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Study of chemical profile for essential oil of *Salvia officinalis* L. plants by using CO_2 supercritical extraction

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Abstract

This study shows chemical profile of *Salvia officinalis* L. plants by using CO2 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.

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, 4, 5, 6].

Material and Method

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. *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, 6, 7].

Isolation of essential oils by CO2 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].

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, 7, 8, 10, 11, 12].

Results

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 Clevengar 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 above figures.

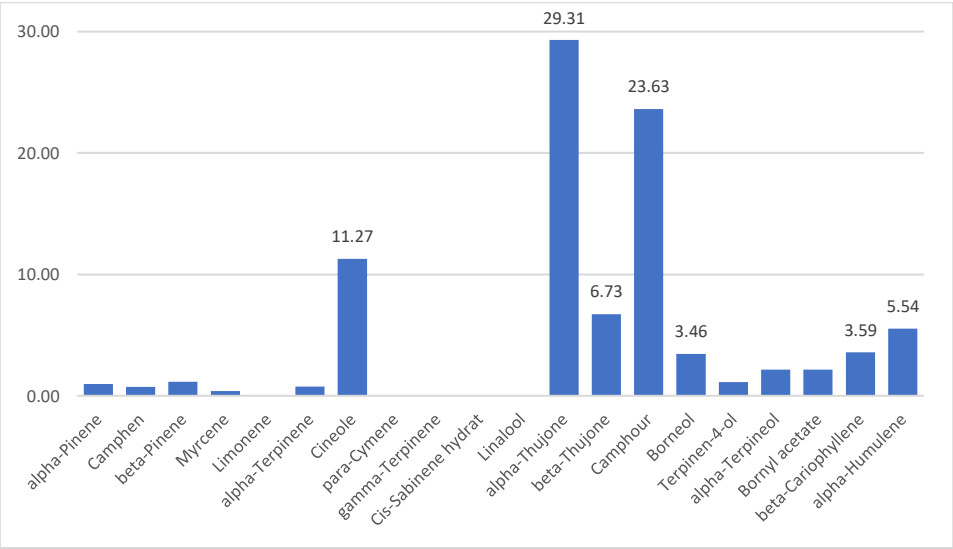

Figure 1. Chemical profile of Salvia officinalis samples

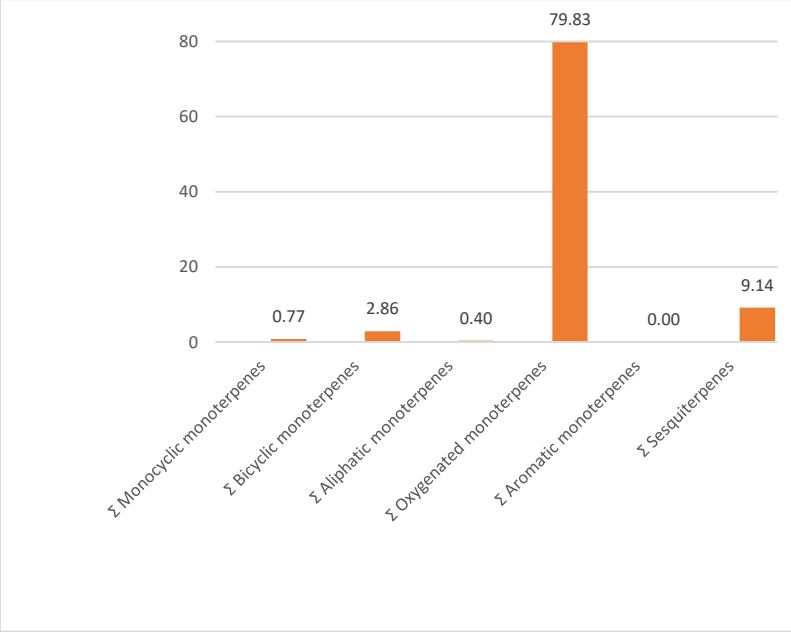

Figure 2. Terpene groups in sage samples

Discussion

Chemical profile of *Salvia officinalis* from Kosovo area by using CO_2 supercritical extraction techniques was: alpha-Thujone > Camphour > Cineole > beta-Thujone > alpha-Humulene > beta-Cariophyllene > Borneol. Other compounds were found lower than 2%. The main compound was alha-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, 7, 8, 10].

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. 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%. The higher percentage was found because the higher values for alpha + beta-Thujones and Camphor. Sesquiterpenes (beta-Cariophyllene and alpha-Humulene) was the second group of terpenes with 9.2%. Bicyclic monoterpenes (alpha + beta-Pinene, Camphene and Sabinene hydrate) was third group of terpenes with 2.9%. Monocyclic, aliphatic and aromatic monoterpenes were found lower than 1%. This profile was the same with other reported studies from Balkan and Mediterranean area [5, 6, 7, 9, 10].

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 alha-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 was 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, 6, 7, 9, 10].

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