

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF SAGE FROM SERBIA

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Abstract. *The water-distilled essential oil of sage (Salvia officinalis L.) grown in Serbia was analyzed by GC/MS. The major constituents of oil were α -thujone (24,88%), camphor (16,03%) and 1,8-cineole (9,79%). The essential oil (2% and 1% in ethylene glycol) from the sage leaves was found to be active against Bacillus subtilis S, Staphylococcus aureus 6538, Escherichia coli 95, Salmonella enteritidis and Aspergillus niger.*

Key words: *Salvia officinalis; Sage; Essential oil; Antimicrobial activity; α -thujone.*

1. INTRODUCTION

Sage (*Salvia officinalis* L.) is a typically Mediterranean plant. Sićevo gorge (Southeast Serbia) is an important natural habitat of sage in the continental part of the Balcanic peninsula. High content of different biological active compound as essential oil [1] and antioxidant compounds [2-4] are reason for scientific interest of sage since long time ago. Essential oil of *S. officinalis* L. is effective microbicides against the pathogens [5]. In the present study we have investigated the antimicrobial activity of essential oil of *S. officinalis* L. in comparison with its chemical composition.

2. EXPERIMENTAL

Plant material. Leaves of *S. officinalis* L. were collected during the blooming stage (June 1998) from the locality Sićevo gorge (near Niš). Air-dried leaves were hydrodistilled for 2.5 h using a Clevenger-type procedure [6]. The pure oil was kept at cca. 4⁰C until analysed.

Analytical procedure for oil. The oil was analyzed on an analytical GC and GC/MS. Identification of the constituents was achieved by comparison of their mass spectra to

those from the MS library [7,8], and of retention indices with authentic samples.

Analytical GC. A Varian model 3700 Gas Chromatograph, equipped with a 2.5 m x 1.8" column packed with 10% OV-101, and FID was used for GC measurements. The operating conditions were shown as follows: temperature program 70-200 °C at 3 °C/min, and an injector and detector temperature of 220 °C; carrier gas N₂ (20 ml/min). Peak areas were calculated electronically by a Varian CDS-111 data calculator.

GC/MS analyses were performed on an HP 5890 Gas Chromatograph equipped with a fused silica 25 m x 0,25 mm, SE-30 capillary column, and a HP 5971 series Mass Selective Detector. The column was temperature programmed from 100-290 °C at 3 °/min with a carrier gas (He) at a flow rate 1 ml/min and a 40:1 split ratio.

Antimicrobial screening. The activity was measured by the standard disc diffusion method [9]. All pure cultures of the microorganisms were taken from the Laboratory of Microbiology, Pharmaceutical Industry "Zdravlje", Leskovac, Yugoslavia. Inocula of *Bacillus subtilis* S, *Staphylococcus aureus* 6538, *Pseudomonas aeruginosa*, *Escherichia coli* 95, *Candida albicans*, *Salmonella enteritidis* and *Aspergillus niger* were applied to agar plates (Petri dish) and each of them had been mixed with a concentration of *Salvia* essential oil (2% and 1%, v/v in ethylene glycol). Zones of inhibition were determined by Fisher-Lilly zone reader. The concentration of the reference antibiotic ampicillin was 1 mg/ml. All measurements were made in triplicate. The values are expressed as mean ± standard error.

3. RESULTS AND DISCUSSION

The amount of the essential oil of *S. officinalis* L. from the continental part of Serbia (2,00 %, v/w) was in accordance with literature data [10]. Ecological factors (climatic and soil conditions) have strong influence on the essential oil content [11].

Table 1. Chemical composition of oil derived from leaves of *S. officinalis* L.

<i>Constituents</i>	Content (%)
α -thujene	0,10
α -pinene	3,50
camphene	3,14
2- β -pinene	0,58
β -myrcene	0,59
α -terpinene	0,89
1,8-cineole	9,79
γ -terpinene	0,15
α -thujone	4,88
β -thujone	8,08
camphor	6,03
1-borneol	4,31
1,4 terpineol	0,81
endobornyl acetate	2,68
caryophyllene	0,82
β -selinene	3,90
veridiflorol	7,89
manool	3,22

The results of the analysis of the chemical composition of *S. officinalis* L. oil can be seen in Table 1. In the essential oil from Serbian sage the domination of monoterpenoids of thujone class was established. The main components are (α -thujone (24,88%), camphor (16,03%) and 1,8-cineole (9,79%). In our opinion it seems that these components represent a possible chemotype for *S. officinalis* L., and could be used as specific compounds for determination of the characteristics of *S. officinalis* L. from Serbia. Based of the content of total ketones (40%), oil from Serbian sage corresponds to the standards of Pharmacopoeia Yugoslavica [12]. Similar results have been published for Dalmatian and Monte Negro sage [10]. On the other hand, major component of oil from Cuba sage is manool, what emphasize the role of ecological factors on amount of essential oil and its composition [13].

Table 2. Antimicrobial activity of the essential oil from leaves of *S. officinalis* L.

Microorganisms species	Dilution		Ampicillin (1 mg/ml)
	1:50 (2%)	1:100 (1%)	
<i>B. subtilis</i> S	20.20±0.84	18.70±0.42	32.56±0.56
<i>S. aureus</i> 6538	19.05±0.23	17.70±0.42	29.48±0.82
<i>P. aeruginosa</i>	0	0	31.42±0.12
<i>E. coli</i> 95	19.20±0.57	18.00±0.21	33.56±0.78
<i>C. albicans</i>	0	0	21.87±0.48
<i>S. enteritidis</i>	18.80±0.56	17.40±0.49	20.32±0.56
<i>A. niger</i>	15.50±0.39	14.45±0.71	24.32±0.41

The results of antimicrobial activity of the essential oil from leaves of *S. officinalis* L. are presented in table 2. Both concentrations of oil showed antibacterial activity against *Bacillus subtilis* S, *Staphylococcus aureus* 6538, *Escherichia coli* 95 and *Salmonella enteritidis*, as well as antifungal activity against *Aspergillus niger*.

Higher samples concentration (2%) exhibited higher activity against the microorganisms used, compared with the lower sample concentration (1%). The carriers of antimicrobial activity of the sage oil were α -thujone and camphor what is according to literature data [14]. The reference antibiotic ampicillin showed the highest antimicrobial activity against all tested microorganisms.

This study confirms that essential oil of *S. officinalis* L. from Si}evo gorge (southeast Serbia) corresponds to standards of Pharmacopoeia Yugoslavica. The oil was found to have significant antibacterial and antifungal activity and therefore can be used as a strong antimicrobial agent.

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ANTIMIKROBNA AKTIVNOST ETARSKOG ULJA ŽALFIJE IZ SRBIJE

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Ispitivana je antimikrobna aktivnost etarskog ulja izolovanog iz lista žalfije (Salvia officinalis L.) sa lokaliteta Sićevačka klišura u poređenju sa njegovim hemijskim sastavom. Najzastupljenije komponente etarskog ulja su: α -tujon (24,88%), kamfor (16,03%) i 1,8-cineol (9,79%). Etarsko ulje je ispoljilo antimikrobnu aktivnost na: Bacillus subtilis S, Staphylococcus aureus 6538, Escherichia coli 95, Salmonella enteritidis and Aspergillus niger.