PHARMACIA

Pharmacia 72: 1–13
DOI 10.3897/pharmacia.72.e140155

Research Article

Comparison between the chemical composition of essential oils isolated from biocultivated *Salvia rosmarinus* Spenn. (*Rosmarinus officinalis* L.) and some commercial products

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Received 24 October 2024 ♦ **Accepted** 1 December 2024 ♦ **Published** 20 January 2025

Citation: Ivanova S, Nalbantova V, Benbassat N, Dzhoglova V, Dzhakova Z, Koleva N, Vasilev V, Grekova-Kafalova D, Ivanov K (2025) Comparison between the chemical composition of essential oils isolated from biocultivated *Salvia rosmarinus* Spenn. (*Rosmarinus officinalis* L.) and some commercial products. Pharmacia 72: 1–13. https://doi.org/10.3897/pharmacia.72.e140155

Abstract

Salvia rosmarinus Spenn. (*Rosmarinus officinalis* L.) is an aromatic medicinal plant associated with rich phytochemical composition and significant pharmacological potential. The aim of the current study was to evaluate the chemical profile of eight samples containing essential oil (EO) from biocultivated *Rosmarinus officinalis* L. from different locations and various harvesting periods. Another key point of this study was the evaluation of the chemical profile of commercial products containing EO isolated from bio *Salvia rosmarinus* Spenn. It was observed that the harvest period may not have a significant effect on some of the bioactive components that are present but may affect their percentage content. Furthermore, not only the time of harvesting the plant material influences essential oil component composition, but also its location may have an effect.

Keywords

essential oils, herbal plants, medicinal plants, *Rosmarinus officinalis* L., *Salvia rosmarinus* Spenn., traditional medicine

Introduction

Rosmarinus officinalis L. (syn. *Salvia rosmarinus* Spenn.) or rosemary belongs to one of the largest and most widespread families of flowering plants—Lamiaceae (de Macedo et al. 2020). The Lamiaceae family includes about 230 genera and over 6,000 species (Andrade et al. 2018). Essential oils (EOs) isolated from species belonging to this family, such as mint, sage, basil, lavender, rosemary, and thyme, are highly valued worldwide and associated with a great variety of biological activity.

Salvia rosmarinus Spenn. (Fig. 1) is an aromatic plant that originates from the Mediterranean region (Andrade et al. 2018), with one of the largest importers and processors of rosemary being the province of Murcia, Spain (Nieto et al. 2018). Currently, it is cultivated all over the world. The plant plays an important role in the traditional medicine of different nations and nowadays represents the main key point of many studies. It is associated with high nutritional value, a great variety of pharmacological activities, and diverse applications in various fields, including food flavoring and preservatives (Borges et al. 2019).

The name "rosemary" originates from the Latin word for dew, "ros", and sea, "marinus" (Begum et al. 2013). Rosemary is an evergreen, dense, and branching shrub that reaches about 1 m in height. The leaves are leathery, sessile, opposite, and curved-edged, linear or linear-lanceolate, characterized by 2–4 mm wide and 1–4 cm long. The leaves have a very specific and characteristic smell (Andrade et al. 2018). Their upper surface is dark green, and the lower surface is grey and covered with numerous trichomes (Begum et al. 2013; Andrade et al. 2018). The inflorescences are white or blue. *Salvia rosmarinus* Spenn. could be cultivated in gardens by sowing the seeds on calcareous soils, and after flowering, the plant is cut 10 cm above the ground (Begum et al. 2013).

The main compounds found in the composition of rosemary EO are oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons, aromatic compounds, and their derivatives. Their percentage content is affected by a number of factors, including climatic conditions. According to the European Pharmacopoeia, two types of rosemary EO could be distinguished: the Spanish type and the Moroccan and Tunisian type, which differ in the percentage content of the components in their composition: α-pinene, camphene, β-pinene, β-myrcene, limonene, cineole, *p*-cymene, camphor, bornyl acetate, α-terpineol, borneol, and verbenone (Andrade et al. 2018).

The main phytochemicals found in rosemary extracts include camphor, rosmarinic acid, caffeic acid, carnosic acid, carnosol, betulinic acid, and ursolic acid. Phenolic compounds, and especially rosmarinic acid, are known for their significant antioxidant activity, as they act as hydrogen atom donors and affect free radicals in different phases (Andrade et al. 2018; Katanić Stanković et al. 2020). In addition to the known terpenoid glycosides in rosemary extracts, five new species were obtained in China: officinoterpenosides A1, A2, B, C, and D (Zhang et al. 2014).

Rosemary is mostly known for its pleasant aroma and usage as a spice in the culinary (Zhang et al. 2014; Andrade et al. 2018). It is also proven effective and safe as a natural antioxidant for the preservation of food (Andrade et al. 2018). EO isolated from *Salvia rosmarinus* Spenn. is widely used in traditional medicine for the prevention and treatment of colds, muscle and joint pains, as well as rheumatism (Andrade et al. 2018). EOs and extracts obtained from *Salvia rosmarinus* Spenn. leaves and flowers are used to treat rashes, minor wounds, dyspepsia, circulation problems, headaches, and as an expectorant. Also as a diuretic and antispasmodic in renal colic (al-Sereiti et al. 1999; Ulbricht et al. 2010; Begum et al. 2013). In addition, studies associated the EO with anti-inflammatory, anti-anxiety, anti-Alzheimer's, and memory-improving activity. Moreover, rosemary EO exhibits antibacterial and anticancer properties and could be used as a nerve and brain tonic (Shimira et al. 2022).

Figure 1. *Salvia rosmarinus* Spenn.

Currently, the scientific interest in molecules of plant origin is significant, and it is highly likely that some novel drug candidates will be introduced soon (Doncheva et al. 2013; Tomova et al. 2020, 2021; Petkov et al. 2021; Slavova et al. 2022, 2024; Staynova and Yanachkova 2023; Stavrakeva et al. 2024). *Salvia rosmarinus* Spenn. is a plant with a rich phytochemical composition, containing a wide variety of secondary metabolites, including essential oil. Furthermore, it has significant pharmacological potential. It is widely used in traditional medicine and is included in many commercial products.

The aim of the present study is to evaluate the chemical composition of EOs isolated from biocultivated *Salvia rosmarinus* Spenn. collected from different locations during different seasons. The chemical profile of some commercial products was also evaluated.

Materials and methods

Plant material

The herbs of biocultivated *Salvia rosmarinus* Spenn. were collected from four different locations in Bulgaria shown in Table 1. The plants were authenticated in accordance with the European Pharmacopoeia by Associate Professor Niko Benbassat (European Pharmacopoeia 2020.). Voucher specimens (Table 1) were deposited in the Herbarium of the University of Agriculture-Plovdiv, Bulgaria. The plant material was collected in two different seasons—summer and winter. After collection, the plants were carefully dried at room temperature.

Table 1. Sample origin, coordinates, collection period, and voucher specimens of biocultivated *Salvia rosmarinus* Spenn.

Analysed Sample Origin	Coordinates	Collection period	Voucher Number	Abbreviation
Plovdiv region 1	42.2252508, 24.7304175	Winter	063581	Plov1W
Plovdiv region 1	42.2252508, 24.7304175	Summer	063580	Plov1S
Plovdiv region 2	42.1239160, 24.6885532	Winter	063575	Plov2W
Plovdiv region 2	42.1239160, 24.6885532	Summer	063574	Plov2S
Parvomay region	42.1037600, 25.2304724	Winter	063577	PW
Parvomay region	42.1037600, 25.2304724	Summer	063576	PS
Haskovo region	42.0245947, 25.3275170	Winter	063579	HW
Haskovo region	42.0245947, 25.3275170	Summer	063578	HS

Chemicals and reagents

For the calculation of retention indices (RI), the following hydrocarbons were used: nonane (≥99%), decane (≥99%), undecane (≥99%), dodecane (99%), tridecane (≥99%), tetradecane (≥99%), and hexadecane (≥99%), purchased from Merck KGaA (Darmstadt, Germany). The EO was diluted with hexane of analytical grade, which was purchased from Thermo Fisher Scientific GmbH (Bremen, Germany) and was used for the dilution of the EO.

Isolation of the essential oil

The essential oils of the air-dried flowering aerial parts were obtained by hydrodistillation for 4 hours using a Clevenger-type apparatus. The collected EOs were dried over anhydrous sodium sulfate and stored in dark glass vials at 4 °C until the GC-MS analyses.

Chromatographic conditions

The analyses of the composition of the EOs were performed using gas chromatography with mass spectrometry (GC-MS). For the GC-MS analysis, a Bruker Scion 436-GC SQ MS (Bremen, Germany) equipped with a ZB-5MSplus fused silica capillary column (0.25 µm film thickness and 30 m 0.25 mm i.d.) was used. As a carrier gas, helium was used with a constant flow rate of 1 mL/min. The volume of the injection was 1 µL. The split ratio of the injector was 1:25. In the beginning, the oven temperature was set at 60 °C, held for 1 min, then increased to 90 °C at a rate of 1 °C/min, then increased to 120 °C at a rate of 10 °C/min, and then increased to 220 °C at a rate of 17 °C/min for 1 min. The temperature of the injector was 250 °C, and the detector temperature was set to 300 °C. The collected mass spectra were in a full-scan mode with a mass range of 50–350 m/z. The identification of the separated components of the essential oils was achieved by comparing their MS spectra and retention indices (RI) with spectral data within the Wiley NIST11 Mass Spectral Library (NIST11/2011/EPA/NIH) and data in the literature. The retention times of the C8-C30 n-alkane series injected under the same conditions, which are described above, were used for the calculation of the RI values.

Results and discussion

Volatile constituents of the essential oils from biocultivated *Salvia rosmarinus* Spenn. from different locations

The chemical composition of the EOs obtained from the biocultivated *Salvia rosmarinus* Spenn. was determined by GC-MS. A total of 28, 26, 35, and 17 compounds representing 99.78%, 98.36%, 98.96%, and 99.0% of the total EO, respectively, were identified in the winter samples, while 29, 24, 28, and 30 compounds representing 99.96%, 94.96%, 99.19%, and 99.5% of the total EO, respectively, were identified in the summer samples.

The chemical composition of the EOs is presented with retention indices, formulas, class of the compound, and % of the total EO in Table 2**.**

The dominant class of terpenes in the EOs from the biocultivated *Salvia rosmarinus* Spenn. are oxygenated monoterpenes (MO). The percentage MO of the total oil content of winter samples is from 50.46% to 65.94%, while for the summer samples, the percentage MO of the total oil content is from 47.71% to 73.65%. Monoterpene hydrocarbons (MH) are also one of the main components in the composition of both winter and summer EOs—21.17% to 45.82% of the total oil content. Followed by the sesquiterpene hydrocarbons (SH)—1.15%-2.8%. The content of oxygenated sesquiterpenes (SO) is minimal, 0.25%–0.76%.

Of the representatives of MO, the highest percentages in all EOs are eucalyptol, camphor, and endo-borneol. The highest percentage of eucalyptol (30.04%) is in the Plov1S EO, camphor in the HS sample—29.55%, and endo-borneol in the HW sample—8.39%.

α-Pinene and camphene are the leading MH representatives, with the highest content in the Plov2W EO—19.12% and 8.46%, respectively. Of the other compounds, the percentage of 3-octanone in the HW sample was the highest (2.59%).

Different factors could affect the chemical composition of rosemary EO, including the drying process (Mohammed et al. 2020), the harvest stage, the growing media, and the usage of fertilizers (Miguel et al. 2007; Singh and Guleria 2013; Mwithiga et al. 2022). Mohammed et al. reported a significant change in the composition and yield, as well as the antioxidant activity of EOs, after drying the herb at different times (Mohammed et al. 2020). It has also

Table 2. Volatile constituents of the EOs from biocultivated *Salvia rosmarinus* Spenn. from different locations in Bulgaria as a percentage of the total EO.

№	Compound	RT	RI	Formula	Class of comp.	Plov1W	Plov1S	Plov2W	Plov2S	HW	HS	PW	PS
1	Tricyclene	6.621	920	$C_{10}H_{16}$	MH	0.18	0.15	0.30	0.28	0.14	0.13	–	0.13
2	*α*-Thujene	6.774	926	$C_{10}H_{16}$	MH	–	0.15	0.22	0.19	–	0.18	–	0.18
3	*α*-Pinene	7.11	932	$C_{10}H_{16}$	MH	18.08	12.92	19.12	18.60	7.51	5.34	17.08	5.62
4	Camphene	7.771	935	$C_{10}H_{16}$	MH	5.6	4.51	8.46	7.44	4.42	3.73	7.34	3.93
5	Dehydrosabinene	7.919	939	$C_{10}H_{14}$	MH	0.27	0.21	0.19	0.19	0.52	0.39	–	0.40
6	*β*-Pinene	9.07	948	$C_{10}H_{16}$	MH	1.85	3.09	3.53	2.67	1.28	3.44	3.88	3.56
7	1-Octen-3-ol	9.294	953	$C_8H_{16}O$	O	0.28	0.22	–	–	0.23	0.17	–	0.20
8	3-Octanone	9.514	968	$C_8H_{16}O$	O	0.15	0.13	0.42	0.10	2.59	2.04	–	2.10
9	*β*-Myrcene	9.75	975	$C_{10}H_{16}$	MH	3.13	3.25	1.09	1.00	0.81	0.79	–	0.78
10	3-Octanol	10.252	977	$C_8H_{18}O$	O	–	–	–	–	0.43	–	–	0.18
11	*α*-Phellandrene	10.67	987	$C_{10}H_{16}$	MH	0.32	0.36	2.84	3.55	1.45	2.44	–	2.48
12	3-Carene	10.795	990	$C_{10}H_{16}$	MH	0.98	1.17	–	–	0.59	0.6	1.52	0.61
13	*α*-Terpinene	11.328	997	$C_{10}H_{16}$	MH	0.71	0.66	1.15	1.38	0.68	0.84	0.74	–
14	4-Carene	11.428	1003	$C_{10}H_{16}$	MH	–	–	–	–	–	–	–	0.84
15	*p*-Cymene	11.854	1016	$C_{10}H_{14}$	MH	1.58	0.64	2.11	0.78	4.46	1.37	2.71	1.37
16	D-Limonene	12.201	1025	$C_{10}H_{16}$	MH	3.21	–	4.63	4.85	4.13	–	2.74	3.45
17	D-Sylvestrene	12.258	1027	$C_{10}H_{16}$	MH	–	–	–	–	0.61	–	–	–
18	Eucalyptol	12.449	1031	$C_{10}H_{18}O$	MO	27.12	30.04	17.19	17.49	20.20	25.82	23.04	23.09
19	*γ*-Terpinene	14.183	1065	$C_{10}H_{16}$	MH	0.84	1.09	1.49	2.08	0.46	1.21	–	1.19
20	α-Terpinolene	16.269	1070	$C_{10}H_{16}$	MH	0.74	0.97	0.69	0.92	0.29	0.71	0.45	0.69
21	Linalol	17.855	1094	$C_{10}H_{18}O$	MO	2.96	2.10	0.24	0.22	0.28	0.21	2.66	0.17
22	Chrysanthenone	19.324	1110	$C_{10}H_{14}O$	MO	0.16	0.38	0.11	–	0.35	–	–	–
23	Camphor	21.72	1143	$C_{10}H_{16}O$	MO	16.64	17.97	18.17	16.14	28.86	29.55	17.08	29.19
24	Isopinocamphone	22.921	1159	$C_{10}H_{16}O$	MO	–	–	–	–	0.48	–	–	–
25	Pinocarvone	23.086	1161	$C_{10}H_{14}O$	MO	–	0.20	–	–	0.31	0.31	–	0.29
26	endo-Borneol	24.399	1168	$C_{10}H_{18}O$	MO	5.75	5.35	5.93	5.25	8.39	7.15	7.59	6.74
27	Pinocamphone	24.527	1171	$C_{10}H_{16}O$	MO	0.14	0.46	–	–	0.73	1.15	–	1.32
28	Terpinen-4-ol	25.125	1190	$C_{10}H_{18}O$	MO	1.07	2.15	1.24	1.1	0.99	1.1	1.04	1.05
29	*α*-Terpineol	26.851	1205	$C_{10}H_{18}O$	MO	3.40	3.88	1.83	2.15	2.65	2.5	3.16	2.35
30	Verbenone	27.787	1212	$C_{10}H_{14}O$	MO	2.68	5.70	2.96	–	1.95	2.54	4.53	2.43
31	Bornyl acetate	34.004	1278	$C_{12}H_{20}O_2$	MO	0.52	1.43	2.79	5.36	0.75	3.32	2.09	3.22
32	*α*-Caryophyllene	36.932	1421	$C_{15}H_{24}$	SH	0.97	1.19	1.15	2.39	1.10	1.41	1.35	1.33
33	Humulene	37.415	1465	$C_{15}H_{24}$	SH	0.18	0.20	0.19	0.41	0.34	0.39	–	0.36
34	*β*-Bisabolene	38.002	1505	$C_{15}H_{24}$	SH	–	–	–	–	0.08	–	–	–
35	*δ*-Cadinene	38.116	1519	$C_{15}H_{24}$	SH	–	–	–	–	0.14	–	–	–
36	Caryophyllene oxide	38.767	1581	$C_{15}H_{24}O$	SO	0.27	0.39	0.32	0.42	0.33	0.36	–	0.25
37	Isoaromadendrene epoxide	39.376	1641	$C_{15}H_{24}O$	SO	–	–	–	–	0.43	–	–	–
					Terpene classes								
	Monoterpene hydrocarbons (MH)					37.49	28.17	45.82	43.93	27.35	21.17	36.46	25.23
	Oxygenated monoterpenes (MO)					60.44	69.66	50.46	47.71	65.94	73.65	61.19	69.85
	Sesquiterpene hydrocarbons (SH)					1.15	1.39	1.34	2.8	1.66	1.8	1.35	1.69
	Oxygenated sesquiterpenes (SO)					0.27	0.39	0.32	0.42	0.76	0.36	–	0.25
	Others (O)					0.43	0.35	0.42	0.1	3.25	2.21	–	2.48
	Total identified (%)					99.78	99.96	98.36	94.96	98.96	99.19	99.0	99.5

been reported that the chemical composition of rosemary EOs and yields vary more depending on collection period, photoperiod, and temperature, rather than with fertilizers and growing media used (Miguel et al. 2007). Singh and Guleria also found that the composition and content of the EO were not affected by the organic and inorganic fertilizers used, but only by the harvesting period (Singh and Guleria 2013). It was investigated by Mwithiga et al. the effect of soil amendments on EO yield and quality, as well as on rosemary growth, and oil quality was found to be significantly affected by cow manure (Mwithiga et al. 2022).

Annemer et al. demonstrated differences in the identified components during the different harvest periods. The amount of 1,8-cineole was present in a higher percentage during the May harvest compared to October. On the other hand, the amount of *α*-pinene identified was found to be greater during October. Although with minor differences, the present study confirms this correlation with respect to the different seasons and the harvest time (Annemer et al. 2022). Moreover, the amount of EO originating from the Plovdiv region is slightly higher than in other regions.

The chromatograms from the GC-MS analyses of the EOs are presented in Figs 2–9**.** The percentage of the relative peak area is the average value of three measurements. The standard error of the mean has been removed, and it was not more than 2%.

The GC-MS chromatogram from Fig. 2 shows that the main compounds in the Plov1W sample are α-pinene (18.08%), eucalyptol (27.12%), camphor (16.64%), and endo-borneol (5.75%), as well as for the HW sample (Fig. 4), 7.51%, 20.20%, 28.86%, and 8.39%, respectively. The Plov2W sample also contains α-pinene (19.12%), eucalyptol (17.19%), and camphor (18.17%), but also another main compound, camphene—8.46% (Fig. 3). The main compounds in the PW sample are α-pinene—17.08%, camphene—7.34%, eucalyptol—23.04%, and camphor—17.08% (Fig. 5).

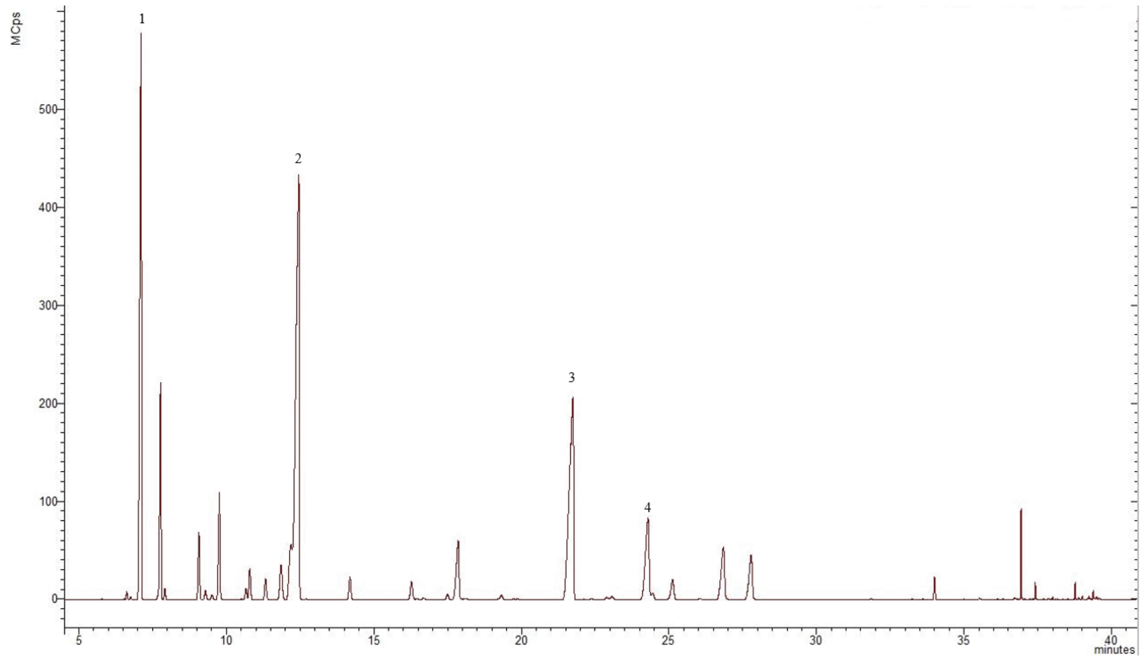

Figure 2. GC-MS chromatogram of the Plov1W EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-eucalyptol, 3-camphor, and 4-endo-borneol.

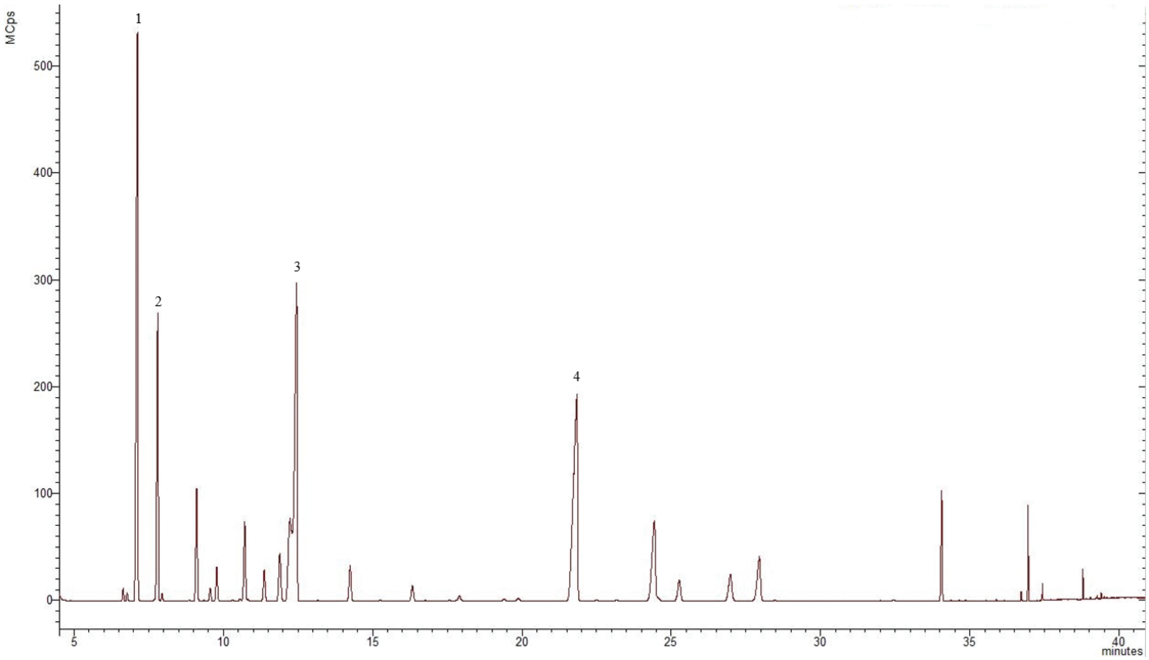

Figure 3. GC-MS chromatogram of the Plov2W EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-camphene, 3-eucalyptol, and 4-camphor.

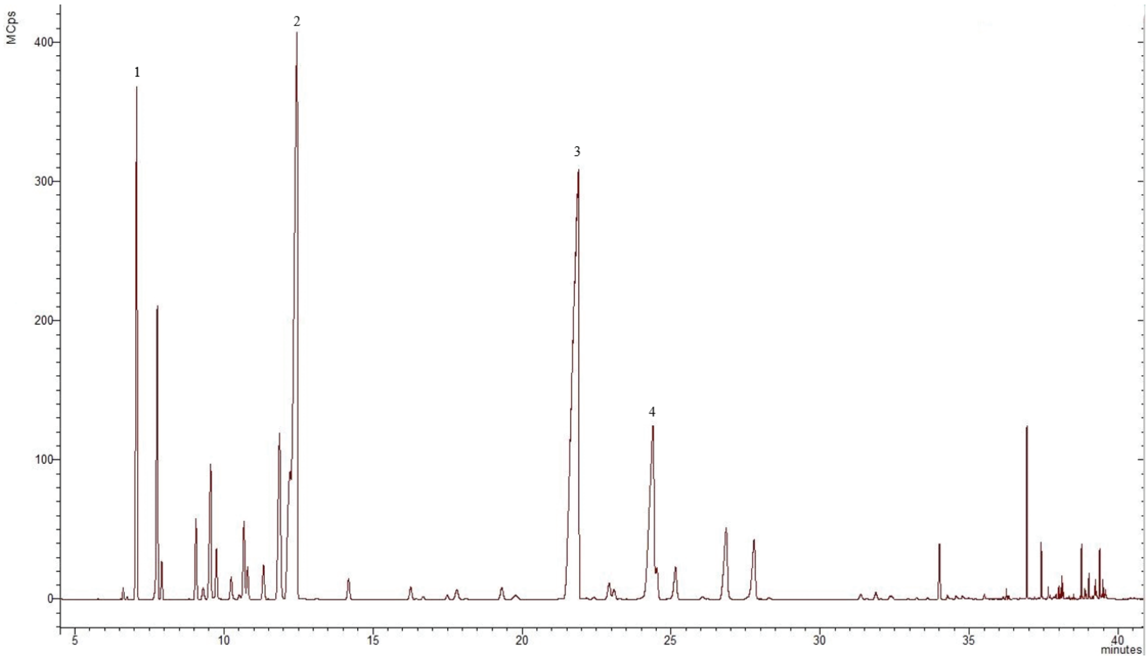

Figure 4. GC-MS chromatogram of the HW EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-eucalyptol, 3-camphor, and 4-endo-borneol.

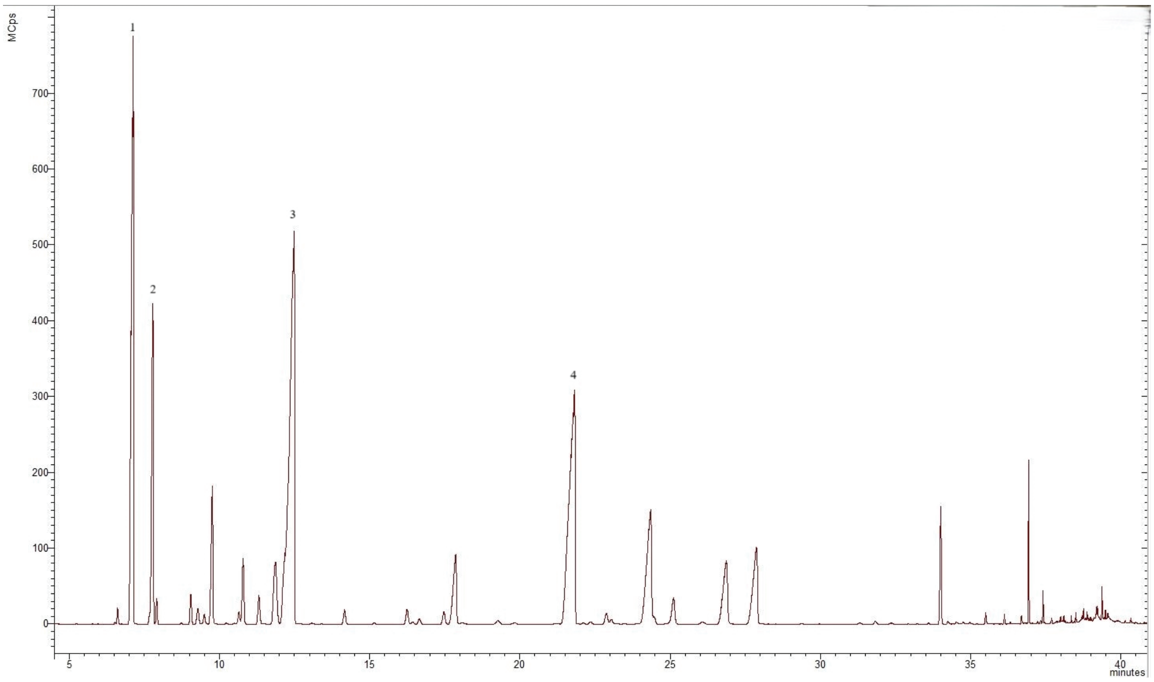

Figure 5. GC-MS chromatogram of the PW EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-camphene, 3-eucalyptol, and 4-camphor.

The GC-MS chromatograms from Figs 6–9 represent the main components in the chemical composition of Plov1S, Plov2S, HS, and PS EOs as α-pinene (12.92%, 18.60%, 5.34%, and 5.62%), eucalyptol (30.04%, 17.49%, 25.82%, and 23.09%), and camphor (17.97%, 16.14%, 29.55%, and 29.19%). Also, endo-borneol for HS sample (7.15%) and PS sample (6.74%), verbenone for Plov1S sample (5.70%), and camphene for Plov2S sample (7.44%).

The main compounds found in the EOs from biocultivated *Salvia rosmarinus* Spenn. were eucalyptol, α-pinene, and camphor.

Eucalyptol, also known as 1,8-cineole, is a monoterpene that is mainly isolated from *Eucalyptus* EO, in which it is found to be in the highest concentration (Cai et al. 2021). *Eucalyptus* EO has been used in folk medicine for decades, and today it is widespread in the food, cosmetic,

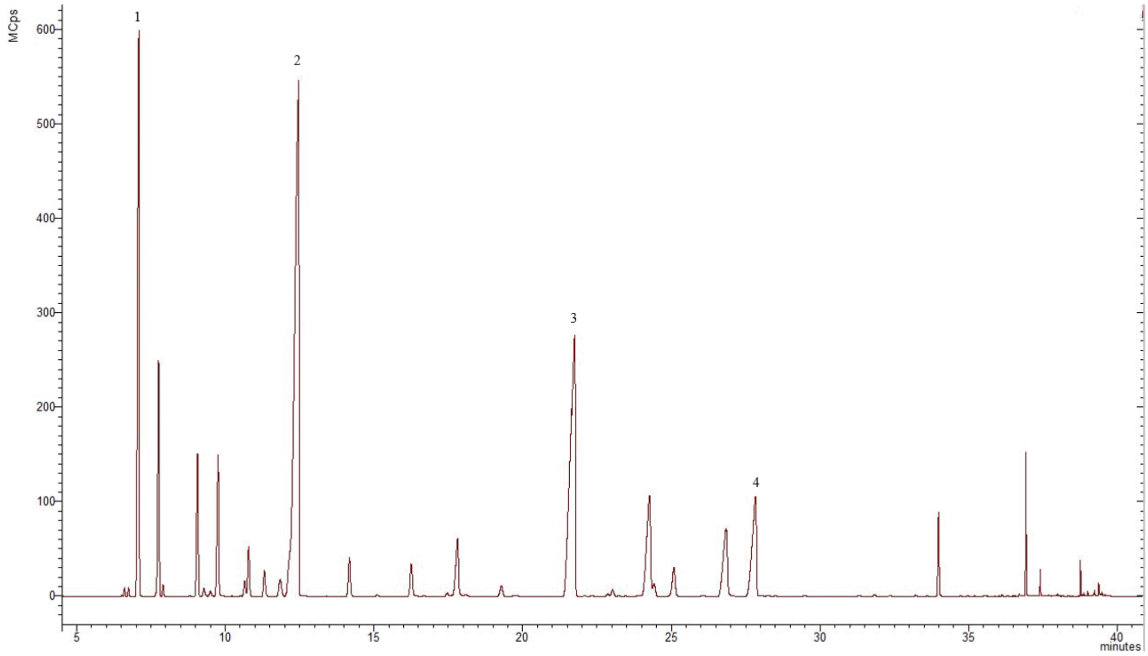

Figure 6. GC-MS chromatogram of the Plov1S EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-eucalyptol, 3-camphor, and 4-verbenone.

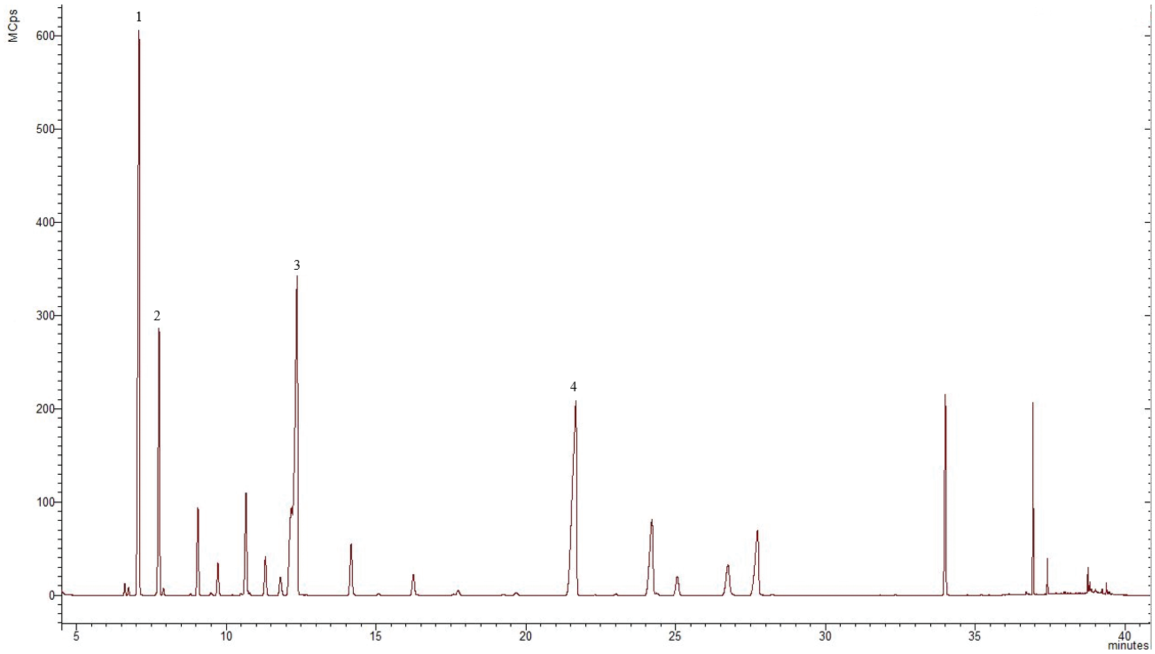

Figure 7. GC-MS chromatogram of the Plov2S EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-camphene, 3-eucalyptol, and 4-camphor.

and perfume industries (Cai et al. 2021). It is included in the composition of mouthwashes with low activity against *K. pneumonia*, MRSA, and others (Masadeh et al. 2013), as well as in repellents, precisely because of the high content of eucalyptol (Cai et al. 2021). Some of the mechanisms of action of 1,8-cineole are well studied, and the substance is used for the treatment of gastrointestinal, respiratory, and cardiovascular diseases, as well as in influencing the progression of Alzheimer's disease (Seol and Kim 2016; Cai et al. 2021; Hoch et al. 2023). Other beneficial effects of eucalyptol are analgesic, sedative, anti-inflammatory, antimicrobial, and antioxidant effects (Seol and Kim 2016; Hoch et al. 2023). 1,8-cineole activates the PI3K-AKt signaling pathway, delays the progression of liver cirrhosis and fibrosis, and is therefore suitable for the treatment of non-alcoholic steatohepatitis (Murata et al. 2015; Cai et al. 2021). Rosemary 1,8-cineole has been tested against hepatitis virus A (HAV) in berries and was proven effective

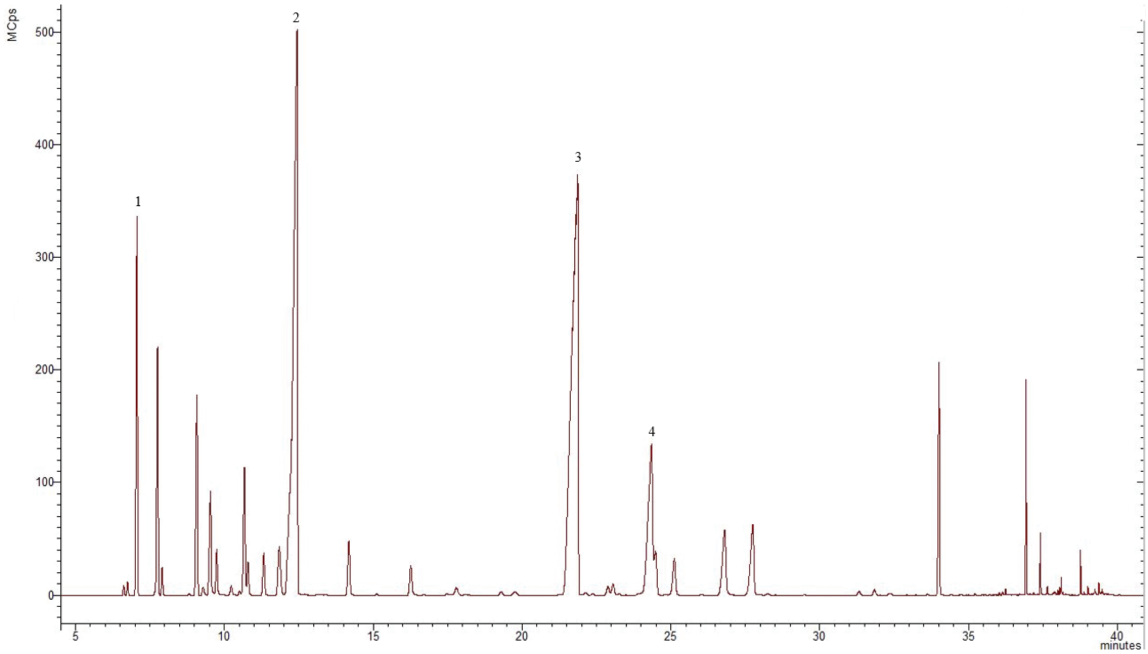

Figure 8. GC-MS chromatogram of the HS EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-eucalyptol, 3-camphor, and 4-endo-borneol.

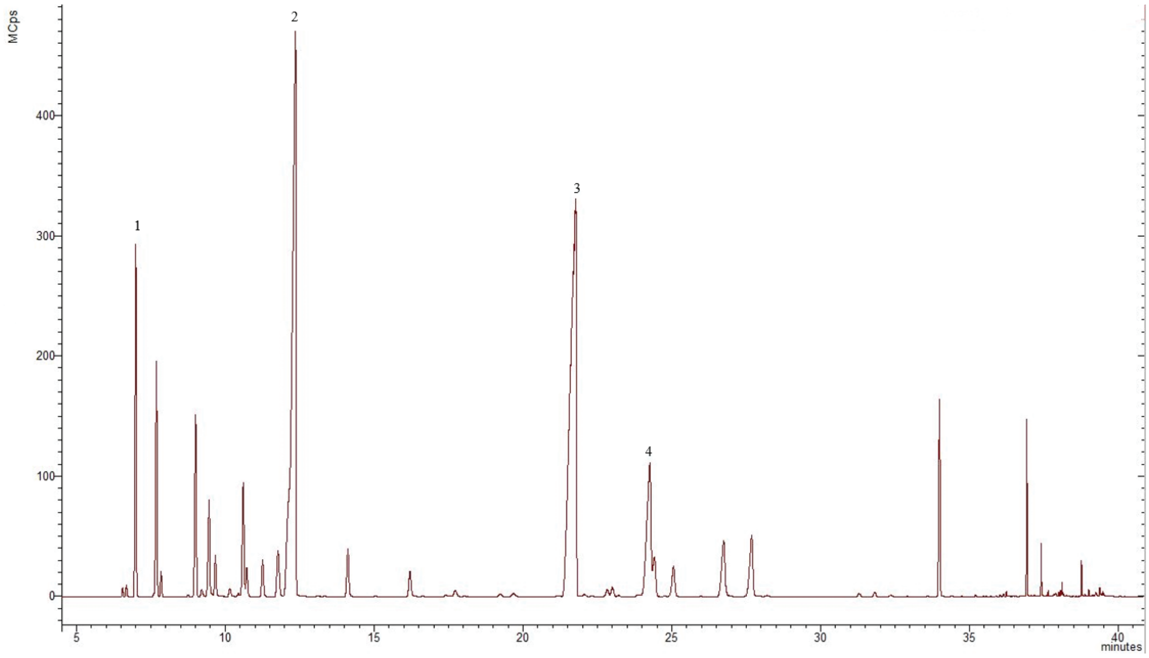

Figure 9. GC-MS chromatogram of the PS EO, where GCps—Giga Counts per second, and the numbers refer to the following compounds: 1-α-pinene, 2-eucalyptol, 3-camphor, and 4-endo-borneol.

by a significant reduction of the virus titer on the surface of these fruits (Battistini et al. 2019). The use of the EO in combination with the HAV vaccine may be a prospect for future studies on the control of the spread of HAV, especially in risk groups (Dimitrova et al. 2014).

Cineol also has effects on acute pancreatitis (Lima et al. 2013), diarrhea (Jalilzadeh-Amin and Maham 2015), and colitis (Santos et al. 2004). Eucalyptol is a peroxisome proliferator-activated receptor-γ (PPARγ) agonist that regulates inflammation in the colon (Venkataraman et al. 2023). The gastroprotective activity of 1,8-cineole has been proven by several studies (Santos and Rao 2001; Caldas et al. 2015; Azab et al. 2017) and is due to three mechanisms of action: cytoprotective activity by increasing the amount of mucus in the stomach, stimulating the regeneration of cells in the stomach, and decreasing myeloperoxidase activity and the related antioxidant activity (Cai et al. 2021). 1,8-cineole, alone or in combination with other products and drugs, is used in the treatment and relief of symptoms of diseases affecting the respiratory system, such as asthma, bronchitis, pneumonia, flu, rhinosinusitis, and others. Effects on the respiratory system are due to its anti-inflammatory activity by binding to NF-jB (Yu et al. 2018), muscle relaxation (Bastos et al. 2009), as well as inhibition of mucus hypersecretion by affecting tumor necrosis factor-a (TNF-a) and interleukin-1b (Sudhoff et al. 2015).

α-Pinene and its structural isomer β-pinene are representatives of the monoterpenes class that are common in the chemical composition of many plants. These two compounds are widely used due to their diverse biological effects. α-Pinene is found to have anticoagulant (Yang et al. 2011; Salehi et al. 2019), antioxidant, anti-inflammatory, neuroprotective (Goudarzi and Rafieirad 2017; Lee et al. 2017; Salehi et al. 2019; Zamyad et al. 2019; Khoshnazar et al. 2020; Allenspach and Steuer 2021), antimicrobial, analgesic, antitumor (Salehi et al. 2019), and gastroprotective activity (Pinheiro et al. 2015). In vitro, α-pinene exhibited anticoagulant activity, significantly suppressing platelet aggregation due to thromboxane A2 inhibition or stimulation of platelet Ca^{2+} (Yang et al. 2011; Salehi et al. 2019). Moreover, the target compound—α-pinene—exhibits anti-inflammatory and antioxidant activity. Inhibits the expression of nuclear factor-kappa B (NF-κB), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), which are inflammatory mediators in human skin, and therefore the formation of reactive oxygen species (ROS) is reduced. α-Pinene reduces the oxidative and inflammatory responses induced by UVA exposure (Allenspach and Steuer 2021). These activities of α-pinene are also related to its neuroprotective effect in affecting the symptoms of neurodegenerative diseases, such as Parkinson‘s disease, Alzheimer‘s disease, and epilepsy (Goudarzi and Rafieirad 2017; Lee et al. 2017; Zamyad et al. 2019; Khoshnazar et al. 2020). α-Pinene showed antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Yang et al. 2015; Utegenova et al. 2018; Allenspach and Steuer 2021) and *Escherichia coli* (Yang et al. 2015; Allenspach and Steuer 2021); also antifungal activity was observed against *Candida* species (Nóbrega et al. 2021).

The compound was used in a study with mice to demonstrate its gastroprotective activity by being orally administered prior to the induction of gastric lesions with ethanol and indomethacin. α-Pinene has been found to reduce the acidity and volume of gastric juice, to stimulate mucus secretion, and to protect the gastric mucosa (Pinheiro et al. 2015).

Camphor is a monoterpenoid that occurs naturally in nature in the dextrorotatory form, and the levorotatory form is obtained synthetically or is included in small amounts in the composition of specific plants. It is widely distributed as a flavoring agent in the food, perfumery, and cosmetic industries. Also, the compound is included in the composition of cleaning agents. Camphor exhibits analgesic, antiviral, antimicrobial, antitussive, anticancer, insecticidal, and antinociceptive activity (Chen et al. 2013; Zielińska-Błajet and Feder-Kubis 2020). The antispasmodic activity and the control of bronchospasm and cough are due to the anticholinergic and antihistaminergic activity of the compound (Zuccarini and Soldani 2009). Nowadays, camphor is included in rubefacients and topical analgesics used to treat muscle pain. Moreover, the compound is also used as a topical antipruritic and anti-infective and internally as a carminative and stimulant. However, ingestion toxicity has been demonstrated and results in dizziness, confusion, irritability, convulsions, and neuromuscular hyperactivity, with a lethal dose of 50–500 mg/kg (Chen et al. 2013). Camphor is proven to relieve itching in contact dermatitis by having a direct effect on the nerve endings of the skin (Zuccarini and Soldani 2009). Rosemary has antimicrobial activity against microorganisms that cause meat spoilage. This activity is due precisely to the high content of camphor in the essential oil, which affects Gram-negative (*Serratia liquefaciens* and *Pseudomonas fluorescens*) and Gram-positive (*Lactobacillus curvatus*, *Lactobacillus sake*, *Carnobacterium piscicola*, and *Brochothrix thermosphacta*) bacteria (Ouattara et al. 1997).

Other compounds included in the composition of all analyzed samples in high percentages are endo-borneol and camphene.

The secondary alcohol endo-borneol, from the group of bicyclic terpenes, is known for its efficacy in treating coughs, colds, and bronchitis. It also improves blood circulation, affects swelling and pain, and is included in the composition of repellents (Manilal et al. 2021). Borneol and its derivatives exhibit anti-inflammatory, antiviral, and antimicrobial effects. In China and India, it is used to treat gastrointestinal diseases (Zielińska-Błajet and Feder-Kubis 2020).

The bicyclic monoterpene camphene is present in the essential oils of plants such as *Rosmarinus officinalis*, *Salvia lavandulifolia*, *Valeriana officinalis*, *Piper cernuum*, *Thymus satureoides*, *Thymus camphoratus*, and *Thymus carnosus*. It is widely used in the food and cosmetic industries as a flavor and a fragrance additive. A number of in vitro and in vivo studies prove the antioxidant, antibacterial, antifungal, hypolipidemic, and anti-inflammatory activity of camphene (Hachlafi et al. 2023).

Chemical profiles of the EOs from commercial products

The chemical composition of the EOs obtained from the commercial products was determined by GC-MS. A total of 16, 19, and 24 compounds, representing 97.76%, 96.97%, and 99.08% of the total EO, respectively, were identified.

The chemical composition of the EOs is presented with retention indices, formulas, class of the compound, and % of the total EO in Table 3.

The dominant class of terpenes in the EOs from commercial products are oxygenated monoterpenes (MO). The percentage MO of the total oil content of the samples is from 41.77% to 61.42%. Monoterpene hydrocarbons (MH) are the main components in the composition of all three EOs—42.34%, 53.16%, and 31.53%, respectively, of the total oil content. Followed by the sesquiterpene hydrocarbons (SH): 1.94%–5.82%. The content of oxygenated sesquiterpenes (SO) is minimal, and they persist just in EO3: 0.18%.

Table 3. Volatile constituents of the EOs from commercial products as a percentage of the total EO.

№	Compound	RT	RI	Formula	Class of compound	EO1	EO2	EO3
1	Tricyclene	6.621	920	$C_{10}H_{16}$	MH	–	–	1.16
2	*α*-Thujene	6.774	926	$C_{10}H_{16}$	MH	–	0.42	1.19
3	*α*-Pinene	7.11	932	$C_{10}H_{16}$	MH	1.54	18.13	13.89
4	Camphene	7.771	935	$C_{10}H_{16}$	MH	0.64	11.50	4.47
5	*β*-Pinene	9.07	948	$C_{10}H_{16}$	MH	0.81	5.18	5.13
6	1-Octen-3-ol	9.294	953	$C_8H_{16}O$	O	–	–	0.13
7	β-Myrcene	9.75	975	$C_{10}H_{16}$	MH	–	–	0.13
8	*α*-Phellandrene	10.67	987	$C_{10}H_{16}$	MH	0.40	1.01	0.19
9	3-Carene	10.795	990	$C_{10}H1_6$	MH	0.22	1.38	–
10	*α*-Terpinene	11.328	997	$C_{10}H_{16}$	MH	–	1.17	0.61
11	4-Carene	11.428	1003	$C_{10}H_{16}$	MH	18.39	–	–
12	*p*-Cymene	11.854	1016	$C_{10}H_{14}$	MH	10.50	5.69	1.92
13	D-Limonene	12.201	1025	$C_{10}H_{16}$	MH	8.90	8.14	1.67
14	Eucalyptol	12.449	1031	$C_{10}H_{18}O$	MO	27.32	12.67	37.21
15	*γ*-Terpinene	14.183	1065	$C_{10}H_{16}$	MH	0.94	0.54	0.84
16	*α*-Terpinolene	16.269	1070	$C_{10}H_{16}$	MH	–	–	0.33
17	Linalol	17.855	1094	$C_{10}H_{18}O$	MO	0.92	–	0.84
18	Camphor	21.72	1143	$C_{10}H_{16}O$	MO	15.75	20.64	14.74
19	endo-Borneol	24.399	1168	$C_{10}H_{18}O$	MO	4.72	0.90	4.24
20	Terpinen-4-ol	25.125	1190	$C_{10}H_{18}O$	MO	–	–	0.74
21	*α*-Terpineol	26.851	1205	$C_{10}H_{18}O$	MO	4.01	5.19	2.96
22	Verbenone	27.787	1212	$C_{10}H_{14}O$	MO	–	0.76	–
23	Linalyl formate	32.619	1262	$C_{11}H_{18}O_2$	MO	–	0.21	–
24	Bornyl acetate	34.004	1278	$C_{12}H_{20}O_2$	MO	0.76	1.40	0.69
25	*α*-Caryophyllene	36.932	1421	$C_{15}H_{24}$	SH	1.94	1.61	4.97
26	Humulene	37.415	1465	$C_{15}H_{24}$	SH	–	0.43	0.66
27	*β*-Bisabolene	38.002	1505	$C_{15}H_{24}$	SH	–	–	0.19
28	Caryophyllene oxide	38.767	1581	$C_{15}H_{24}O$	SO	–	–	0.18
	Terpene classes							
	Monoterpene hydrocarbons (MH)					42.34	53.16	31.53
	Oxygenated monoterpenes (MO)					53.48	41.77	61.42
	Sesquiterpene hydrocarbons (SH)					1.94	2.04	5.82
	Oxygenated sesquiterpenes (SO)					–	–	0.18
	Others (O)					–	–	0.13
	Total identified (%)					97.76	96.97	99.08

Of the MO representatives, the highest percentage in all EOs are eucalyptol (EO1: 27.32%, EO2: 12.67%, and EO3: 37.21%) and camphor (EO1: 15.75%, EO2: 20.64%, and EO3: 14.74%). Other MO that are in the composition of the EOs, but in a lower percentage of the total oil content, are endo-borneol (in EO1 and EO3 samples: 4.72% and 4.24%, respectively) and α-terpineol (in EO1 and EO2 samples: 4.01% and 5.19%, respectively).

However, the three EOs differ in their MH content. For the EO1 sample, the main ones are 4-carene (18.39%) and *p*-cymene (10.50%). For the EO2 sample, the main MH compounds are *α*-pinene (18.13%), camphene (11.50%), *β*-pinene (5.18%), and *p*-cymene (5.69%), while for the EO3 sample are *α*-pinene (13.89%) and *β*-pinene (5.13%). *α*-Caryophyllene, a SH, persists just in the EO3 sample—4.97%.

The data obtained from the comparative analysis of the chemical composition of EOs from biocultivated *Salvia rosmarinus* Spenn. and commercial products indicate that the contents of the major components are identical with minor differences in their percentages. The compounds that are about 5% of the composition are also similar, with a few exceptions.

Conclusion

The conducted GC-MS analyses showed the presence of oxygenated monoterpenes, monoterpene hydrocarbons, and sesquiterpene hydrocarbons in a lower percentage, and a minimal percentage of the total oil content of oxygenated sesquiterpenes in the EOs from the biocultivated *Salvia rosmarinus* Spenn. and the commercial products. 17–35 volatile constituents representing 94.96–99.96% of the total oil were detected in the EOs from the biocultivated rosemary, while for the commercial products the identified volatile constituents were 16–24, representing 96.97–99.08% of the total oil content. The main components isolated from EOs are α-pinene, eucalyptol, and camphor. The percentage of eucalyptol in biocultivated rosemary oil is from 17.19–30.04%, while for commercial products it ranges from 12.67–37.21%. In addition, it was observed that the harvest period may not have a significant effect on some of the bioactive components that are present but may affect their percentage content. Furthermore, not only the time of harvesting the plant material influences essential oil multi-component composition, but also its location may have an effect.

Eucalyptol is well studied and widely used to treat gastrointestinal and respiratory diseases, as well as being used in the food and cosmetic industries. However, further examinations could be done regarding the effect on bacteria, as well as the effect on neurodegenerative diseases such as Alzheimer's disease. On the other hand, the topical application of camphor as an antipruritic, anti-infective, and topical analgesic agent for the treatment of muscle pain could be more researched. The results indicate the potential opportunity for further studies of the biological activity of *Salvia rosmarinus* Spenn. The EO has the potential to be included in novel drugs for the treatment of gastrointestinal, respiratory, and cardiovascular diseases, as well as in local remedies for the treatment of dermatitis.

Acknowledgements

This study was supported by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C03.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.
The authors declared that no experiments on humans or human tissues were performed for the present study.
The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.
The authors declared that no experiments on animals were performed for the present study.
The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Funding

This research received no external funding.

Author contributions

Conceptualization, S.I.; methodology, S.I, K.I. and N.B..; software, V.N.; investigation, N.B., V.N. V.D and Z.D., K.I., N.K.; writing—original draft preparation, S.I., Z.D., V.N.; writing—review and editing S.I., D.G-K..; visualization V.N., V.V.; supervision, D.G-K and S.I..

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Data availability

All of the data that support the findings of this study are available in the main text.

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