



Research Article

Similarities and differences of chemical profile for *Rosmarinus officinalis* plants when different extraction techniques were used

Blerina Xhaferaj^{1,3}, Anila Kripa², Sokol Kociaj³, Lindita Vrushi⁴, Kleva Shpati⁵ , Aida Dama⁵ , Erda Qorri⁵ , Kozeta Vaso⁶, Altin Mele⁶ and Aurel Nuro^{6*}

1. Department of Pharmacy, Faculty of Medicine, University of Medicine, Tirana, Albania.
2. Department of Food Science and Biotechnology, Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania.
3. ALTREChem (Private Company on Medicinal Plants), Tirana, Albania.
4. Esencial (Private Company on Medicinal Plants), Elbasan, Albania.
5. Department of Pharmacy, Faculty of Medicinal Sciences, Albanian University, Tirana, Albania.
6. Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Tirana, Albania.

Abstract

The essential oil of *Rosmarinus officinalis* L. was analyzed from a cultivated population in the Durres area using three different extraction techniques: hydro-distillation with a Clevenger apparatus (HD), Soxhlet extraction (SE) and headspace technique by PDMS fiber (HS-SPME). *Rosmarinus officinalis* can be found easy (naturally and cultivated) in all Albanian territories. It is very popular in Albania for culinary, traditional medicine, and as an ornamental plant. The samples of *Rosmarinus officinalis* (branches and leaves) were collected from different stations of Lalez Bay (Durres area), in May 2025. The air-dried samples were subjected to extraction processes by Clevenger and Soxhlet apparatuses as well as the HS-SPME technique. The chemical compositions of the extracts obtained using all methods were determined by GC/FID technique. The chemical profiles of *Rosmarinus officinalis* obtained using the two methods were almost identical. Oxygenated monoterpenes were found in higher percentages in all samples. Cineole, alpha-pinene, caphour and verbenone were found at higher level. Some differences were observed between the extraction techniques. HD and HS-SPME techniques showed a higher number of isolated compounds, while SE showed a lower number of compounds, but in some cases, a higher percentage for some individuals. Although all these techniques can be used to investigate the chemical profile of aromatic and medicinal plants, the use of HD and/or HS-SPME is suggested as they provide a clear and more complete chemical profile of plants compared to SE. Chemical profile of *Rosmarinus officinalis* samples from the Durres area, was similar to that reported in other reported studies from the Balkan and Mediterranean areas.

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Prof. Dr. Radosław Kowalski

Corresponding Author

Prof. Dr. Aurel Nuro
E-mail: aurel.nuro@fshn.edu.al
Tel.: +00355684056176

Keywords

Rosmarinus officinalis, essential oil, hydro-distillation, HS-SPME, Soxhlet extraction, GC/FID.

1. Introduction

Rosmarinus Officinalis is a species of the Lamiaceae family. This plant, commonly known as rosemary, is fragrant and perennial. It is spontaneous in almost all Albanian territories and the Mediterranean region. It

is also easy to find because it is frequently cultivated in many houses for culinary and ornamental purposes [1-3]. Rosemary plants can withstand drought, and survive prolonged water shortages. The branches of

rosemary are usually straight and can reach 1.5 m. The leaves are evergreen, 2-4 cm long and 2-5 mm wide, green above and white below, with dense and short hairs. The plants produce flowers in spring and summer, but can continuously bloom in warm climates. Rosemary also has a tendency to bloom outside its normal flowering season (in December, and February). Rosemary is cultivated in gardens (ornamental plant) because it is thought to have effects in insect control. The leaves (occasionally branches with leaves) are used to enhance the flavor of various foods [4, 5]. They have a slightly bitter (pine) taste and characteristic aroma that complements the aroma of cooked foods [1-5]. Rosemary has been known for centuries as a medicinal plant and in culinary as a spice with a characteristic aroma. It is one of the oldest plants known and used by humans. It is thought to have arrived from England in the Mediterranean regions from the Romans. Rosemary tea can be used to improve the health of the entire body, including the brain, heart and liver. Rosemary contains a large number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid, etc. In traditional medicine, extracts and essential oils extracted from flowers and leaves are used to treat various disorders. Rosemary essential oil contains 10-20% camphor, although its chemical composition can vary greatly in different regions [4-11]. The components of essential oils are usually compounds with boiling points from 120-150°C and most of them are very slightly soluble in water. Therefore, steam distillation is the most widely used technique for obtaining essential oils from aromatic and medicinal plants. As limitation of this technique is that, polar and relatively hydrophilic compounds cannot be completely obtained from the plant. In addition, the hydro-distillation (HD) technique is a common method used in laboratories and/or industrial scales for the production of essential oils from aromatic/medicinal plants. For research purposes and also recommended by the Pharmacopoeia, hydro-distillation by Clevenger apparatus, is the most commonly used technique. Continuous extraction was performed using this apparatus. The extraction process using a Soxhlet (SE) apparatus is similar, but there is no solvent in the

contact with the plant; instead, its vapors are used. Soxhlet extraction has been modified by using gases (instead of water), such as carbon dioxide, which is more efficient than water vapor extraction. Other methods used for the extraction of essential oils include maceration, squeezing, ultrasonic baths, and more recently, headspace (HS) techniques. HS is based on the isolation (extraction) of volatile compounds from plants or other substances in the upper part of a closed bottle. Air sampling from this bottle can be performed directly using a syringe (classic Head space) or using a high-porosity polymer fiber that binds well to organic compounds [3, 5, 10, 11].

2. Materials and methods

2.1. Sampling of rosemary samples

The samples of *Rosmarinus officinalis* were collected at six (6) different stations in the Durres area (Lalez Bay). Plants were collected in May 2025. At each station, the branches and leaves of the cultivated plants were collected. Samples were selected with branches and its leaves because rosemary use in most cases, both in cooking and traditional medicine, is done in this way (with both of them, branches and leaves). Samples were air-dried in the shade for conserving their morphological characteristics. After drying, the plant material was cut into small pieces of 0.5-2 cm for further analysis.

2.2. Chemical profile of *Rosmarinus officinalis* by using HS/SPME technique

Rosemary leaves (2 g) were put in a SPME bottle with a volume of 10 mL. The bottles were equipped with Teflon stoppers suitable for the Head-space technique. A manual SPME syringe equipped with a 100 µmPDMS (polydimethyl siloxane) fiber was inserted through the Teflon stopper in the upper part of the bottle. The absorption process was performed at 50°C for 40 minutes. The PDMS fiber was then transferred to the injector of a Varian 450 gas chromatograph equipped with a FID detector. Desorption process was done at 280°C for 20 s [6, 7].

2.3. Isolation of essential oils by using Clevenger apparatus

Branches and leaves of rosemary samples (50 g of plant material) were subjected to hydro-distillation for 4 h without interruption using a Clevenger-type apparatus (recommended by European Pharmaco-



Figure 1. Extraction of rosemary by using HS-SPME, HD and SE techniques.

peia, 2004) for the isolation of the essential oil. The essential oil was collected in 2 mL of toluene as the extraction solvent. The extract was then dehydrated by adding 1 g of anhydrous sodium sulfate. It was stored in dark vials at +4 °C. The essential oil of *Rosmarinus officinalis* was subjected to analysis using the GC/FID technique [1,3,4,6,8,11-13].

2.4. Isolation of essential oils by using Soxhlet apparatus

Rosemary samples (10 g of dry branches and leaves) were put on the thimble. Filter paper was used to cover the samples. A thimble was inserted into the extraction chamber, and the extraction process was initiated. A mixture of 200 mL ethanol/water (50/50) was used as the extracting solvent. The extraction process was continued for 6 h by Soxhlet apparatus. Subsequently, the rosemary extracts were concentrated to 20 mL by using a rotary evaporator. The essential oil of *Rosmarinus officinalis* was analysed using the GC/FID technique [12,13]. Fig. 1 shows laboratory photos of HS-SPME, HD and SE techniques used for the isolation of chemical constituents from cultivated rosemary samples.

2.5. Apparatus and gas chromatographic analyses

Gas chromatographic analysis of the essential oil samples of *Rosmarinus officinalis* (for all methods) was performed on a Varian 450 GC apparatus, equipped with a split/splitless injector and a flame ionization detector (FID). The injector and detector temperatures were set at 280°C and 300°C, respectively. Essential oil (2 µL) dissolved in toluene was injected in split mode (1:50) for HD and SE extracts. Nitrogen was used as the carrier gas (1 mL/min) and as the make-up gas (25

mL/min). Hydrogen and air were the detector flame gases at 30 mL/min and 300 mL/min, respectively. A VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was used to isolate the essential oil compounds. The compounds were identified based on their retention times (RT) and Kovats indices (KI) data. The quantitative data of the analyzed compounds are expressed as percentage [3, 4, 13-16]. Fig. 2 shows the chromatograms obtained using the HD, SE and HS-SPME techniques.

3. Results and discussion

From the GC/FID analyses of rosemary samples (its chromatograms) using the hydro-distillation technique (Clevenger apparatus), it was detected up to 108 compounds by Soxhlet extraction and up to 25 compounds were detected. While for the same samples analyzed with the Headspace technique up to 100 compounds were detected. These differences could be due to the different processes used for each extraction method. It is evident that HD and HS-SPME identified the majority of the compounds in the same sample. The 22 main compounds that constitute about 91.9% of the total amount using HD technique, 95.9% using SE and 82.7% using HS-SPME technique, were considered in this study. The higher percentage of the same compounds was observed in the SE method because of the lower number of identified compounds for this technique. The main compounds identified in the majority of all analyzed samples (6 parallel samples for each method), for all techniques were: cineol (eucalyptol), camphor, alpha-pinene, verbenone, borneol and camphene. Table 1 lists the

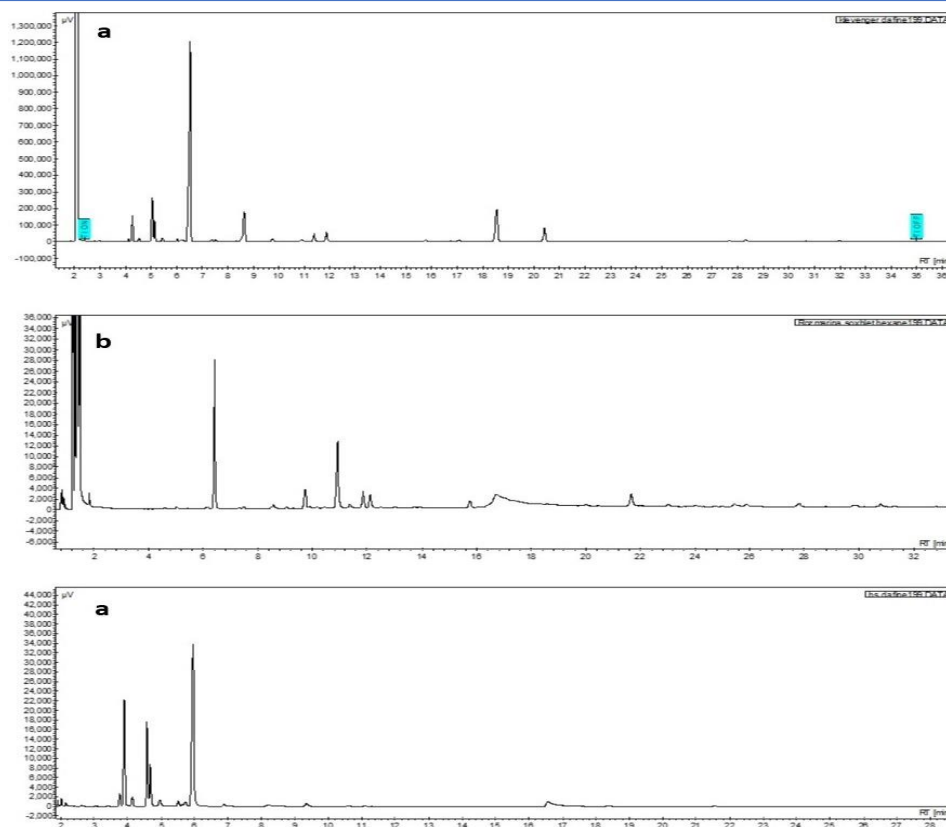


Figure 2. Chromatograms of rosemary samples from Durres area. (a) Hydro-distillation (HD) by using Clevenger apparatus, (b) Soxhlet extraction (SE), (c) Head space – Solid Phase Micro-extraction (HS-SPME).

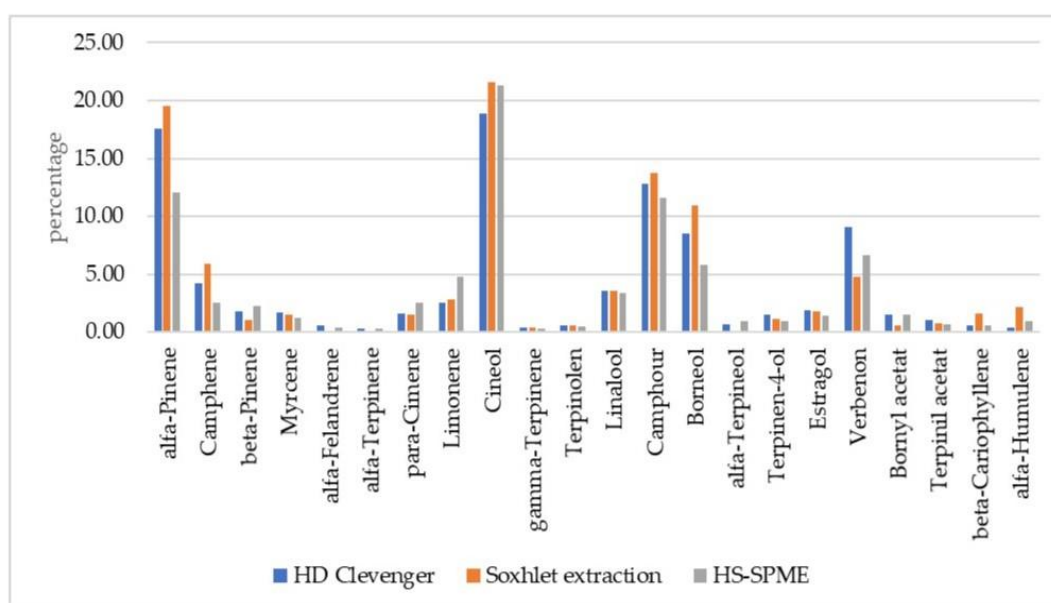
average percentages of the main compounds analyzed in the rosemary samples (branches and leaves) for all methods. Peaks with areas lower than 0.05% were not considered in this study.

Fig. 3 shows the chemical profiles of the main compounds obtained by HD, SE and HS-SPME extraction techniques. It was almost the same chemical profile for all extraction techniques. The main compounds found in the rosemary samples were: cineole > alpha-pinene > camphor, borneol > verbenone > camphene > linalool > limonene > paracymentene. The differences in the percentages of the compounds are related to the extraction processes applied in each method. To evaluate the similarities and differences between the extraction techniques, the results were interpreted according to the terpene classes. In Fig. 4, the percentages of the main terpene classes are shown. For all methods, the highest level was for oxygenated monoterpenes, with more than 50 % for all extraction techniques. The second group comprised bicyclic monoterpenes (17% to 26%). Other

groups (monocyclic, aromatic, aliphatic monoterpenes and sesquiterpenes) were found to be lower than 5% for HD, SE and HS-SPME techniques. Note that, similarities and differences between techniques were evident; it was the same profile but not the same percentages. Fig. 5 shows the percentages of bicyclic monoterpenes in the rosemary samples. Alpha-Pinene was the most abundant compound in all samples. Camphene was also found in higher concentration in the SE extracts. In the essential oil extracted by HD, bicyclic monoterpenes (alpha-pinene, camphene and beta-pinene) were found to be second technique that isolate these compounds. Beta-pinene was found higher in HS-SPME method. These compounds have lower boiling points than the other compounds. It was expected that these compounds would be present in higher percentages in the HS technique. Their lower levels in this method are related on the one hand to the large number of volatile compounds identified by the HD and HS-SPME methods and the fact that during the

Table 1. Average percentages (%) and standard deviation of main components for *Rosmarinus officinalis* samples from Durres area

Compounds	KI	Clevenger app. (HD)		Soxhlet extraction (SE)		HS-SPME (PDMS fiber)	
		Mean	STDEV	Mean	STDEV	Mean	STDEV
α -Pinene	985	17.59	1.12	19.50	1.51	12.05	2.85
Camphene	991	4.20	1.05	5.87	1.02	2.59	0.48
β -Pinene	994	1.82	1.27	1.03	1.23	2.28	0.35
Myrcene	1007	1.67	0.43	1.50	0.33	1.27	0.56
α -Felandrene	1012	0.61	0.55	0.00	0.00	0.44	0.05
α -Terpinene	1019	0.35	0.12	0.00	0.00	0.26	0.07
<i>p</i> -Cimene	1124	1.65	0.10	1.48	0.20	2.51	0.45
Limonene	1057	2.56	0.98	2.85	0.58	4.78	1.05
Cineol	1165	18.89	0.21	21.57	3.21	21.28	4.20
γ -Terpinene	1077	0.36	0.02	0.36	0.02	0.33	0.12
Terpinolene	1096	0.61	0.10	0.61	0.10	0.47	0.13
Linalool	1121	3.57	1.14	3.57	0.72	3.35	0.67
Camphor	1146	12.79	1.81	13.76	2.51	11.60	3.58
Borneol	1186	8.56	1.61	10.94	1.73	5.83	1.69
α -Terpineol	1197	0.71	0.54	0.00	0.00	0.94	0.37
Terpinen-4-ol	1214	1.55	0.54	1.13	0.36	0.95	0.34
Estragol	1236	1.86	0.38	1.83	0.34	1.40	0.27
Verbenone	1247	9.05	3.04	4.75	1.44	6.64	1.26
Bornyl acetate	1251	1.48	0.67	0.62	0.27	1.48	0.37
Terpinil acetate	1289	1.04	0.55	0.79	0.29	0.73	0.26
β -Cariophyllene	1295	0.57	0.29	1.59	0.30	0.56	0.13
α -Humulene	1318	0.41	0.11	2.17	0.69	0.99	0.45
Total		91.88	-	95.92	-	82.73	-

**Figure 3.** Chemical profile of terpenes in rosemary samples by using HD, SE and HS-SPME extraction techniques.

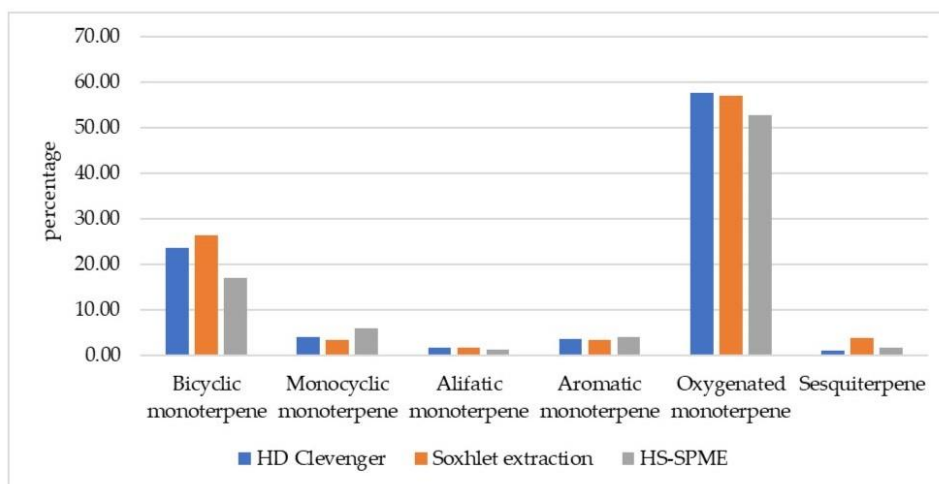


Figure 4. Terpene classes in rosemary samples by using different techniques.

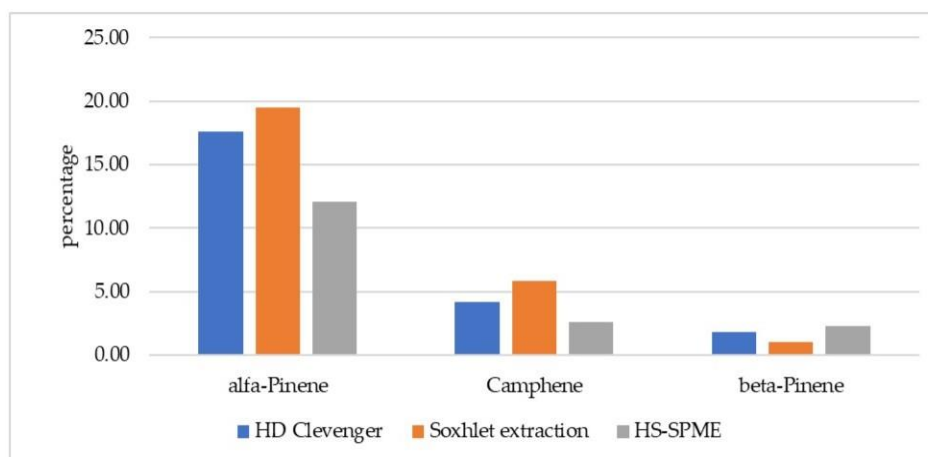


Figure 5. Bicyclic monoterpenes in rosemary samples from Durres area.

fiber transfer from the sample to the injector of the apparatus, these volatile compounds can evaporate, reducing their percentage. This is supported by the results of the monocyclic monoterpenes, which were found in greater abundance using HS-SPME technique (Fig. 6). Limonene was the main compound of this group from 2.5–4.8%. alpha-Phellandrene and alpha-terpinene were identified only by the HD and HS-SPME techniques but not by SE. gamma-Terpinene was found at similar levels for all three methods. Again, for this group of compounds, HD and HS-SPME seem to be the best extraction techniques, as SE discriminates compounds of this group. Fig. 7 shows the percentages of aliphatic and aromatic monoterpenes isolated using all the extraction techniques. Myrcene (aliphatic monoterpene) and estragol (aromatic) were found in

higher level for HD technique followed by SE and the HS-SPME, while for para-cymene (aromatic) HS-SPME was the most preferred technique. This is likely related to the solvents used for extraction (polarity) and the boiling points of the compounds being analyzed. Fig. 8 shows the respective percentages of oxygenated monoterpenes. This was the main group of compounds found in samples of *Rosmarinus officinalis*. The main compounds of this group (cineol, camphor, borneol, linalool and verbenon) were isolated using all methods higher percentage. Higher levels of these compounds were found in SE extracts, except for verbenon which was found in greater amounts in HD essential oil. Alpha-terpineol, terpinen-4-ol, bornyl acetate and terpinyl acetate were isolated in HD and HS-SPME techniques but not in Soxhlet technique. Water, ethanol and PDMS fibers

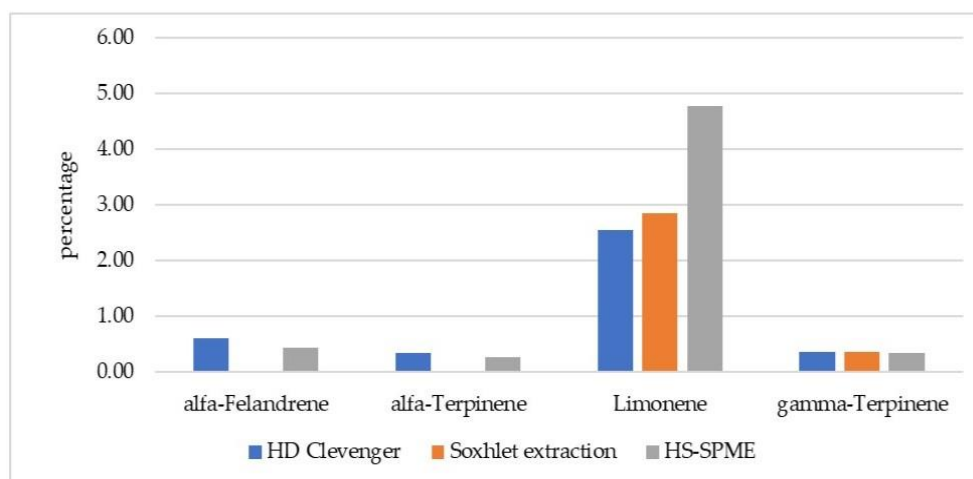


Figure 6. Monocyclic monoterpenes in rosemary samples from Durres area.

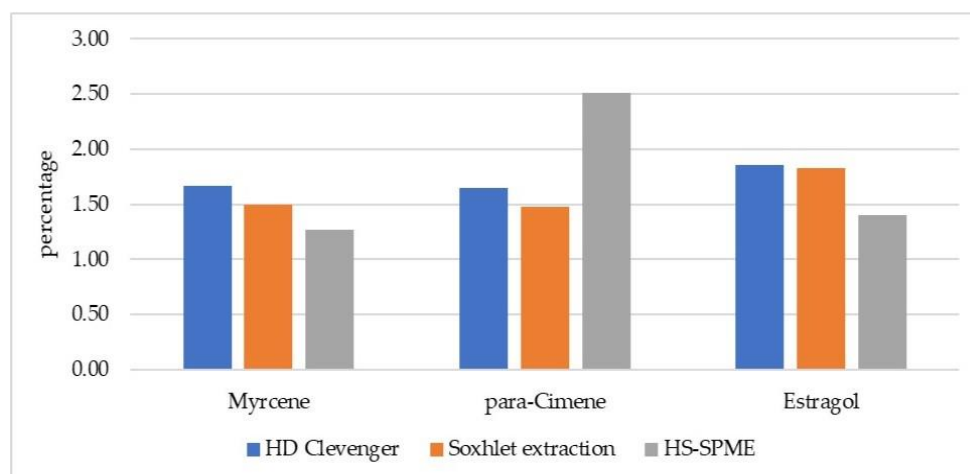


Figure 7. Aliphatic and aromatic monoterpenes in rosemary samples from Durres area.

seem to be suitable for their isolation due to their physicochemical properties (polarity, boiling point, etc.). A smaller number of extracted compounds favors an increase in the percentage of SE. For HD and HS-SPME methods, a higher number were isolated compounds (up to 4 times higher) and for this, sometimes their percentages could be lower compared to SE. This fact is supported by the higher levels of sesquiterpenes in SE method 2-5 higher than HD or HS (Fig. 9).

alpha-Humulene and beta-Caryophyllene are usually found at levels lower than 1% [3-8]. Therefore, HD and HS-SPME could be considered the most appropriate methods for investigating the chemical profiles of rosemary samples and other aromatic/medicinal plants. Data obtained from HD and HS analyses of dried rosemary samples (branches

with their leaves) from the Durres area showed that their chemical composition was the same as the reported in other studies works from other areas of Albania and wider in the Balkan and/or Mediterranean areas [1, 3-6, 8].

4. Conclusions

In this study, samples of *Rosmarinus officinalis* L. from the Durres area were analyzed. Rosemary is an aromatic/medicinal plant widely used in cooking (as a spice) and its active compounds have been found to have very good pharmacological effects. Therefore, it is often used in traditional medicine to treat certain symptoms and improve the quality of life.

The chemical analysis of rosemary plants was performed using three different extraction techniques, hydro-distillation (Clevenger apparatus), Soxhlet

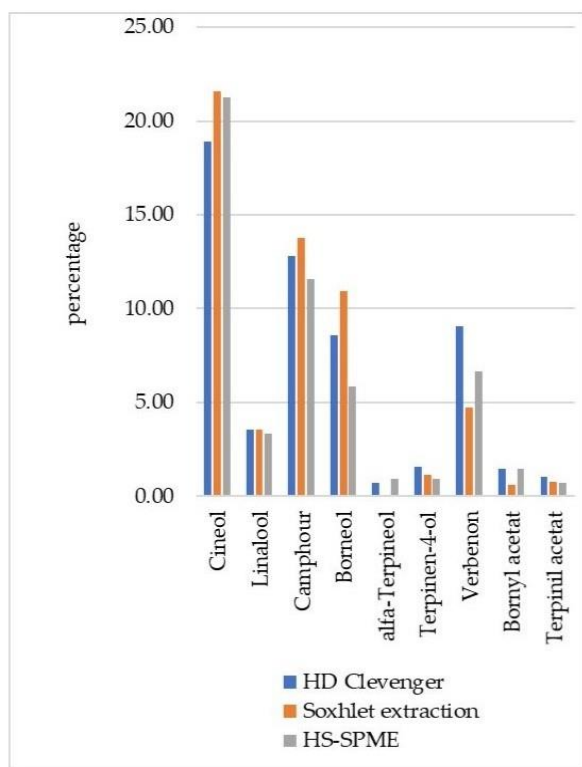


Figure 8. Oxygenated monoterpenes in analyzed rosemary samples.

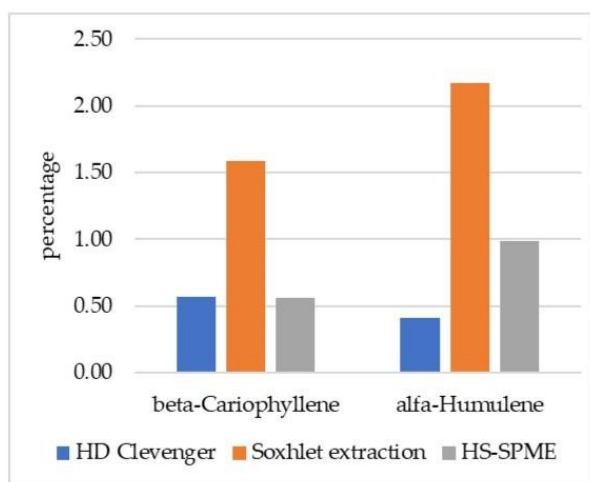


Figure 9. Sesquiterpenes in rosemary samples by using HD, SE and HS-SPME extraction techniques.

extraction and Headspace (assisted by PDMS fiber), followed by GC/FID quantification. The HD and HS-SPME techniques showed an advantage in the number of identified compounds (up to 100) compared to the SE technique (up to 25). This is likely related to the preference/discrimination of compounds (from solvents and/or PDMS fiber), the extraction processes and the physicochemical properties (molecular mass, polarity, boiling point,

etc.) of compounds that were found in the rosemary samples. Note that, all extraction techniques showed the same chemical profile. The main compounds found for all techniques (in all samples) were: cineole, alfa-pinene, camphor, verbenone, camphene and beta-pinene. Oxygenated monoterpenes were found in more than 50% of all samples followed by bicyclic monoterpenes. All methods can be used to determine the chemical profile of essential oils of aromatic/ medicinal plants, including rosemary plants, however, HD and HS-SPME methods have advantages and could be considered the most appropriate. They can isolate a greater number of compounds in the extraction process. Also, HD and HS-SPME showed good reproducibility and repeatability (low values for STDEV in parallel measurements). Generally, they do not use organic solvents (green techniques), are low cost and shorter analysis time. The chemical profile of *Rosmarinus officinalis* was the same as that reported in other studies from other areas of our country (Albania) and wider (Balkan and Mediterranean areas).

Authors' contributions

Conceptualization, B.X., A.K., S.K. K.S., A.D., E.Q., A.N.; methodology, B.X., S.K., L.V., K.V., A.N.; formal analysis, B.X., S.K., A.M., A.N.; investigation, B.X., K.S., A.N.; resources, S.K., B.X., L.V.; writing—original draft preparation, B.X., A.N., K.S.; writing—review and editing, supervision, A.N. and B.X.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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