Chemical Composition and Antimicrobial Activity of Essential Oil of Western Romanian *Salvia officinalis*

COSMIN ILIE[1], IONUT GOLET[2], MIHAELA CRACIUNESCU [1], ELENA HOGEA[1]*, ROXANA POPESCU[1]*, FLORIN GEORGE HORHAT[1]

[1] Victor Babes University of Medicine and Pharmacy, Faculty of Medicine, Timisoara, Romania, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2] West University of Timisoara, Faculty of Economics and Business Administration, Str. J. H. Pestalozzi 16, 300115, Timisoara, Romania

This study has focused on determining the chemical composition and antimicrobial properties of the essential oil (EO) of S. officinalis grown in Romania. The oil was isolated by steam distillation in 1.9% yield and then analyzed by GC-MS. The major compounds were camphor (23.76%), alpha-thujone (14.60%) and alpha-humulene (10.13%). The disk diffusion method was used to evaluate the antimicrobial properties of sage EO. The EO exhibited antibacterial activity against all tested strains, especially against Staphylococcus aureus and Klebsiella pneumoniae. The results obtained suggest that the analyzed oil can represent a new cheap and natural source of antiseptic compounds with potential applications against food-borne pathogens.

Keywords: sage, essential oil, steam distillation, GC-MS, antimicrobial activity

The genus *Salvia* is one of the largest among the *Labiatae* genera; it includes over 900 species and has an almost cosmopolitan distribution. *Salvia officinalis L.* or Dalmatian sage is a perennial low shrub which is native of the western part of the Balkan Peninsula [1]. In Romania, the *Salvia* genus contains 15 species including *Salvia officinalis* [2].

According to European Pharmacopoeia 5.0, the minimum content of EO in *S. officinalis* is 15 mL/kg [3]. The chemical composition of the *S. officinalis* essential oil (EO) has been previously investigated, alpha- and beta-thujone, 1,8-cineole, camphor and humulene being the main major components reported [4-9]. A number of studies, however, indicate significant variations in the concentration of these components and/or the presence of other major compounds, such as: alpha-pinene, viridiflorol, borneol, manool and limonene [10-13]. The amount of EO in the plant as well as its chemical composition are dependent on a number of factors, such as environment, growth region, phenological cycle and cultivation practices [10, 12-14].

The International Organization for Standardization (ISO) regulates by ISO 9909:1997 the level of several compounds in the sage EO: alpha-thujone, 18.0-43.0%; beta-thujone, 3.0-8.5%; camphor, 4.5-24.5%; 1,8-cineole, 5.5-13.0%; alpha-humulene, 0-12%; alpha-pinene, 1.0-6.5%; camphene, 1.5-7.0%; limonene, 0.5-3.0%; linalool, <1%; and bornyl acetate, <2.5% [15].

In addition to its flavoring properties, the sage EO presents significant bioactive properties: antimicrobial [8, 16-19] and antifungal activity [18, 20], together with strong antioxidant properties [16, 21]

The purpose of this study was to determine: i) the chemical composition and ii) the antimicrobial properties of the EO from *S. officinalis* grown in Romania, towards the accession of new sources of natural antiseptics with potential applications in the pharmaceutical and food industry.

Experimental part

Materials and methods

Raw material

The plant material was collected from the Cicir Fruit-Tree Nursery, Arad County, in July 2014; dried at room temperature and stored at temperatures of 3-5°C until distillation. A voucher specimen (V.FPT-521) was identified and deposited in the herbarium of the Victor Babes University of Medicine and Pharmacy, Timisoara, Romania.

Isolation of essential oil

The EO was extracted by steam distillation for 4 h, using a Clevenger-type apparatus, as prescribed in the 5th European Pharmacopoeia [3]. The oil obtained was dried on anhydrous Na_2SO_4 and stored for future analyses at 4°C.

Gas chromatography-mass spectrometry

The chemical composition of the *S. officinalis* EO was performed by GC-MS, using a gas-chromatograph coupled with a quadrupole mass spectrometer, model CLARUS 500 - PERKIN ELMER, fitted with a flame-ionization detector (FID) and an Elite-1701 15-m capillary column (0.53 mm i.d. and 1.00 µm film thickness, Perkin Elmer, USA). The temperature of FID was 250°C, 70-260°C (5° C/min) for the injector and 60-250°C (5° C/min) for the oven. The carrier gas was helium at 6 mL/min. The composition of the EO was calculated as percentage. The identification of EO components was carried out by comparison of the obtained mass spectra with mass spectra in the NIST 98 library (USA National Institute of Science and Technology software).

Determination of antimicrobial activity

Sage EO was tested against 6 common food-related bacteria: *Staphylococcus aureus* (ATCC25923), *Salmonella typhimurium* (ATCC14028), *Pseudomonas aeruginosa* (ATCC27853), *E. coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC13882) and *Enterococcus faecalis* (ATCC 29212), using the disk diffusion method as previously described by Jianu C. et al. [22]. Briefly, a suspension of the tested microorganism (10^6 cells·mL^{-1}) was spread on solid media plates (Mueller-Hinton agar). The paper discs (6 mm diameter - Whatman No 1 filter paper) with 5, 10, 15 and 20µL EO was placed on the inoculated plates. The inoculated plates were incubated for 24 h at 37°C. As positive control was used ciprofloxacin (30 µg/disk). After incubation, the zone of inhibition (the diameter) was

* *email: ehogea70@yahoo.com; popescu.roxana@umft.ro*

RT (min)	Area % of Total	Constituents
5.39	0.54	alpha-Thujene
5.63	6.10	alpha-Pinene
6.19	8.47	Camphene
6.74	0.25	beta-Thujene
6.90	3.57	beta-Pinene
6.98	0.91	beta-Myrcene
7.53	0.08	alpha-Phellandrene
7.75	0.20	4-Carene
8.05	2.49	D-Limonene
8.26	0.21	beta-Phellandrene
8.51	7.81	Eucalyptol
8.95	0.43	alpha-Terpinene
9.48	0.13	Terpineol
9.68	0.54	alpha-Terpinolen
10.13	0.34	Linalool
10.84	**14.60**	**alpha-Thujone**
11.07	2.10	beta-Thujone
12.54	**23.76**	**Camphor**
12.59	2.80	Borneol
14.96	5.99	Bornyl acetate
17.32	4.19	Caryophyllene
18.22	**10.13**	**alpha-Humulene**
21.68	4.14	Viridiflorol
	99.78	**Total**

Table 1
COMPONENTS OF ESSENTIAL OIL OF WESTERN ROMANIAN *SALVIA OFFICINALIS*

Test microorganism	Amount of essential oil [µL]/disk			
	5	10	15	20
Staphylococcus aureus ATCC 25923	11.22 ± 0.97	22.22 ± 1.2	22.89 ± 0.6	26 ± 0.71
Salmonella typhimurium ATCC 14028	7.89 ± 0.6	10.44 ± 0.53	10.78 ± 0.67	11.33 ± 0.5
Pseudomonas aeruginosa ATCC27853	6 ± 0	6.56 ± 0.53	9.56 ± 0.53	9.78 ± 0.44
E. coli ATCC 25922	8.11 ± 0.33	8.89 ± 0.33	10.78 ± 0.67	11.33 ± 0.5
Klebsiella pneumoniae ATCC 13882	15.67 ± 0.5	18 ± 0.87	18.44 ± 0.73	20.56 ± 0.53
Enterococcus faecalis ATCC 29212	6.56 ± 0.53	8.11 ± 0.33	9.89 ± 0.6	15 ± 0.87

Inhibitions are expressed in mm including the diameter of the paper disc (6 mm). Data distributions were presented as mean values and standard deviations (SD) (n = 9). Ciprofloxacin was used as positive control.

Table 2
EFFECTS OF SAGE OIL AGAINST BACTERIA, EXPRESSED BY THE MEAN SIZES OF THE INHIBITORY ZONES

measured in millimeters (mm). The tests was performed in triplicate on at least three separate experiments.

Statistical analysis

Statistical analysis was performed using SPSS Version 21 (IBM Corp.). The mean inhibition zone for all nine experiments was compared with the value of the disc diameter (6 mm) using the *t*-test. The GLM procedure was used to perform a two-way analysis of variance (ANOVA) on inhibition zones, with the microorganism and the amount of EO as factors in the full factorial model. Post-hoc tests for each amount of EO were performed, using Tukey's HSD method in order to compare the effect on different types of microorganism.

Results and discussions

The isolation yield of EO from dried plant material was 1.9% (v/w). The sage EO's chemical composition determined by GC/MS is presented in table 1.

Twenty-three components comprising 99.78% of the total area were identified. The major components were camphor (23.76%), alpha-thujone (14.60%) and alpha-humulene (10.13%). The EO is also rich in camphene (8.47%), eucalyptol (7.81%) and alpha-pinene (6.10%). The following constituents having quantities higher than 4% in the analyzed EO were identified: viridiflorol (4.14%), caryophyllene (4.19%) and bornyl acetate (5.99%). Chalcat et al. have found a similar content for EOs isolated from samples originating in Romania [4]. However, previous studies in Romania have reported a high content of alpha-thujone (21.85-52.86%) for EOs isolated from *S. officinalis*; camphor, a major component, being present in a lower amount (11.25-13.47%) [6, 7], while Lamien-Meda et al. report for Romanian sage EOs, viridiflorol as the dominant compound, with low amounts of alpha- and beta-thujone [12]. This variations can be attributed mainly to the different chemotypes reported in the literature [4, 5, 11, 12].

The antimicrobial activity of sage oil against the food-related bacteria tested is shown in table 2.

The null hypothesis that the inhibition zone is equal to the disc diameter (6 mm) was rejected for almost all microorganisms and at each amount of EO ($p = 0.00$). The exception is *P. aeruginosa* at the amount of five with an inhibition zone of exactly 6 mm. The main finding of ANOVA analysis is a strong interaction effect between the type of microorganism and the amount of EO ($p = 0.00$). The interaction effect, being highly significant, invalidates any general conclusion on the main effects, even if the two factors are also highly significant ($p = 0.00$). In order to compare more thoroughly the effect of sage oil on each microorganism (fig. 1), the results of multiple comparisons, at each amount of oil, have been considered. Tukey' HSD test reveals that the microorganisms with similar response

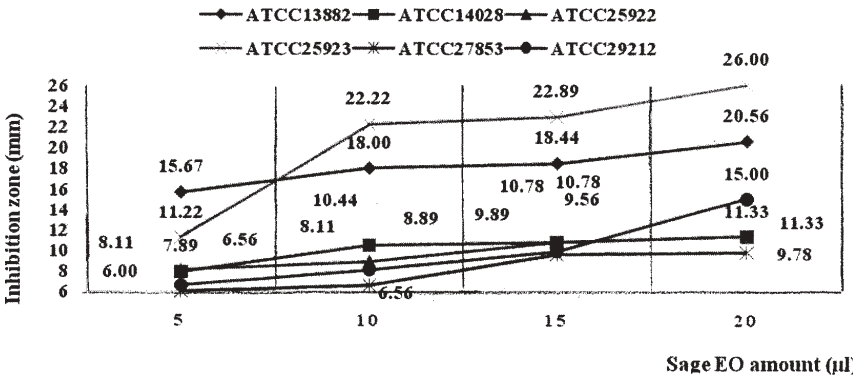

Fig. 1. The antimicrobial activity of sage oil, at 5, 10, 15 and 20 µL EO, expressed as mean inhibition zone

are: *S. typhimurium* and *E. coli* at the amount of 5 (p = 0.96), 15 (p = 1), and 20 (p = 1); *P. aeruginosa* and *E. faecalis* at the amount of 5 (p = 0.32) and 15 (p = 0.87); *E. coli* and *E. faecalis* at the amount of 10 (p = 0.197); *E. faecalis* and *S. typhimurium* at the amount of 15 (p = 0.5); *E. faecalis* and *E. coli* at the amount of 15 (p = 0.5) The observed p-value for the pairwise differences in the above-mentioned cases do not pass the 5% significance level.

The antimicrobial effectiveness of sage EO against *E. coli* [17, 19, 23, 24], *S. typhimurium* [19, 23], *S. aureus* [19, 23, 24], *E. faecalis* [17] and *K. pneumoniae* [17] has been previously reported. A number of studies, however, report the ineffectiveness of sage EO against *P. aeruginosa* [17, 19, 24, 25], in contrast with the results obtained by us and Delamare et al. [23]. The antimicrobial activity recorded can be attributed mainly to the major components of *S. officinalis* EO, i.e., camphor, alpha-thujone and alpha-humulene, recognized for their biological activities [26-28]. The presence of eucalyptol, alpha-pinene, caryophyllene, borneol and camphene also contributes to a considerable degree to the observed activity [19, 26-30]. These observations suggest possible synergistic effects between the different constituents of the EO, the literature in this area repeatedly noting a stronger antimicrobial activity of EOs than that of their major constituents [16, 31, 32]. Jianu et al. [33], citing Nemeth E. and Bernath J. [34], assert that that beside the major constituents, the total composition should be taken into consideration, because of the synergistic role of the compounds which can modify the biological activity of the oil.

Conclusions

The antibacterial activity recorded for the *S. officinalis* EO studied recommends accessing it as a new accessible natural source of antiseptic substances with potential applications against food-borne pathogens.

References

1.KINTZIOS, S.E., Sage: The Genus Salvia, ed.: Taylor & Francis. 2000.
2.CIOCÂRLAN, V., Flora ilustratã a României: Pteridophyta et Spermatophyta, ed.: Ed. Ceres. 2009. p. 656-660.
3.PHEUR_5.0, European pharmacopoeia. 5 ed, ed. 5. Strasbourg: Council of Europe E.P.C. 2004. p. 217-218.
4.CHALCHAT, J.C., MICHET, A., PASQUIER, B., Flavour Frag. J., 13, 1, 1998. p. 68-70.
5.BÖSZÖRMÉNYI, A., HÉTHELYI, É., FARKAS, Á., HORVÁTH, G., PAPP, N., LEMBERKOVICS, É. , SZÕKE, É., J. Agric. Food Chem., 57, 11, 2009. p. 4663-4667.
6.RADULESCU, V., CHILIMENT, S., OPREA, E., J Chromatogr A, 1027, 1–2, 2004. p. 121-126.
7.ONIGA, I., OPREAN, R., TOIU, A., BENEDEC, D., Rev. Med. Chir. Soc. Med. Nat. Iasi, 114, 2, 2010. p. 593-595.
8.LONGARAY DELAMARE, A.P., MOSCHEN-PISTORELLO, I.T., ARTICO, L., ATTI-SERAFINI, L., ECHEVERRIGARAY, S., Food Chem., 100, 2, 2007. p. 603-608.
9.DOB, T., BERRAMDANE, T., DAHMANE, D., BENABDELKADER, T., CHELGHOUM, C., Chem. Nat. Compd., 43, 4, 2007. p. 491-494.
10.HOSSEIN MIRJALILI, M., SALEHI, P., SONBOLI, A., MOHAMMADI VALA, M., Chem. Nat. Compd., 42, 1, 2006. p. 19-23.
11.COULADIS, M., TZAKOU, O., MIMICA-DUKIÆ, N., JANÈIC, R., STOJANOVIC, D., FLAVOUR FRAG J, 17, 2, 2002. p. 119-126.
12.LAMIEN-MEDA, A., SCHMIDERER, C., LOHWASSER, U., BÖRNER, A., FRANZ, C., NOVAK, J., Flavour Frag. J., 25, 2, 2010. p. 75-82.
13.BERNOTIENË, G., NIVINSKIENË, O., BUTKIENË, R., MOCKUTË, D., Chemija, 18, 4, 2007. p. 38-43.
14.PUTIEVSKY, E., RAVID, U., DUDAI, N., J Nat Prod, 49, 2, 1986. p. 326-329.
15. *** ISO. ISO 9909:1997. Oil of Dalmatian sage (*Salvia officinalis* L.). 1997.
16.BOZIN, B., MIMICA-DUKIC, N., SAMOJLIK, I., JOVIN, E., J. Agric. Food Chem., **55**, 19, 2007. p. 7879-7885.
17.HAYOUNI, E.A., CHRAIEF, I., ABEDRABBA, M., BOUIX, M., LEVEAU, J.-Y., MOHAMMED, H., HAMDI, M., Int. J. Food Microbiol., **125**, 3, 2008. p. 242-251.
18.SOKOVIC, M., VAN GRIENSVEN, L.J., Eur J Plant Pathol, **116**, 3, 2006. p. 211-224.
19.SOKOVIC, M., GLAMOÈLIJA, J., MARIN, P.D., BRKIC, D., VAN GRIENSVEN, L.J., Molecules, **15**, 11, 2010. p. 7532-7546.
20.PINTO, E., SALGUEIRO, L.R., CAVALEIRO, C., PALMEIRA, A., GONÇALVES, M.J., Ind Crop Prod, **26**, 2, 2007. p. 135-141.
21.MILIAUSKAS, G., VENSKUTONIS, P.R., VAN BEEK, T.A., Food Chem., **85**, 2, 2004. p. 231-237.
22.JIANU, C., MISCA, C., POP, G., RUSU, L.-C., ARDELEAN, L., GRUIA, A.T., Rev. Chim. (Bucharest), **63**, no. 6, 2012. p. 641
23.DELAMARE, A.P.L., MOSCHEN-PISTORELLO, I.T., ARTICO, L., ATTI-SERAFINI, L., ECHEVERRIGARAY, S., Food Chem., **100**, 2, 2007. p. 603-608.
24.VELIÈKOVIC, A.S., RISTIC, M.S., VELIÈKOVIC, D.T., ILIC, S.N., MITIC, N.D., J. Serb. Chem. Soc., **68**, 6, 2003. p. 435-445.
25.NASCIMENTO, G.G., LOCATELLI, J., FREITAS, P.C., SILVA, G.L., Braz. J. Microbiol., **31**, 4, 2000. p. 247-256.
26.MAGIATIS, P., SKALTSOUNIS, A.-L., CHINOU, I., HAROUTOUNIAN, S.A., Zeitschrift für Naturforschung C, **57**, 3-4, 2002. p. 287-290.
27.SIVROPOULOU, A., NIKOLAOU, C., PAPANIKOLAOU, E., KOKKINI, S., LANARAS, T. , ARSENAKIS, M., J Agr Food Chem, **45**, 8, 1997. p. 3197-3201.
28.NISSEN, L., ZATTA, A., STEFANINI, I., GRANDI, S., SGORBATI, B., BIAVATI, B., MONTI, A., Fitoterapia, **81**, 5, 2010. p. 413-419.
29.SCORTICHINI, M. , ROSSI, M.P., J Appl Bacteriol, **71**, 2, 1991. p. 109-112.
30.DORMAN, H. , DEANS, S., J. Appl. Microbiol., **88**, 2, 2000. p. 308-316.
31.GILL, A.O., DELAQUIS, P., RUSSO, P., HOLLEY, R.A., Int. J. Food Microbiol., **73**, 1, 2002. p. 83-92.
32.MOUREY, A., CANILLAC, N., Food Control, **13**, 4-5, 2002. p. 289-292.
33.JIANU, C., MIªCÃ, C., MUNTEAN, S.G., GRUIA, A.T., Hem. Ind., **69**, 4, 2015. p. 381-386.
34.NEMETH, E., BERNATH, J., Curr. Pharm. Des., **14**, 29, 2008. p. 3151-3167

Manuscript received: 14.09.2015