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Chemical profile of wild salvia officinalis population from Kosovo by using CO_2 supercritical extraction

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Abstract

This study shows chemical profile of Salvia officinalis L. plants by using CO_2 supercritical extraction method. Samples of sage plants were selected by natural population of Gjilan, Kosovo. Sage samples were obtained in August 2022. The dried and ground plants were subjected of CO_2 supercritical extraction techniques in a modified Clevenger apparatus, to obtain the essential oil of Salvia officinalis plants. Chemical profiles for all sage samples were performed using the GC/FID technique. Capillary column VF-1ms (30 m x 0.33 mm x 0.25 um) was used for the separation and isolation of compounds found in sage plants. In the analyzed chromatograms from sage samples, 30-45 compounds were identified. In the study, the 20 main compounds that make up over 90% of the total identified compounds were considered. Oxygenated monoterpenes were the main group of monoterpenes because of higher values for alpha + beta-Thujones and Camphor. Sesquiterpenes were the second group while bicyclic monoterpenes were third group of terpenes. Percentages of monocyclic, aliphatic and aromatic monoterpenes were found in non-considerable level. Chemical profile of Salvia officinalis plants from Kosovo was the same with other reported studies from Balkan and Mediterranean area.

1. Introduction

Kosovo is located on the Balkan Peninsula. As part of this area and due to its favorable geographical position, it has a rich vegetation, with over 2500 different types of plants. This development of the flora is favored by the great diversity of plain, hilly, and mountainous relief forms, as well as by its climate. From this diverse vegetation, about 300 species are aromatic and medicinal plants, which constitute a good and important natural economic resource for Kosovo [1,2]. Medicinal plants of Kosovo are distinguished by their active ingredients and essential oils. These plants are known and used since ancient times. They are widely used in traditional medicine and culinary [1,2]. The activity of gathering aromatic-medicinal plants constitutes one of the main incomes for the livelihood of poor families in rural areas. Salvia officinalis, known as sage, is a perennial plant of the pre-mountainous Mediterranean area. It grows from 25-80 cm in height. It blooms in the May-July period, depending on the climatic subzone where the plant's biotope is located. This plant is found on stony soils, rising above limestone karst rocks with slightly basic to slightly acidic pH. It grows in dry, cool soils that stretch along the submontane Mediterranean zone. It is found at altitudes of 150-1200 m above sea level.

Sage has found many uses in the cosmetic industry, the essence of the leaves is used as a flavoring agent for perfumes and soaps. In the kitchen, it is used as a spice in various meat dishes. Sage has different properties such as: astringent, antiseptic, aromatic, carminative, estrogenic, antisudorific, tonic, stimulant, antispasmodic, etc. Cures nervous disorders, dizziness, fainting, depressive states. It stimulates the entire organism, regulates the secretions of the digestive tract, soothes diarrhea due to its astringent properties, regulates menstrual flow and soothes painful reactions by fighting the disorders brought about by menopause. The combination of antiseptic, soothing and astringent properties make it ideal for almost all types of sore throats and widely used for gargling. It is also used for mouth and gum ulcers [1, 3-6].

2. Material and Method

2.1. Sampling of *Salvia officinalis*

The samples of Salvia officinalis plants (aerial parts) were taken from the wild populations of the area of Anamorava, Gjilan in the southern area of Kosovo (Figure 1). Salvia officinalis plants were collected in August 2022. Sage sampling stations were at an altitude of 1000 – 1200 m above sea level. At each station, the aerial parts of the Salvia officinalis plant were selected. The plants of each station were dried in the shade so as not to lose morphological characteristics. The plant material after drying was chopped in a grinder into small pieces < 0.5 cm for further analysis [5-7].

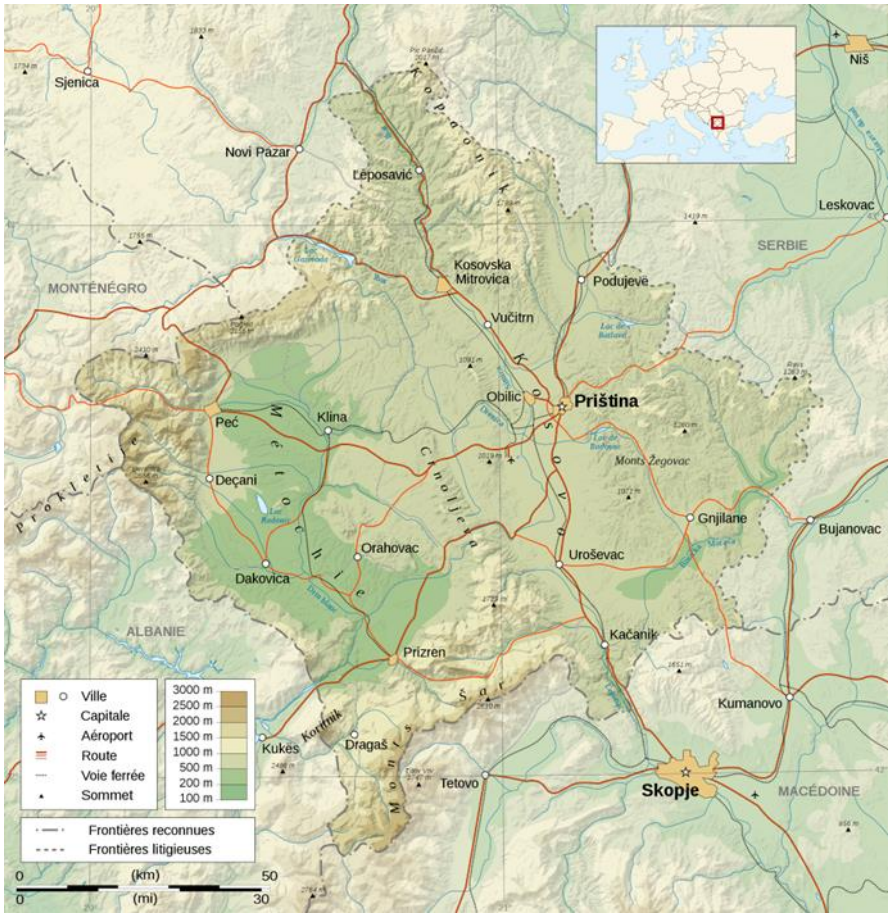

Figure 1. Sampling of Salvia officinalis samples from Gjilan area, Kosovo.

2.2. Isolation of essential oils by CO_2 supercritical technique

The plant material of Salvia officinalis (50 g of plants from the aerial parts of Salvia officinalis) was subjected to CO_2 supercritical in a modified Clevenger apparatus. CO_2 temperature was 33°C and the pressure was 100 bar. The isolation of the essential oil was done for 30 minutes. The essential oil was collected in 2 ml of Toluene as extraction solvent. The extract was treated with 1 g of anhydrous sodium sulfate. It was stored in dark vials at +4 °C. The essential oil of Salvia officinalis in organic solvents was subjected to GC/FID analysis [6, 8, 9].

2.3. Gas chromatographic apparatus and analysis

Gas chromatographic analysis of the essential oil of Salvia officinalis was performed on a Varian 450 GC apparatus, equipped with a PTV injector and a flame ionization detector (FID). Injector and detector temperatures were set at 280 °C and 300 °C, respectively. 2ul of essential oil of Salvia officinalis dissolved in Toluene was injected in split mode (1:250). Nitrogen was used as carrier gas (1 ml/min) and as make-up gas (25 ml/min). Hydrogen and air were the flame gases in the detector at 30 ml/min and 300 ml/min, respectively. VF-1ms capillary column (30 m x 0.33 mm x 0.25 mu) was used to isolate the essential oil compounds. The oven temperature was programmed as follows: from 40°C (held for 2 minutes at 40 °C) to 150°C at 4°C/min, further to 280°C at 10°C/min, at 280°C held for 2 minutes. The identification of compounds was based on the comparison of retention time (RT) with Kovats indices which together with literature data were used to identify the main compounds. The quantitative data of the analyzed compounds are given in % against the total areas of the piks [6-8, 10-12].

3. Results and Discussion

In this study, sage plants from the area of Gjilan (Anamorava), Kosovo, were analyzed. The chemical components of sage plants have been analyzed using the CO_2 supercritical technique with the Clevenger apparatus. The chemical profile of the Salvia officinalis samples was performed using the GC/FID technique. In the analyzed chromatograms from sage samples, 30-45 compounds were identified. In the study, the 20 main compounds that make up over 90% of the total identified compounds were considered. Some of the main compounds identified to the all samples were: alpha-Thujone, beta-Thujone, Camphor, Cineole and Camphene. Chemical profile for 20 main compounds and for the main groups of terpenes were shown in Table 1.

Table 1. Statistical data for main compounds find to *Salvia officinalis* samples from Gjilani area.

	Mean	Median	STDEV	Min	Max
alpha-Pinene	2.18	0.97	2.16	0.89	4.67
Camphen	2.83	2.36	2.35	0.74	5.38
beta-Pinene	2.39	2.94	1.07	1.15	3.07
Myrcene	0.46	0.43	0.08	0.40	0.55
Limonene	0.49	0.65	0.43	0.00	0.81
alpha-Terpinene	0.97	1.04	0.18	0.77	1.11
Cineole	12.73	12.94	1.37	11.27	13.99
para-Cymene	0.14	0.00	0.24	0.00	0.41
gamma-Terpinene	0.59	0.58	0.60	0.00	1.20
Cis-Sabinene hydrat	0.15	0.00	0.25	0.00	0.44
Linalool	0.03	0.00	0.06	0.00	0.10
alpha-Thujone	28.70	29.31	2.20	26.26	30.53
beta-Thujone	6.56	6.73	0.46	6.03	6.91
Camphour	21.54	22.02	2.37	18.96	23.63
Borneol	2.96	2.99	0.52	2.42	3.46
Terpinen-4-ol	0.73	0.67	0.36	0.40	1.12
alpha-Terpineol	1.72	1.53	0.37	1.49	2.15
Bornyl acetate	1.66	1.52	0.44	1.31	2.16
beta-Cariophyllene	2.99	2.87	0.56	2.50	3.59
alpha-Humulene	4.27	3.70	1.10	3.57	5.54

Figure 2 shows the average values for 20 main compounds find to Salvia officinalis samples (Gjilani area, Kosovo 2023) extracted by using CO_2 supercritical technique. Chemical profile of *Salvia officinalis* was: alpha-Thujone > Camphor > Cineole > beta-Thujone > alpha-Humulene > beta-Caryophyllene > Borneol. Other compounds were found lower than 2%. The main compound was alpha-Thujone for all sage samples with average values of 29.3%. Total of Thujones (alpha + beta isomers) was 36%. Thujone values were the same with reported values for classical techniques of hydro-distillation technique based on Pharmacopeia instructions. The second compound found in sage samples was Camphor (23.6%). This value was higher than hydro-distillation process. This difference could be because of the affinity for the CO_2 supercritical for this compound. The same was noted for alpha-Humulene (5.6%) but for the other compounds was the opposite. This profile was almost the same with reported values for *Salvia officinalis* plants from Kosovo and other countries of Balkan areas [6-8, 10].

Figure 3 shows the percentage levels for each of main terpene groups found in sage samples from Gjilani area, Note that, the main place in all samples was occupied by monoterpenes. The total of monoterpenes in the analyzed samples was 83.4%. The profile of monoterpenes was: oxygenated monoterpenes were found in most abundance, followed by bicyclic monoterpenes, monocyclic monoterpenes, aliphatic monoterpenes and aromatic monoterpenes. Sesquiterpenes (beta-Caryophyllene and alpha-Humulene) were identified in sage samples as the second group of terpenes. The profile of main terpene grups to sage samples from Kosovo area was almost the same with reported studies from other Balkan and Meditereanen areas [4, 5, 7, 9].

Oxygenated monoterpenes (Cineole, Linalool, alpha-Thujone, beta-Thujone, Borneol, Terpinen-4-ol, alpha-Terpineol and Bornyl acetate) was the main group of monoterpenes with 79.8% (Figure 4). The higher percentage was found because the higher values for alpha + beta-Thujones and Camphor. Total for alpha + beta-Thujones (35.3%) was almost the same with the values of sage samples for the same area but using hydro-distillation process in a Clevenger apparatus (recommended as Pharmacopoeia standard techniques for obtain essential oils of medicinal and aromatic plants). This was not the same for the Camphor which was found in higher percentage than hydro-distillation process. CO_2 in supercritical conditions is more preferable for extracting this compound compare to other oxygenated monoterpenes.

Figure 5 shows the percentages for each individual of bicyclic monoterpene. Bicyclic monoterpenes (alpha + beta-Pinene, Camphene and Sabinene hydrate) was third group of terpenes with 2.9%. Percentages for these compounds were 2-3 times lower than hydro-distillation process (Clevenger apparatus) for the same samples. CO_2 in supercritical condition is not the best extracting "solvent" for this class of terpenes.

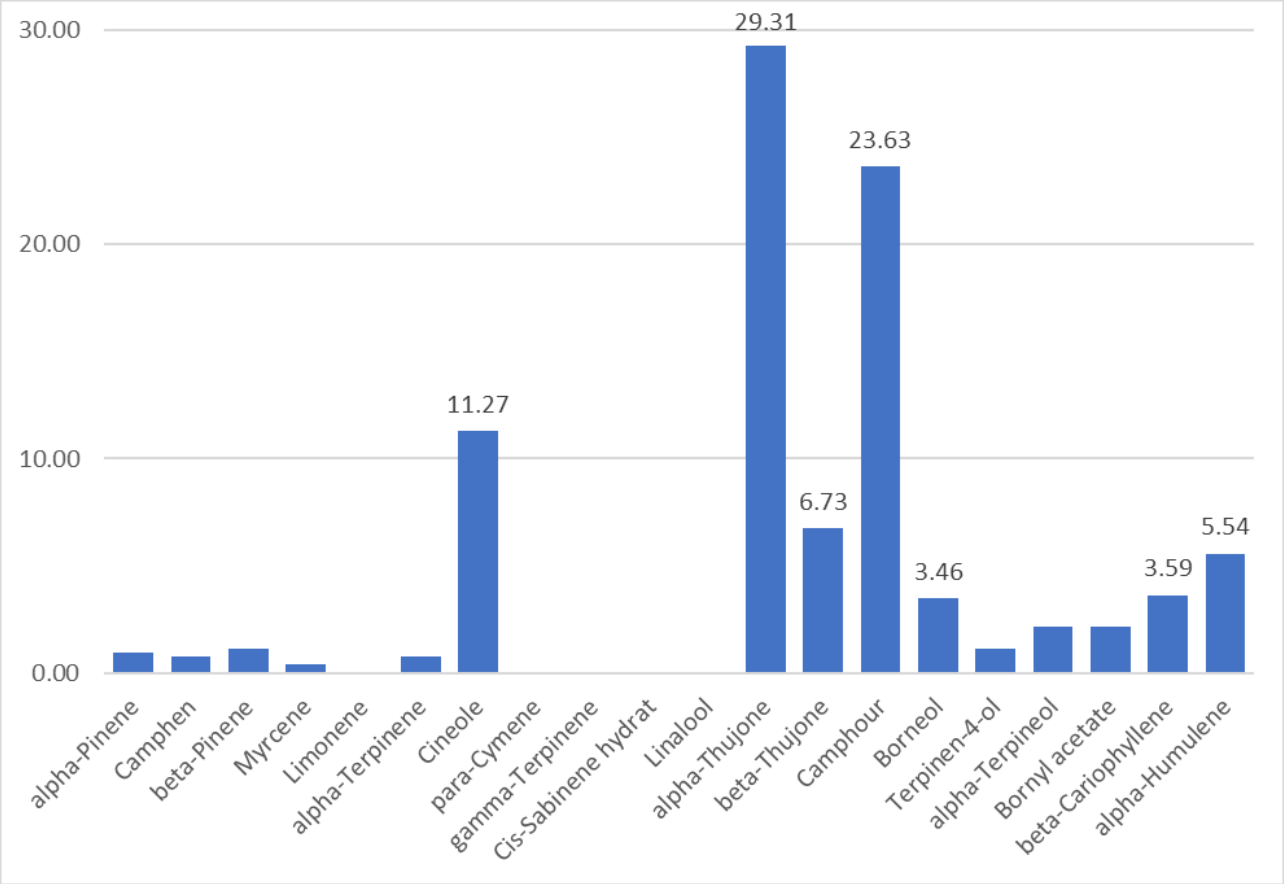

Figure 2. Chemical profile of Salvia officinalis samples.

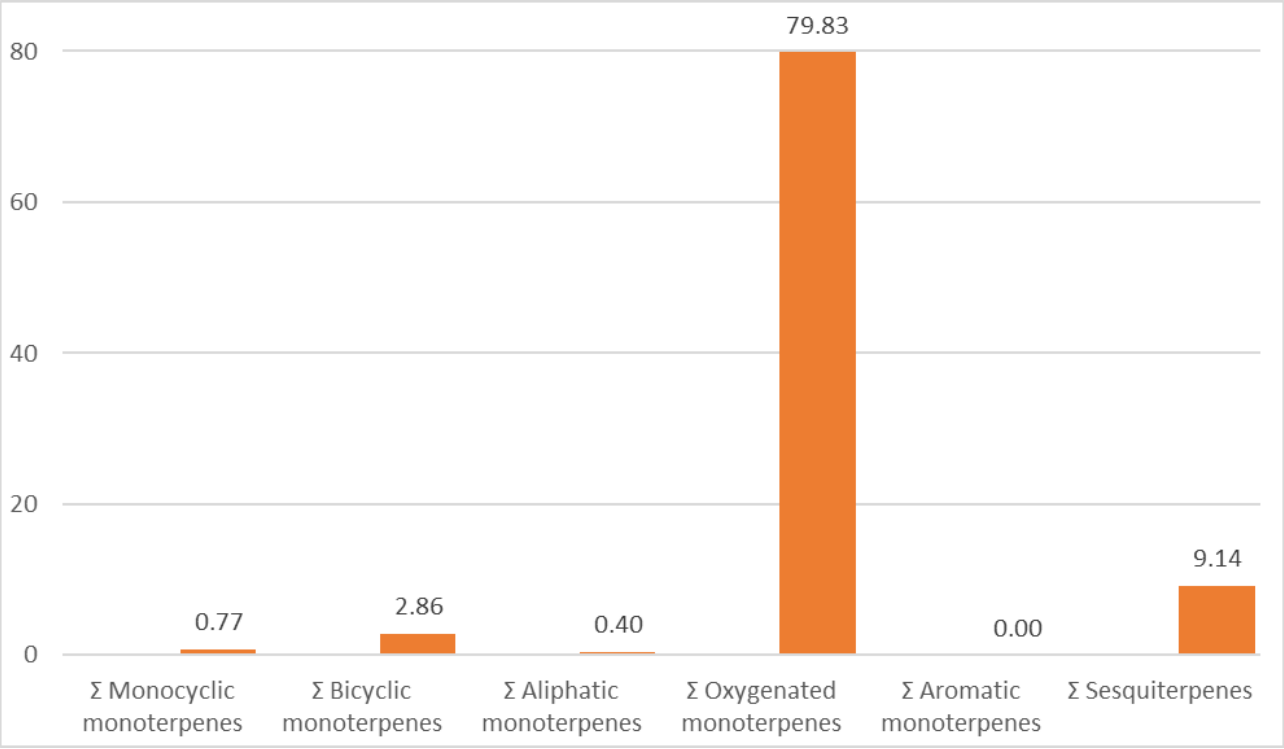

Figure 3. Terpene groups in sage samples from Gjilani area, Kosovo 2023.

Figure 6 shows percentages for Limonene, alpha + gamma-Terpinene (monocyclic monoterpenes) in sage samples from Gjilani area (Kosovo 2023). Monocyclic monoterpenes were not identified for all sage samples. Their presence was lower than reported percentage by using other techniques (Clevenger apparatus). CO_2 was shown to have not preference for this class of terpenes.

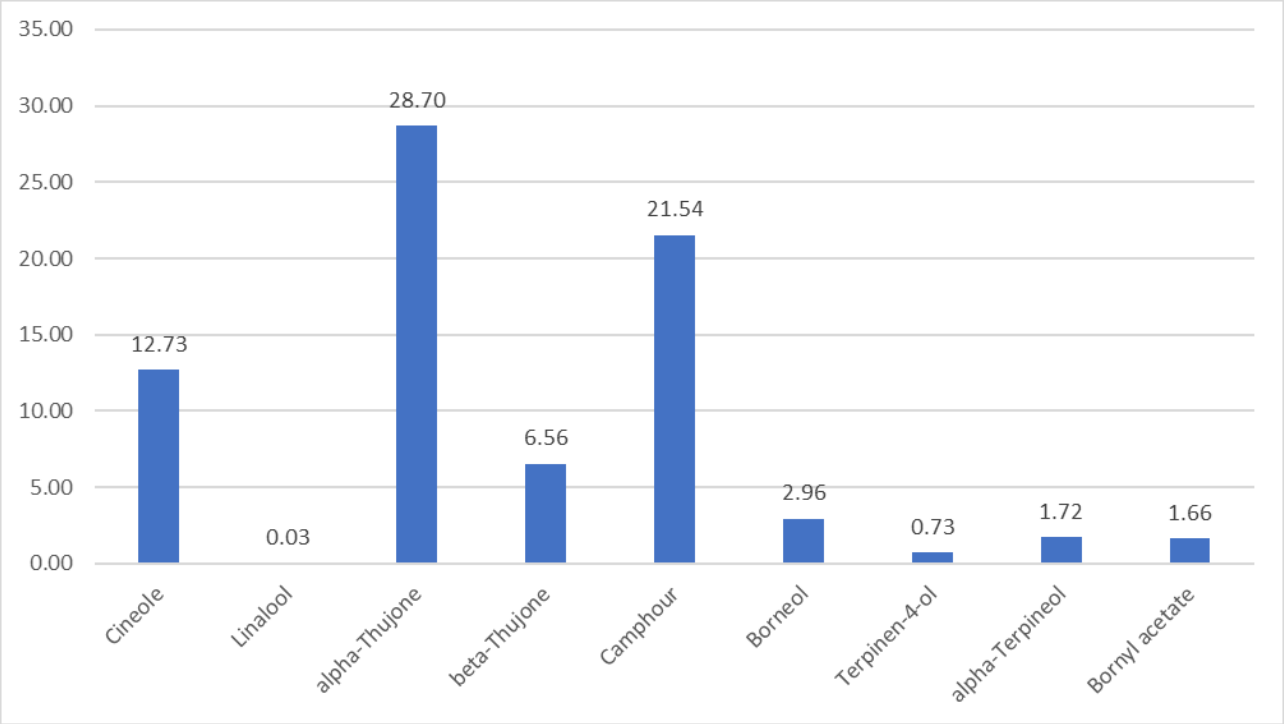

Figure 4. Oxygenated monoerpenes in sage samples from Gjilani area.

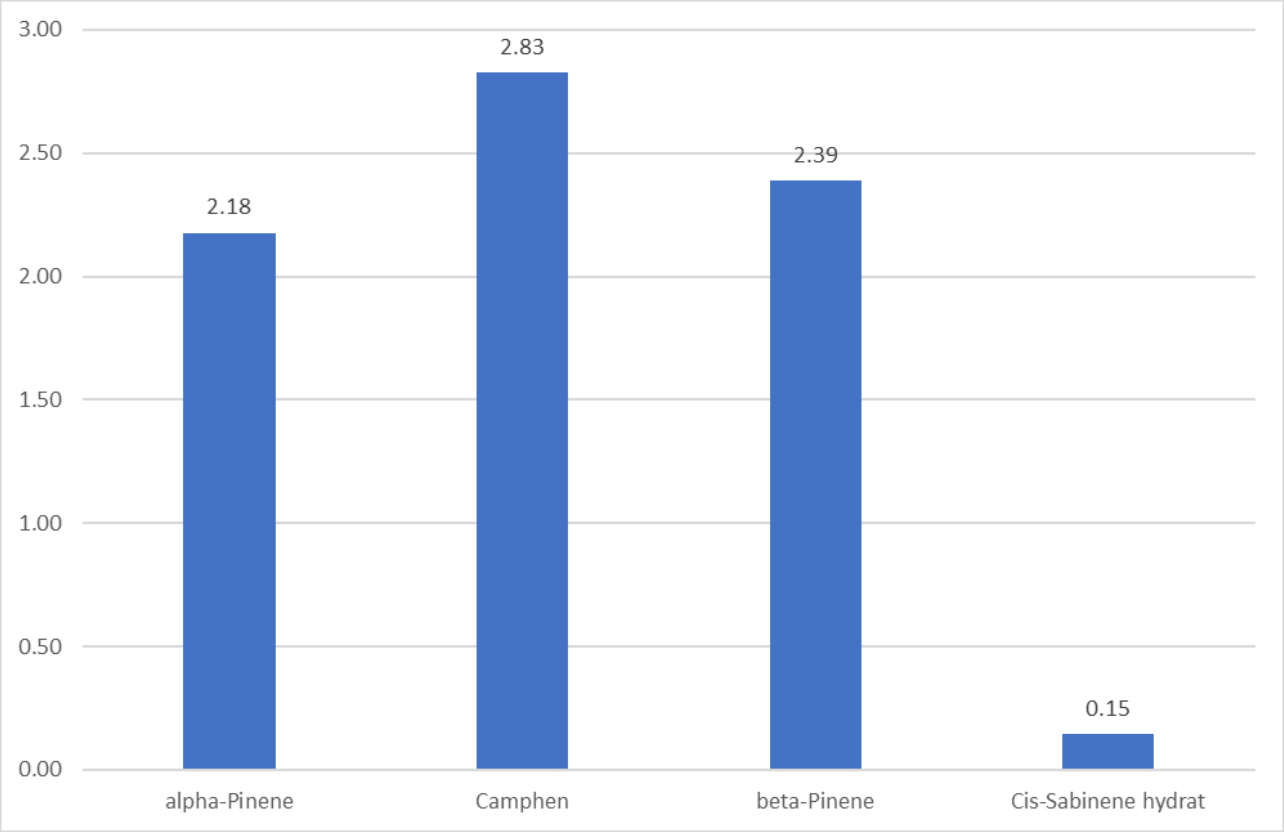

Figure 5. Bicyclic monoterpenes in Salvia officinalis samples from Kosovo.

Myrcene (aliphatic monoterpene) and p-Cymene (aromatic monoterpene) were found lower than 0.5%. Note that their presence was not detected in more than 60% of sage samples. CO_2 is not preferable for extracting these classes of terpenes in sage samples.

Figure 7 shows percentages of sesquiterpenes in sage samples from Gjilani area (Kosovo). Sesquiterpenes (beta-Caryophyllene and alpha-Humulene) was the second group of terpenes with 9.2%. Note than percentage for alpha-Humulene was higher than reported percentages for other techniques (Clevenger apparatus).

Profile of individual terpenes and main groups of terpenes were shown to be the same with other reported studies from Balkan and Mediterranean area [5-10].

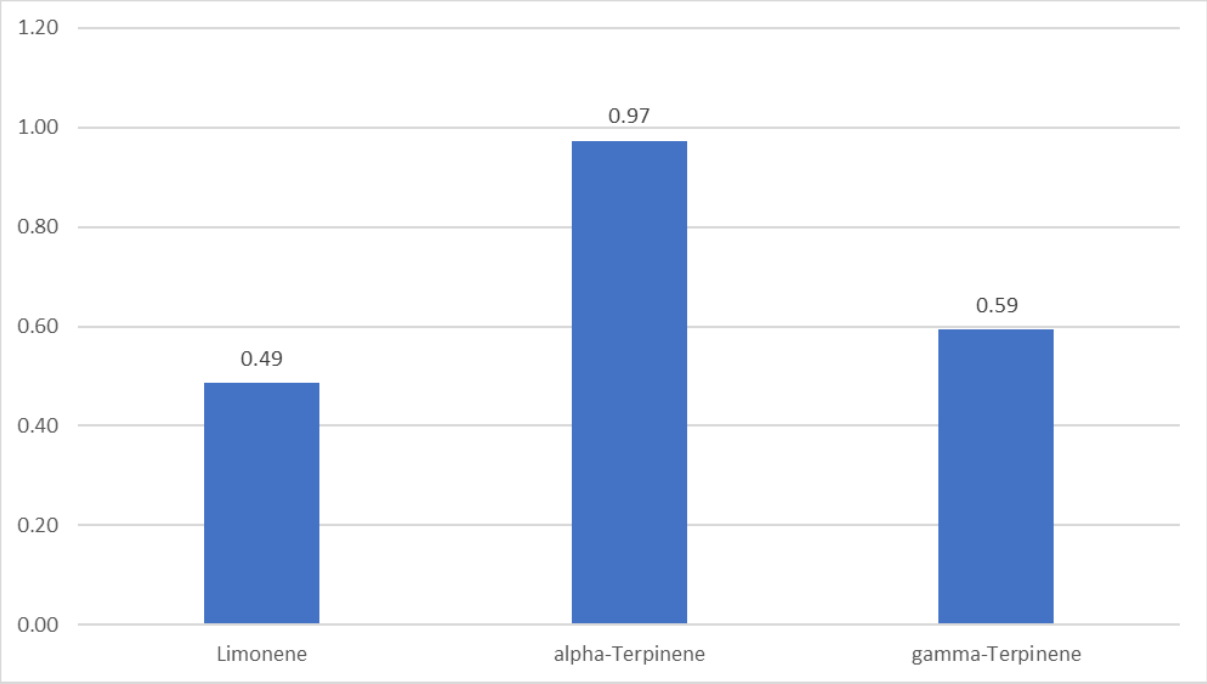

Figure 6. Monocyclic monoerpenes in sage samples.

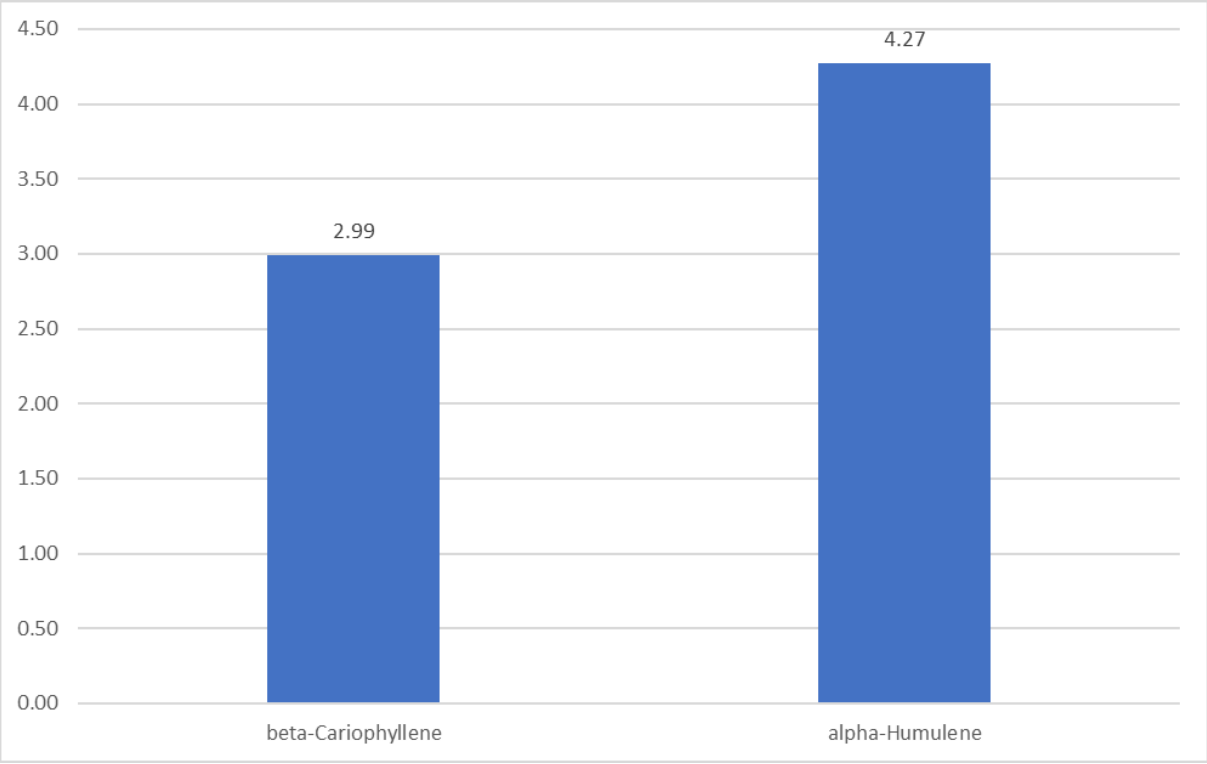

Figure 7. Sesquiterpenes in sage samples from Gjilani area, Kosovo 2023.

4. Conclusion

Salvia officinalis essential oil samples from the area of Anamorava (Gjilan), Kosovo 2022 were analyzed using the CO_2 supercritical technique with the modified Clevengar apparatus. Their quantification was performed by GC/FID. In the analyzed chromatograms from sage samples, 30-45 compounds were identified. In the study, the 20 main compounds that make up over 90% of the total identified compounds were considered. Some of the main compounds identified in all solvents were: alpha-Thujone, beta-Thujone, Camphor, Cineole and Camphene. The main compound was alpha-Thujone for all sage samples. Total of Thujones (alpha + beta isomers) was the same with reported values for sage samples from this area measured by hydro-distillation. Camphor and alpha-Humulene were found higher than hydro-distillation process but for the other compounds was the opposite. The main compounds in all samples were monoterpenes. The profile of monoterpenes was: oxygenated monoterpenes were found in most abundance, followed by bicyclic monoterpenes, monocyclic monoterpenes, aliphatic

monoterpenes and aromatic monoterpenes. Oxygenated monoterpenes were the main group of monoterpenes because of higher values for alpha + beta-Thujones and Camphor. Sesquiterpenes were the second group while bicyclic monoterpenes were third group of terpenes. Percentages of monocyclic, aliphatic and aromatic monoterpenes were found in non-considerable level. Chemical profile of Salvia officinalis plants from Kosovo was the same with other reported studies from Balkan and Mediterranean area [5-10].

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Author contributions

Miranda Misini: Methodology, Writing-Original draft preparation **Arben Haziri:** Editing Software, Visualization **Fatmir Faiku:** Data curation, Validation **Aurel Nuro:** Conceptualization, Writing-Reviewing and Last Editing

Conflicts of interest

The authors declare no conflicts of interest.

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