.2	0.7	0.9						
n-Tricosane	2300	0.8								
n-Tetracosane	2400	1.1			2.6	1.6				
n-Pentacosane	2500	2.5	7.9	4.3	3.1	2.0				
Number of recorded components		57	41	39	57	44	38	63	74	38
Number of identified components		22	23	25	17	16	19	29	32	24
% of identified constituents		56.2	41.0	46.1	34.3	22.1	52.7	79.2	75.4	90.8
No.of identified monoterpenoids		2	8	7	2	2	8	13	15	11
% of identified monoterpoenoids		12.1	21.8	29.1	8.1	3.1	42.6	56.6	42.0	53.9
No. of identified sesquiterpenoids		5	4	6	6	6	4	12	15	10
% of identified sesquiterpenoids		15.9	1.3	5.0	10.1	5.7	4.0	21.2	31.1	30.4
No. of identified diterpenoids		–	–	–	–	–	–	1	1	1
% of identified diterpenoids		–	–	–	–	–	–	0.6	2.1	5.7

RI - retention index (Kovats); tr. - traces; m - monoterpenoids; s - sesquiterpeoids, d - diterpenes

Antimicrobial activity, as investigated by the diffusion method, was only noticed for some extracts on the mould *A. niger*. The diameters of the zones of inhibition were: 6.8 mm for the leaf extract of *S. pratensis*, 7.2 mm for the flower extract of *S. glutinosa*, 6.2 mm for the leaf extract of *S. glutinosa* and 6.4 mm for the leaf extract of *S. aethiopis*. All other microbial cultures were resistant. The values of MIC and MLC (μL of extract/mL of inoculum) of all the investigated extracts were constant throughout the experiment (Table II). The extracts of *S. aethiopis* had the best action on the Gram (+) bacterium *S. aureus*. However they had the weakest action on yeast *C. albicans*. No MLC values were recorded neither for yeast *S. cerevisiae* and mould *A. niger*, nor for bacterium *S. enteritidis* (stem extract of *S. pratensis* and *S. glutinosa*).

Also, the essential oils of *S. pratensis*, *S. glutinosa* and *S. aethiopis* have antimicrobial activity.[21]

CONCLUSION

The ethanol extracts from the flower, leaf and stem of *S. pratensis*, *S. glutinosa* and *S. aethiopis* have some common components, such as: 1,8-cineole, beta-caryophyllene, alpha- and beta-pinene, camphene, alpha- and beta-thujone, camphor, *n*-dodecane, alpha-copaene, alpha-humulene and caryophyllene oxide. The investigated extracts have antimicrobial activity on: Gram (+) and Gram (-) bacteria, yeast and mould. Antimicrobial activity of the extracts was not registered for individual microorganisms by the diffusion method. It is noticeable that the dilution method is much more precise.

TABLE II. Values of MIC and MLC (μL/mL) of the extracts of *Salvia pratensis*, *Salvia glutinosa* and *Salvia aethiopis*

Microorganisms	CFU*/ml inoculum	*Salvia pratensis*						*Salvia glutinosa*						*Salvia aethiopis*					
		Flower		Leaf		Stem		Flower		Leaf		Stem		Flower		Leaf		Stem	
		MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
E. coli ATCC 25922	4.5×10^7	140	160	140	180	140	180	140	160	120	140	160	180	140	160	100	140	160	180
S. enteritidis ATCC 13076	4×10^7	180	200	160	180	200	-	160	180	160	180	200	-	140	180	120	140	180	200
P. aeruginosa ATCC 9027	1.2×10^7	140	160	160	180	160	180	140	160	140	160	180	200	160	180	140	160	180	200
B. subtilis ATCC 6633	1×10^6	80	100	80	100	140	160	100	120	120	140	160	180	80	100	80	100	120	140
S. aureus ATCC 6538	4×10^7	100	120	80	120	100	140	100	120	80	120	120	140	80	100	60	80	100	140
S. lutea ATCC 9341	8.5×10^6	80	100	80	100	140	160	120	140	100	120	140	160	100	120	80	120	140	160
C. albicans ATCC 10231	2.4×10^6	140	160	140	160	160	180	160	180	140	160	160	200	180	200	140	160	180	200
S. cerevisiae ATCC 9763	6×10^6	200	–	200	–	200	–	200	–	200	–	200	–	200	–	200	–	200	–
A. niger	1.7×10^6	140	–	140	–	180	–	160	–	140	–	180	–	120	–	120	–	140	–

* - number of Colony Forming Units

MIC - minimal inhibitory concentration; MLC - minimal lethal concentration

- there was no lethal activity (200 μL/mL)

ИЗВОД

ХЕМИЈСКИ САСТАВ И АНТИМИКРОБНО ДЕЛОВАЊЕ ЕТАНОЛНИХ ЕКСТРАКАТА *Salvia pratensis* L., *Salvia glutinosa* L. и *Salvia aethiopis* L.

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У овом раду је извршено испитивање хемијског састава и антимикробних особина етанолних екстраката цвета, листа и стабљике *Salvia pratensis* L., *Salvia glutinosa* L. и *Salvia aethiopis* L. Испитивани екстракти углавном у највећем проценту садрже монотерпене, осим екстракта цвета *S. pratensis* и листа *S. glutinosa*, где преовладавају сесквитерпени. Дитерпени су идентификовани само у екстрактима *S. aethiopis*. У свим екстрактима присутни су 1,8-цинеол (највише у екстракту стабљике *S. glutinosa*, 19,1 %) и бета-кариофилен (највише у екстракту цвета *S. pratensis*, 7,0 %). Антимикробна активност екстраката одређена је дифузионом и дилуционом методом.

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