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ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF SALVIA OFFICINALIS L. CULTIVATED IN KOSOVO

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Chemical composition of *Salvia officinalis* L. cultivated in Kosovo

Abstract

The essential oil composition of the cultivated plant species of Sage (*Salvia officinalis* L.) originating from Kosovo was investigated. The water and ethanolic extracts of cultivated Sage were also tested for their antibacterial activity against *Staphylococcus aureus*, *Escherichia Coli* and *Candida albicans*. Extraction of essential oil was performed with water distillation method using Clevenger apparatus. Essential oil composition was analyzed by GC/MS. The result for the essential oil content was 1.57%, and the GC/MS analysis of the essential oil resulted in 7 principle components: α-thujone (22.0%), β-thujone (3.03%),1,8 cineole (12.68%), camphor (10.75%), β-caryophyllene (6.28%), α-humulene (5.68%), and α-pinene (2.30%).The antibacterial activity of the water and ethanolic extracts of Sage against *Staphylococcus aureus, Escherichia Coli* and *Candida albicans* resulted in significant activity, especially regarding the ethanolic extracts.

Conclusion: The cultivated Sage originating from Kosova complied with the standard requirements for the plant species, so that it can be used as a high quality raw materials for the production of herbal medicines. Most significant antimicrobial activity was resulted with the ethanolic extract of Sage against all three tested microorganisms.

Keywords: Chemical composition, GC/MS, essential oil, *Salvia officinalis* L., Sage;

Introduction

Main indications for *Salvia officinalis* L. (Sage) use are inflammation and infection of mouth and throat. Because of its vitalizing and antiseptic action on mucosal tissue (the effect of tannins and essential oil respectively), sage is used in many herbal preparations as gargle, in inflammation and abscess. Sage's dried herb, its extract and essential oil have an antimicrobial, spasmolytic, carminative, antioxidant and astringent action [1].

According to the *Salviae officinalis aetheroleum* monograph of German Medicines Codex (DAC), the Sage essential oil chemical composition criteria for five main constituents based on gas chromatography identification are: cineol (6.0 – 16%), α – thujone + β – thujone (at least 20% in total), camphor (14-37%), bornyl acetate and borneol (not more than 5%) [2].

In Kosovo, medical herbs are also being cultivated and put in the market in the form of teas, and are being exported as raw material. Cultivation of some medical herbs is almost impossible without altering their characteristics [2]. In this research we aim to gain information on the overall quality of cultivated Sage in Kosovo, and to also determine the antimicrobial activity of some of its extracts. International standards and sophisticated techniques have been applied for evaluation. The study results have been compared to other similar publications.

Materials and methods

Plant material

The analized material consisted of dried leaves of *Salvia officinalis* L. cultivated by the company "Agroprodukt" in Syne village, municipality of Istog, Kosovo, at a level of 650m above sea. The plant has been collected in flowering period in June 2010 and leaves have been dried in shade in a well ventilated area.

Essential oil determination

Essential oil was extracted from the plant material through Clevenger distillation apparatus for 2 hours at a rate of 2 to 3ml/min, using xylene as organic solvent. For the procedure 20 g plant material and 400 ml of water have been used.

Essential oil chemical analysis

Determination of chemical constituents of essential

ISSN: 2304-2354
Number 3, Volume 44, 2013

oil has been done by gas chromatography. The solution to be analyzed was prepared by diluting 100μl of essential oil obtained by distillation to volume with xylene in a 5ml flask. 1ml of the solution has been transferred to the gas chromatograph vial. The essential oils were analyzed using a Shimadzu GC-14B (FID detector) fitted with a fused silica column HP-5MS (60m x 0.25mm, film thickness 0.25μm), split 1: 60, temperature programmed from 60°-240°C at 2°C/min with helium as the carrier gas at the flow rate of 1.0 ml/min, sample injection 2 μl. The injector and detector temperatures were kept at 250°C and 270°C respectively. GC/MS analyses of the oils were run on a Hewlett-Packard mass spectrometer HP 5970 B, ion source 70 eV, coupled with a gas chromatograph HP 5890A with fused silica capillary column 50m x 0.21mm, film thickness 0.30μm, coated with SE-54 temperature programmed from 50°-260°C at 2°C/min using Helium as the carrier gas with a flow rate of 0.4 ml/min. The essential oil of sample was analyzed using a GC/MS Agilent 7890A, temperature programmed from 60°-260°C at 5°C/min using Helium as the carrier gas with a flow rate of 0.4 ml/min. Identification of individual components was made by comparison of their retention times with those of analytical standards and by computer searching, matching mass spectral data with those held in Wiley / NBS library of Mass spectra [12]. For quantification purposes were used area percent reports, obtained by GC (FID).

Antimicrobial activity of plant extracts

Water and alcoholic extract of Sage were tested against the microorganisms Escherichia coli, Staphylococcus aureus, and Candida albicans for their antimicrobial activity.

Preparation of extracts

Liquid extracts were prepared by pulverizing 1g of the plant material and moistening it with 10g of extraction liquid. After shaking them periodically in the first hours, the mixtures where left for 24 hours in a dry and dark place. The mixtures were than filtrated.

Tested microorganisms

The antimicrobial activity testing was performed against Escherichia coli, Staphylococcus aureus, and Candida albicans. The microorganisms were clinically isolated, Staphylococcus aureus from the respiratory tract, Escherichia coli from cultivated urine and Candida albicans from vaginal swab of patients.

Testing method

Agar diffusive method has been applied for plant extract antimicrobial activity testing. Microorganism suspensions of 0.5 Mc Farland turbidity were inoculated in cultivation media of a 4mm thickness. The cultivation media used in the method are listed below: Agar blood (BioMerieux, France) for Staphyllococcus aureus, Endo agar (BioMerieux, France) for Escherichia coli, Sabouraund agar (BioMerieux, France) for Candida albicans. In each of the media, a hole of 6.5mm in diameter has been created after the microorganisms have been inoculated. The plant extracts were placed in the holes on each cultivation plate. Ethanol 70% was used as control. Isolated microorganisms were incubated in cultivation media for 24 hours in 37°C, and inhibition zones were measured after 48 hours incubation in 37°C.

Results and discussion

Essential oil yield

The essential oil yield 1.57% from the studied sample resulted 1.57%. According to the published studies, the Albanian sage samples ranged from 1.8% - 2.5% [11] Sage essential oil resulted in 1.66% in Serbia, 1.41% in Montenegro [5] and Bosnia 1.07% [7]. A study from Poland reported a yield of 1.6% [6] Official limits of essential oil content for dried leaves of Sage, according to the literature, are 1 to 2.5% [2]. Based on these data it results that the essential oil content of Sage cultivated in Kosovo complies with the literature standards and can be compared as it is similar to the essential oil content of Sage located in other countries according to consulted publications.

Essential oil chemical analysis

GC/MS analysis results of essential oil constituents of *Salvia officinalis* L. cultivated in Kosovo are presented in table 1.7 main constituents of Sage essential oil have been identified. The major constituent is α- thujone with 22.0%, and if calculated together with β-thujone is 25.03% of total. 1,8 cineole results in 12.68%, camphor in 10.75%, β-caryophyllene in 6.28%, α-humulene in 5.68%, β-thujone in 3.03%, and α-pinene in 2.30%. According to other published studies Sage essential oil from Serbia resulted in thujone (24,88%), camphor (16,03%) and 1,8-cineole (9,79%) [3]. Sage oil from Montenegro and Dalmatia also showed similar results.[8] Albanian sage samples ranged as follows: 1,8 cineole from 12.64% to 23.38% ; α-thujone from 13.12% to 25.41%; β-thujone from 2.10% to 6.67%; borneol

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Number 3, Volume 44, 2013

from 3.12% to 8.28%; β-caryophyllene from 4.26% to 7.20% [10,11]. Close examination of the GC and GC/MS data reveals that sage oil from region of Kosova possessed higher quantity of sesquiterpene and lower quantity of camphor in contrast with the oils from Albanian regions. Meanwhile, similar quantity of 1,8 cineole and α-thujone in essential oils from Kosova and north/south regions of Albania are detected.

Based on these data, we can conclude that essential oil quality of Kosovo Sage is compliant with German Medicines Codex (DAC) monograph criteria [2]. It is comparable or similar with that of other published studies.

Table nr.1. Main chemical constituents of *Salvia officinalis* L. essential oil identified by GC/MS

Peak#	Retention time (min)	Identity	Total %
1	8.994	α-pinene	2.30%,
2.	12.007	1,8cineole	12.68%,
3.	14.336	α- thujone	22.0%
4.	14.660	β-thujone	3.03%
5.	15.547	camphor	10.75%
6.	23.220	α-humulene	5.68%
7.	24.076	β-caryophilene	6.28%

Antimicrobial activity of plant extracts

Results are presented in table nr.2 and illustrated in graphics 1,2,3. From the inhibition zone results we can conclude that the highest antimicrobial activity was recorded with the ethanolic extract of Sage against all three tested. The water extract had a positive but lower activity against Staphylococcus aureus, Candida albicans and Escherichia Coli. Referring to literature, ethanolic extract of Sage leaves has more similarities with essential oil of Sage, thus resulting in higher antimicrobial activity than water extract, because it contains all specific compounds which define the chromatographic profile of essential oil [9]. Water and ethanolic extracts both showed higher antimicrobial activity against all three tested pathogens compared with ethanol 70% as control. The activity against Staphylococcus aureus was shown significantly higher than control for both extracts. For Escherichia Coli and Candida albicans control and water extract showed similar antimicrobial activity, while ethanolic extract showed higher activity. The inhibition zones grew with the amount of extracts applied, as it may be seen in figures nr.1-3. According to the literature, responsible compounds for antimicrobial activity of essential oils are α-thujon and camphor [4]. This is in accordance with the constituents of our studied essential oil resulting with α-thujon and camphor among the main compounds.

Table nr. 2. Antimicrobial activity of Sage water and ethanol extracts, inhibition zones

		Ethanol 70%		Water extract		Ethanol extract	
Nr	Microorganism	µl	Inhibition zone mm	µl	Inhibition zone mm	µl	Inhibition zone mm
1	Staphylococcus aureus	20	Ø	20	8	20	13
		30	Ø	30	9	30	15
		40	Ø	40	10	40	21
		50	Ø	50	11	50	21
		60	Ø	60	13	60	25
2	Escherichia Coli	20	11	20	11	20	13
		30	12	30	11	30	16
		40	13	40	12	40	18
		50	14	50	13	50	21
		60	15	60	13	60	23
3	Candida albicans	20	11	20	7	20	13
		30	14	30	8	30	15
		40	15	40	9	40	18
		50	18	50	11	50	21
		60	20	60	11	60	23

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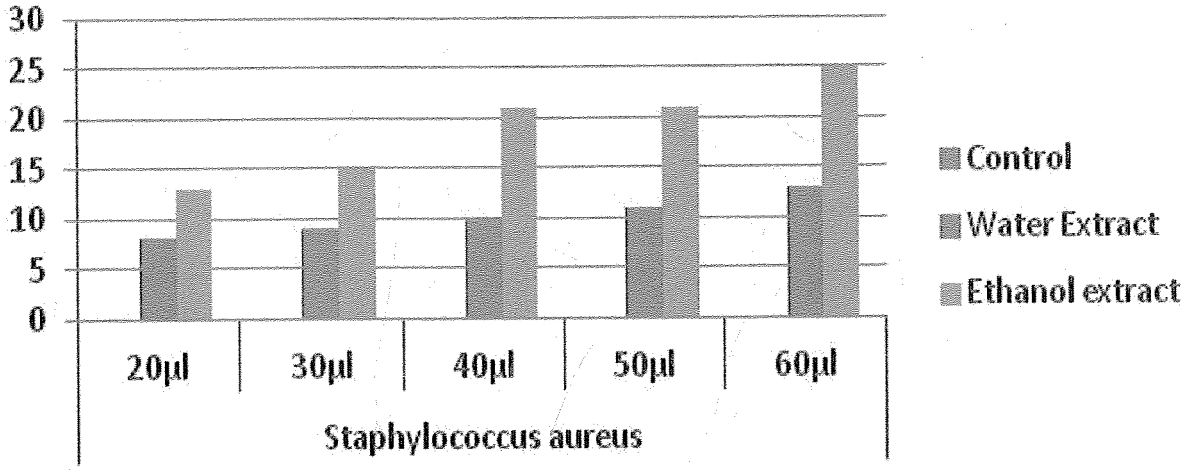
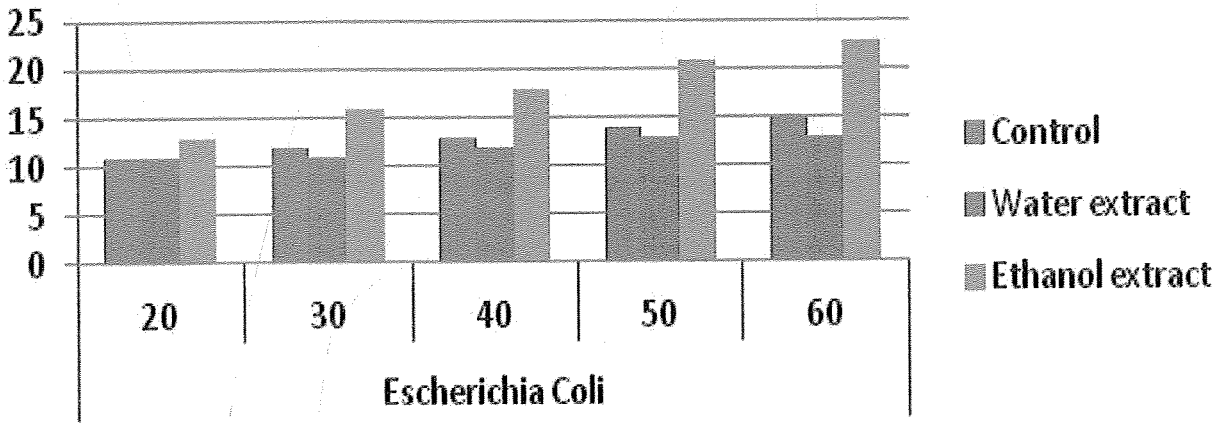
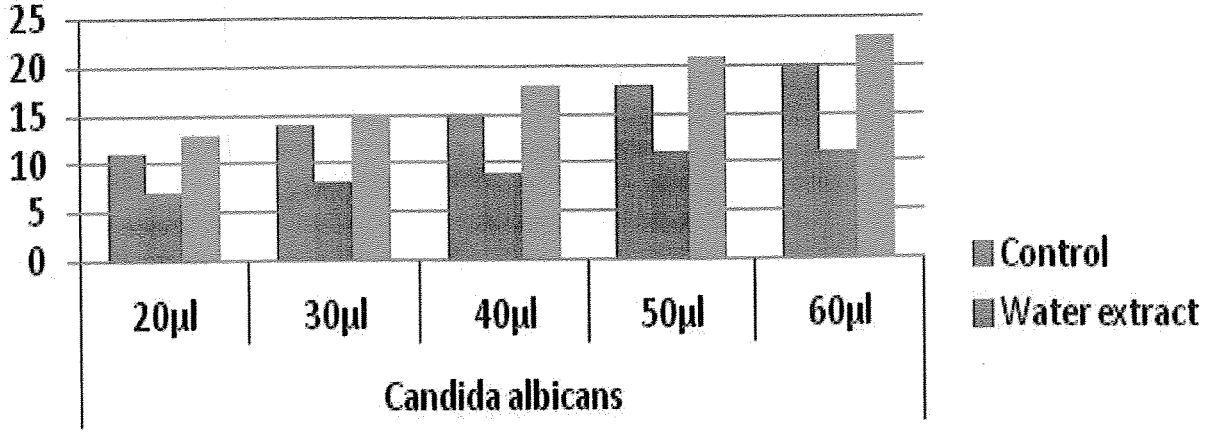
Number 3, Volume 44, 2013



Graphic nr.1. Inhibition zones of extracts against Staphylococcus aureus



Graphic nr.2. Inhibition zones of extracts against Escherichia coli



Graphic nr.3. Inhibition zones of extracts against Candida albicans

ISSN: 2304-2354
Number 3, Volume 44, 2013

Conclusions

As a conclusion, cultivation of Sage in Kosovo can produce a high quality plant material. Essential oil from *Salvia officinalis* L. cultivated in Kosovo possesses higher quantity of sesquiterpenes and lower quantity of camphor in contrast with the oils from Albanian regions; meanwhile, similar quantity of 1,8 cineole and α-thujone are detected.

The highest antimicrobial activity was recorded with the ethanolic extract of Sage against all three tested microorganisms.

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