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Original scientific paper

CHEMICAL COMPOSITION OF ESSENTIAL OILS OF ELDERBERRY (*SAMBUCUS NIGRA* L.) FLOWERS AND FRUITS

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The majority of essential oils obtained from medicinal plants have been demonstrated to be effective in the treatment of different kinds of diseases, and they are increasingly used in the diet. Due to their chemical composition, essential oils are a very interesting product of the secondary metabolism of plants, for both consumers and researchers. Among others, elderberry (Sambucus nigra L.) is mostly a woody plant, while it can rarely be found as a herbaceous perennial plant. This plant species has been used in traditional medicine because it is a very rich source of phytochemicals. The aim of this study was to identify and compare the composition of essential oils obtained from flowers and fruits of this plant, collected from the Balkan Peninsula. The oils were obtained using the Clevenger apparatus, and their composition was evaluated by gas chromatography - mass spectrometry (GC-MS). The oil composition was affected by the part of the plants used: the most abundant bioactive compounds in the essential oil of air-dried elderberry fruits were β-damascenone (35.70%) and linalyl anthranilate (24.15%). β-damascenone was the dominant compound in the essential oil of lyophilized elderberry fruits (38.64%), while linalool was detected in the concentration of 32.80%. In the essential oil of air-dried elderflowers, the most abundant compound was carane (13.19%). The essential oils of S. nigra shown substantial chemical composition and could be used as a potential source of natural products in the cosmetics and food industry.

Keywords: essential oils, *Sambucus nigra* L., chemical composition, hydrodistillation.

INTRODUCTION

The pace of modern life has influenced the development of various diseases that have become the primary concern of contemporary society. The scientific community has focused its research on the treatment of diseases of modern society by using plants. Plant species that grow on the Balkan Peninsula are very available and cheap sources of biopotent molecules and have been used for centuries in traditional medicine. Essential oils are recognized as a very promising product of secondary plant metabolism (1). The use of essential oils in herbal medicine dates back to the early development of civilization. The essential oil has found its application in various cosmetic, pharmaceutical, and food products. Also, in aromatherapy the essential oil is used in pure or diluted form. Plant raw materials are recognized as a source of anti-inflammatory, antimicrobial and antitumor agents, therefore metabolites that are produced in plant metabolism have biological and pharmacological potential in the prevention and treatment of diseases of modern society (2).

One of the unutilized plant species in our region is elderberry (*Sambucus nigra* L). Elderberry is a wild-growing plant species that belongs to the Adoxaceae family. This plant species is characterized by whitish flowers and small dark purple fruits. Of the 30 species

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named in the world, nine have use-value, while only two species, *S. nigra* L. (black elderberry) and *S. canadensis* L. (Canadian, American elderberry), are used for commercial purposes. Three species grow in the Balkans: *Sambucus nigra* L., *Sambucus ebulus* L., and *Sambucus racemosa* L. (3). Black elderberry is widespread in western and central Europe, it can grow at an altitude of 1200 meters, it is also present in the south of Europe, in Sicily, and in the continental regions of Greece. The natural limit for the growth of the plant species *Sambucus nigra* is Scotland and southern Scandinavia. In addition to the European continent, elderberry also grows in Asia, North Africa, and North America. For the suitable growth of the elderberry, fertile humus and moist soil rich in nitrogen are needed, so it can be found in villages, fields, thickets, on the banks of rivers, in lighter forests (4).

Since the development of civilization, it has been used in traditional medicine and nutrition. Elderberry flowers and fruit were used to treat flu and colds, while in traditional nutrition they were used to make syrups, juices, jams, jellies. Numerous studies have shown that elderberry has pronounced biological properties if they are antioxidant, anti-inflammatory, neuroprotective, antimicrobial (5). Based on data from the literature, it was determined that that research focused on methods for isolation of elderberry essential oil is scarce. The studies that have dealt with the isolation and characterization of essential oil from elderberry are Najar et al., 2021 (6) and Agalar et al., 2014 (7). A special contribution and novelty within this research were based on the application of drying techniques, especially lyophilization as a modern drying technology, with the idea of preserving the chemical composition of elderberry, in order to obtain quality products that are not yet available on the market. Hence, the aim of this study was to identify the chemical composition of essential oil, of air-dried elderflowers and elderberry fruits, and lyophilized elderberry fruits collected on the Balkan Peninsula, using the gas chromatography-mass spectrometry (GC-MS) technique.

EXPERIMENTAL

The fresh elderberry flowers and fruits used in this research were collected in June and August 2017 in mountain Ljubišnja, Pljevlja (Montenegro). After collection, part of fresh elderberry flowers and fruits were dried by traditional drying technique and part fresh fruits were dried using lyophilization, as a modern drying technique at the industrial level. Traditional drying was performed in an area that is protected from sunlight and without the influence of temperature, and the drying process lasted 5 days at temperature 22 °C, while lyophilization lasted 48 hours. The dried plant material was prepared for hydrodistillation process. The specimen's voucher (*Sambucus nigra* L., No. 2-1512) was prepared and identified by Milica Rat, Ph.D., and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad, Faculty of Sciences, Republic of Serbia.

HYDRODISTILLATION OF PLANT MATERIAL

The essential oil was isolated from the air-dried fruits and flowers and the lyophilized elderberry fruits, in an apparatus according to Clavenger (1928). The weighed plant material was transferred to a distillation flask, the flask was placed on a heating pad and water was added as a solvent. The ratio of plant material to solvent was 1:10 (g/mL). The distillation process lasted 4 hours, and the essential oil was isolated in 1 ml of n-hexane. The hexane layer was dropped into a beaker and dried over anhydrous sodium sulfate. After 24 hours, the hexane solution was filtered and the filtrate was transferred to a previously measured flask. The residual solvent was evaporated on a vacuum evaporator. The content of easily volatile components in the essential oil is expressed as a relative percentage (%, m/m).

CHEMICAL ANALYSIS OF THE ESSENTIAL OIL OF *SAMBUCUS NIGRA*

The analysis of the essential oils of *S. nigra* included the qualitative and quantitative composition of the oil of air-dried flowers and fruits and lyophilized fruits, which was determined by GC/MS (gas chromatography/mass spectrometry) method (Thermo Fisher, USA). TR WAX-MS (30m x 0.25 mm, 0.25 µm) capillary column was used, while the analyzed samples were dissolved in methylene chloride and injected into GC through TriPlus AS autosampler (2 µL). The temperature program was: initial temperature 45 °C (8 min), then 8.0 °C/min to 230 °C (10 min). Injector, MS transfer line and ion source temperatures were 250 °C, 200 °C and 220 °C, respectively. The compounds were identified combining the NIST 08 MS database and MS spectra of authenticated standards. The final results were expressed as a relative percentage (%) (9).

RESULTS AND DISCUSSION

The results of the research are presented in the tables in the paper.

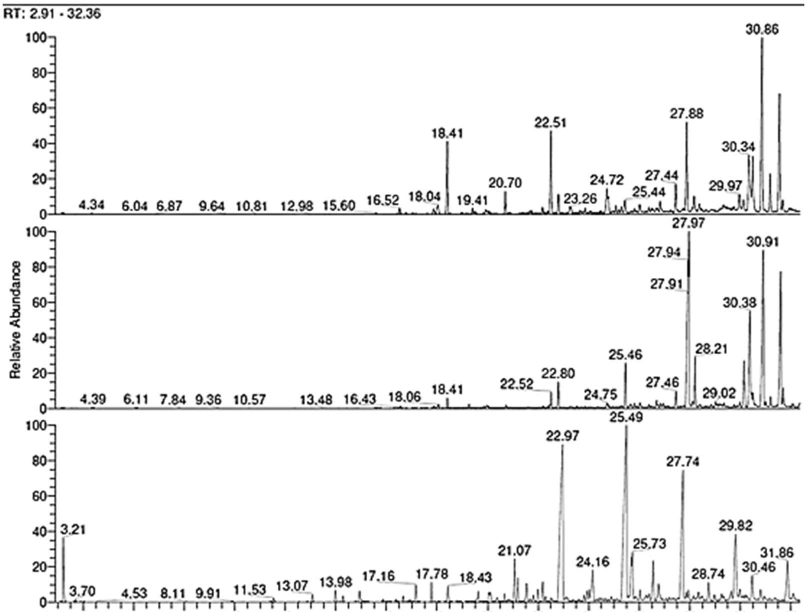

Figure 1. Chromatogram of elderberry and elderflowers essential oils. The numbers refer to those in Tables 1, 2, and 3.

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Table 1. The chemical composition of the essential oil obtained from air-dried elderberry fruits

Retention time	Isolated compounds	Content (%, m/m)
6.12	2-hexenol	5.19±0.21
7.09	4-heptyn-3-ol	0.10±0.01
7.89	isopentyl acetate	0.24±0.01
11.82	2-pentylfuran	0.33±0.02
11.93	E)-ocimene	0.13±0.01
12.73	*p*-cymene	0.10±0.01
14.47	α-lonene	0.53±0.01
14.73	*cis*-rose oxide	0.41±0.01
15.04	*trans*-rose oxide	0.20±0.01
15.60	β-cyclocitral	1.63±0.06
16.46	ethil caprylate	3.22±0.26
16.57	β-lonene	4.46±0.17
17.93	2,5,5,8a-tetramethyl-3,4,4a,5,6,8a-hexahydro-2H-chromene	5.20±0.16
18.44	linalyl anthranilate	24.15±2.31
20.74	α-terpineol	5.71±0.28
22.06	methyl hydrocinnamate	1.66±0.07
22.55	β-damascenone	35.70±3.41
24.01	indane-4-carboxaldehyde	2.45±0.15
25.82	5-methyl-2-phenyl-2-hexenal	8.58±0.26

Table 2. The chemical composition of the essential oil obtained from lyophilized elderberry fruits

Retention time	Isolated compounds	Content (%, m/m)
10.50	limonene	0.04±0.01
11.77	2-pentylfuran	0.03±0.01
11.87	*cis*-β-ocimene	0.04±0.02
12.33	*trans*-β-ocimene	0.11±0.01
12.98	terpinolene	0.12±0.01
14.40	α-lonene	0.25±0.03
14.68	*cis*-rose oxide	0.12±0.01
14.98	*trans*-rose oxide	0.04±0.01
15.54	*trans-p*-mentha-2,8-dien ol	0.19±0.01
16.52	β-lonene	3.25±0.16
18.04	α-lonone	6.87±0.21
18.41	linalool	32.80±3.23
18.98	β-lonone	1.07±0.05
20.70	α-terpineol	9.59±0.18
22.51	β-damascenone	38.64±3.80
31.68	phytol	6.84±0.27

In the essential oil of air-dried fruits, 19 components were identified, which represented 99.99% of the essential oil. The main components of the essential oil of air-dried elderberry fruits were: β-damascenone (35.70%), linalyl anthranilate (24.15%), 5-methyl-2-phenyl-2-hexenal (8.58%) and α-terpineol (5.71%).

In the essential oil of lyophilized elderberry fruits, 16 components were identified, which represented 100% of the composition of the essential oil. The dominant compound in the essential oil of lyophilized fruits was also β-damascenone (38.64%), with a slightly lower percentage of linalool (32.80%). α-Terpineol was present in the percentage of 9.59%, while α-lonone and phytol were recorded in the percentage of 6.87% and 6.84%, respectively. Comparing the obtained results with previous studies, it was noticed that elderberry oil contains the same groups of easily volatile compounds, but in a different percentage. Namely, the essential oil of air-dried elderberry in study (6) as a dominant compound were contained linalyl acetate (26.30%) and linalool (10.20%), while these compounds have not been identified in the essential oil of air-dried elderberry, tested in this paper. However, linalool was detected in the essential oil of lyophilized elderberries in a concentration of 32.80%, which is significantly higher, compared to the research of Najar et al., 2021 (6). These results could be explained by the influence of the drying process on the content of certain components, especially because lyophilization ensures the preservation of the chemical composition of plant materials. Also, the process of hydrodistillation affected the content of easily volatile compounds, but also the composition of the soil on which the plant grew.

In the essential oil of air-dried and lyophilized fruits, the dominant components belong to rose ketones. Damascenones and lonones are compounds found in various essential oils, including rose oil. They significantly contribute to the aroma of roses, despite the relatively low concentration, and are important chemical substances used in the perfumery, and are obtained by the decomposition of carotenoids (10).

Seven of the same components have been identified in the essential oil of air-dried and lyophilized elderberry fruits (2-pentylfuran, α-lonene, *cis*-rose oxide, *trans*-rose oxide, β-lonone, α-terpineol and β-damascenone). With the exception of α-terpineol and β-damascenone, the other compounds identified were in a higher percentage presented in the essential oil of air-dried elderberry fruits compared to the chemical composition of the essential oil of lyophilized elderberry fruits.

The difference in the content of individual components in the essential oils of air-dried and lyophilized elderberry is probably due to the influence of the hydrodistillation process itself, as well as the conditions under which the processes took place. The highest yield of essential oil is expected at the beginning of hydrodistillation until the temperature becomes constant and until equilibrium is established (11). The mechanism of hydrodistillation is closely related to the anatomy of berry fruits and their degree of fragmentation. The process of drying berry fruits affects the chemical composition of the fruits. Air-drying removes water from the plant material, but the dried material is more susceptible to contamination in this case. On the other hand, lyophilization provides the preservation of the chemical composition and quality of dried material. In the process of lyophilization, the anatomy of the berry fruits is more uniform, while air drying does not enable the uniform anatomy of the fruits. Drying and preservation of the plant material are also associated with the process of hydrodistillation. Higher degrees of fragmentation afford a larger contact area and easier isolation of more volatile compounds. During hydrodistillation, the boiling temperature could lead to disturbances or degradation of the chemical structure of thermolabile metabolites, which is directly related to the chemical composition being analyzed. The berry fruits contain essential oil in their structure, and by crushing, the structure of the fruit is destroyed and the essential oil is released on the surface of the particle of plant material. The oil that has reached the surface of the particle is quickly carried away by the steam that is formed during distillation, and that period in hydrodistillation is marked as fast hydrodistillation. The isolation of essential oil from the inner parts of the plant material is not completely ensured by crushing the berries, so the diffusion of the essential oil is difficult and this period of hydrodistillation is marked as slow hydrodistillation (12).

The difference in the content of aromatic components of essential oil also stems from the impossibility of temperature regulation. Thermolabile aromatic compounds are subject to degradation due to the influence of boiling temperature, in addition to hydrodistillation, the composition of the essential oil is also affected by the chosen technique of drying the plant material. The components present in the essential oil of air-dried elderberry fruits were presented in a higher percentage compared to the components found in the essential oil of lyophilized fruits, except α-terpineol and β-damascene which were the most abundant in the essential oil of lyophilized elderberries. Comparison of the drying techniques clearly shows the difference and efficiency of lyophilization in relation to the traditional method of drying, as well as the higher share of compounds in the essential oil of lyophilized fruits. Lyophilization affects the preservation of the structure of fruits, and thus their chemical composition, which is related to the quality of dried raw materials.

In addition to the fruit, for the production of essential oil within this scientific paper, a traditionally dried elderflower was used, and the results of the research are shown in Table 3.

The presence of 35 compounds was determined in the essential oil of the elderflowers, where the basic components of the oil are monoterpenes and sesquiterpenes. The most common compounds in the essential oil of elderflowers were: caran (13.19%), α-limonene diepoxide (7.23%), methyl salicylate (7.00%), caryophyllene (6.55%), benzopyran (5.89%), cis-geraniol (5.78%), and linalyl anthranilate (5.48%), while other aromatic components are presented in a smaller percentage. Compared with the chemical composition of the essential oil of the elderberry fruits, it was noted that monoterpenes cis-rose oxide and trans-rose oxide, as well as linalyl anthranilate belonging to the terpene family, are presented in the essential oil of both the fruits and the flowers of *Sambucus nigra*. Qualitative analysis of the oil showed that monoterpene cis-rose oxide and trans-rose oxide were detected in a higher percentage in the essential oil of the flowers, while linalyl anthranilate was identified four times higher in the essential oil of air-dried elderberry fruits. Based on the conducted examination of the essential oil from the fruits and flowers of *S. nigra*, it could be noticed that the dominant compounds in the essential oil of the fruit were present in a higher percentage, while the components present in the essential oil of the elderflower are identified in up to three times in a lower percentage. The composition and yield of essential oil in different organs of the same plant species depends on biotic and abiotic factors, the genetics of the plant itself, and the influence of the environment (13). In the families, Lamiaceae, Apiaceae, Asteraceae, Rutaceae, Lauraceae, Myrtaceae, plant species rich in essential oil are represented. The isolation of essential oil from the fruits of berries is not particularly interesting, as evidenced by the small number of studies. The essential oil of the elderberry fruits was not the subject of scientific publications, and it is assumed that the main reason is the low content of essential oil in the plant species of the genus Sambucus. Essential oil as a product of secondary metabolism of plants has a number of pharmacological activities, fungicidal, antirheumatic, as well as antiseptic effects.

Table 3. The chemical composition of the essential oil obtained from the traditionally dried elderflower

Retention time	Isolated compounds	Content (%, m/m)
8.44	α-pinene	0.01±0.01
8.94	3-penten-2-ol	0.03±0.01
10.94	2-pentylfuran	0.06±0.02
13.07	4-pentyn-2-ol	2.93±0.02
13.98	*cis*-rose oxide	4.20±0.05
14.28	*trans*-rose oxide	2.09±0.02
14.78	1.2-methyl-1.4-pentadiene	0.39±0.01
14.94	1-undecyn	4.78±0.04
15.86	linalool oxide	0.84±0.02
16.37	3.6-dihydro-4-methyl pyran	0.84±0.02
16.45	1.3-isopentyl-cyclopentene	0.31±0.01
17.16	benzopyran	5.89±0.07
17.78	linalyl anthranilate	5.48±0.05
18.43	caryophyllene	6.55±0.10
20.05	α-terpinol	2.97±0.04
20.67	epoxy-linalool	2.30±0.03
20.80	α-farnesene	0.50±0.02
20.88	β-cadinene	0.18±0.01
21.07	carane	13.19±0.27
21.20	methyl salicylate	7.00±0.37
21.54	α-limonene diepoxide	7.23±0.41
21.81	β-damascenone	1.68±0.12
22.00	6-methyl-5-nonadiene-2-on	3.99±0.23
22.18	*cis*-geraniol	5.78±0.30
22.29	*cis*-geranylacetone	1.39±0.15
23.81	γ-elemene	1.74±0.19
23.93	α-caryophyllene oxide	2.91±0.21
24.02	1-benzyl-1,2,3-triazole	2.51±0.17
24.42	*trans*-2-caren-4-ol	0.86±0.08
24.64	β- caryophyllene oxide	0.93±0.08
24.77	α-copaen-11-ol	0.58±0.03
24.89	β-methyl ionone	1.57±0.10
26.39	methyl-2-hydroxy-1,6-dimethyl cyclohexane carboxylate	2.22±0.15
28.34	α- hexyl cinnamaldehyde	2.18±0.18
29.64	3-*p*-menthen	3.88±0.26

In the food industry, the essential oil is increasingly used as a natural preservative and a potential alternative to synthetic preservatives to improve the taste of products, but also to protect products from oxidation and microorganisms during packaging (14). The obtained results indicate that the essential oils of elderberry are a good source of biopotential aromatic compounds. The presence of rose ketones and terpene molecules as dominant components in the analyzed essential oils provides an opportunity to continue research in this area in the direction of their potential application in the food industry as natural agents for maintaining product freshness and shelf life.

CONCLUSION

The results obtained in this paper showed that elderberry essential oils have a high content of volatile molecules belonging to rose ketones. In this regard, the further work and development of this research could be based on the application of modern technologies in order to isolate the dominant compounds in larger quantities. The dominant compounds detected in the analyzed essential oils are characterized by exceptional biological activity, especially linalool, terpineol, limonene, caryophyllene, which have antihypertensive, anticancer, antimicrobial, antioxidant, and sedative effects. In addition, future research could be based on testing the biological activity of the oil obtained, in order to apply it to existing food or cosmetic products, with the idea of ensuring a better quality of products.

REFERENCES

1. Koné, A. P.; Desjardins, Y.; Gosselin, A.; Cinq-Mars, D.: Guay, F.; Saucier, L. Plant extracts and essential oil product as feed additives to control rabbit meat microbial quality. *Meat sci.* **2019,** *150*, 111-121.
2. Rathod, N. B.; Kulawik, P.; Ozogul, F.; Regenstein, J. M.; Ozogul, Y. Biological activity of plant-based carvacrol and thymol and their impact on human health and food quality. *Food Sci. Technol.* **2021,** *116*, 733-748.
3. Jabbari, M.; Hashempur, M. H.; Razavi, S. Z. E.; Shahraki, H. R.; Kamalinejad, M.; Emtiazy, M. Efficacy and short-term safety of topical Dwarf Elder (*Sambucus ebulus* L.) versus diclofenac for knee osteoarthritis: a randomized, double-blind, active-controlled trial. *J. Ethnopharmacol.* **2016**. *188*, 80-86.
4. Tucakov, J. Lečenje biljem. **2010.** Beograd. Zapis
5. Domínguez, R.; Zhang, L.; Rocchetti, G.; Lucini, L.; Pateiro, M.; Munekata, P. E.: Lorenzo, J. M. Elderberry (*Sambucus nigra* L.) as potential source of antioxidants. Characterization, optimization of extraction parameters and bioactive properties. *Food Chem.* **2020**, *330*, 127266.
6. Najar, B.; Ferri, B.; Cioni, P. L.; Pistelli, L. Volatile emission and essential oil composition of *Sambucus nigra* L. organs during different developmental stages. *Plant Biosyst.* **2021**. *155*(4), 721-729.
7. Ağalar, H. G.; Demirci, B.; Başer, H. C. The volatile compounds of elderberries (*Sambucus nigra* L.). *Nat. Vol. & Essent. Oils*. **2014**. *1*(1), 51-54.
8. Clevenger, J. F. Apparatus for the determination of volatile oil. *J. Am. Pharm. Assoc.* **1928**. *17*(4), 345-349.

9. Micić, D.; Ostojić, S.; Pezo, L.; Blagojević, S.; Pavlić, B.; Zeković, Z.; Đurović, S. Essential oils of coriander and sage: Investigation of chemical profile, thermal properties and QSRR analysis. *Ind. Crops Prod*. **2019**. *138*, 111438.
10. Leffingwell, J. C.; Alford, E. D. Volatile constituents of perique tobacco. *Elec. J. Env. Agricult. Food Chem*. **2005**. *4*(2), 899-915.
11. Milojević, S. Ž.; Stojanović, T. D.; Palić, R.; Lazić, M. L.; Veljković, V. B. Kinetics of distillation of essential oil from comminuted ripe juniper (*Juniperus communis* L.) berries. *Biochem. Eng. J.* **2008**, *39*(3), 547-553.
12. Milojević, S. Ž. Kinetika hidrodestilacije, karakterizacija i frakcionisanje etarskog ulja ploda kleke *(Juniperus communis L.)* **2011**. Doktorska disertacija, Univerzitet u Beogradu, Tehnološko-metalurški fakultet.
13. Simões, C.M.O.; Schenkel, E.P.; Gosmann, G.; Mello, J.C.P.; Mentz, L.A. Farmacognosia: da planta ao medicamento. **2010**. 6. ed. Porto Alegre: UFRGS.
14. Ju, J.; Xu, X.; Xie, Y.; Guo, Y.; Cheng, Y.; Qian, H.; Yao, W. Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. *Food Chem.* **2018**. *240*, 850-855.