Different extracting methods of medicinal herbs

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Abstract

Albania is a well-known country for the diversity and exports of medicinal and aromatic plants. Sage is one of the most popular plants that is exported in USA and in other different countries, due to its scientific and medicinal effects in human body. Sage and its essential oil have been known since the ancient times to be used for the treatment of seizure, ulcers, tremor, hyperglycemia, diabetes, diarrhea, paralysis, etc. According to recent studies sage does not have currently any medical uses but some studies are enthusiast for its use in Alzheimer disease. In this work, essential oil of sage leaves has been obtained with different methods of extraction such as, liquid CO₂ extraction, water distillation method and organic solvent extraction. The essential oil of sage leaves carried out by different extraction methods used in this study, is then characterized with thin layer chromatography. It is obvious from the displayed results that the yield of the extract attained using the near critical conditions (liquid CO₂) is higher than the yield acquired by reflux distillation using hexane as a solvent. In this context, it is relevant to note that in comparison to hexane, carbon dioxide (CO₂) is the most extensively used solvent in fluid extractions, as it is physiologically harmless, environmentally safe, and it can be easily removed from products. This is a good result and very important for its uses in pharmaceutical purposes.

Keywords: *Salvia Officinalis*, Essential Oil, CO₂ Extraction, Hexane Extraction, Thin Layer Chromatography

Introduction

Albania is distinguished for its biological diversity and it is a large manufacturer of aromatic plants such as *Salvia officinalis* L., chamomile, thyme, balsam flower (*Hypericum perforatum*), spearmint (*Mentha spicata* L.) etc (Taraj, 2020; Taraj et al., 2013; Asllani, 2002). Today, it is known that all essential oils of aromatic and medicinal plants are used extensively in detergents, soaps, cosmetics, pharmaceuticals, perfumes and in confectionery food products. *Salvia officinalis* L. has big flowers, in blue-violet color (Asllani, 2002). The leaves of *Salvia officinalis* L. contain essential oil (1-2.5%) (Taraj, 2020) and the main constituents are monoterpenes, sesquiterpenes (thujone (α , β), camphor, borneol etc.). Additionally, the main components of essential oil of *Salvia officinalis* L. plant are thujone (40 - 50%). Pharmaceutical preparations of *Salvia officinalis* L. are used in different therapies, for example treatments of herpes, anxiety, or throat infections etc. In addition, essential oil *Salvia officinalis* L. has bacteriostatic and bactericidal action against *Bacillus cereus* and *Bacillus subtilis*. The most common methods used for obtaining essential oils are water distillation method, distillation with organic solvents method and extraction with liquid CO₂ (under pressure) method (Taraj, 2020; Taraj et al., 2013; Asllani, 2002). The latter is based on the extraction of essential oil from the inner parts of cells onto the surface of the plant. In the present work the essential oil of *Salvia Officinalis* L (leaves) is obtained by hexane distillation and CO₂ extraction (40°C)

methods. The obtained fractions are characterized by thin layer chromatography by means of a standard compound (Çullaj, 2005; Koch, nd). The aim of the current work is to present two complementary methods for extracting essential oils from the leaves of *Salvia officinalis* L. and further characterize the obtained extracts by thin layer chromatography. Additionally, advantages and disadvantages of the methods used for the extractions are indicated as well

Methods

Sage leaves are dried at 40°C and then grounded as finely as possible to be suitable for extraction experiments. Also the leaves have been cleaned by various impurities that may have had during their collection.

Extraction by means of CO₂ solvent: The experiment with liquid CO₂ was conducted in an autoclave, as formerly reported [1,2] by means of a Soxhlet recipient (Soxhlet technique). The extraction occurs in vapor-liquid equilibrium (T=31.1°C and P=73.8 bar). CO₂ is a good solvent for liquids (Taraj, 2020). Under critical conditions (T=40°C, P_{CO₂}=58 bar) CO₂ exhibits properties between those of a liquid and gas.

Extraction by means of hexane solvent: A reflux distillation set up was used, consisting of a cooling system, a condenser, a 2000 ml flask and a heating plate. The amount of the plant used was 100 g dispersed in 1000 ml hexane. The distillation continued for four hours. After filtration was carried out, the solvent was removed using a rotary evaporator.

Thin layer chromatography (TLC): The obtained extracts were characterized by TLC. It is a fast method and has a good resolution and high sensibility. It is reported that for the current conditions used in this work, R_f is characteristic for a single component (Çullaj, 2005). The separation of essential oil in fractions is based on Britannic method of Pharmacopoeia 2007 which includes the test of vanillin (1 % vanillin in alcohol V/V, adding 2 ml H₂SO₄ cc), as identification reagent of compounds containing oil extracts of *Salvia officinalis* L. (Farmacopea ufficiale della Repubblica Italiana, 1981; British Pharmacopoeia, 2007).

Results and Discussion

The overall results of the extracts obtained by the two different extracting methods are presented in table 1. The extract obtained by means of liquid CO₂ was yellow (yellow crystals), whereas in the case of the extraction carried by means of hexane, the acquired extract was green-brown in color (jellylike-gelatinous). It is obvious from the displayed results that the yield of the extract attained using the near critical conditions (liquid CO₂) is higher than the yield acquired by reflux distillation using hexane as a solvent. In this context, it is relevant to note that in comparison to hexane, carbon dioxide (CO₂) is the most extensively used solvent in fluid extractions, as it is physiologically harmless, environmentally safe, and it can be easily removed from products (Taraj, 2020)

Table 1. Experimental data for the CO₂ extraction at 40°C (P_{CO₂}= 58 bar) and hexane extraction (reflux distillation).

	Extraction by CO ₂	
Amount of leaves (g)	Amount of extract (g)	Yield (%)
8.64	0.2	2.3
	Extraction by hexane	
Amount of leaves (g)	Amount of extract (g)	Yield (%)
100	27.61	5.64

Moreover, the chemical composition of the extracts was determined by thin layer chromatography. Fig. 1 represents chromatographic silica gel plates of the standard 1-8 cineole (eucalyptol) (indicated by **A** in Fig. 1) used and of the essential oil of the extract obtained by liquid CO₂ (indicated by **B** in Fig. 1). As eluent was used a mixture of toluene: ethyl acetate (5:1 V/V) and as identification reagent was used vanillin (Farmacopea ufficiale della Repubblica Italiana, 1981; British Pharmacopoeia, 2007).

Figure 1. Chromatographic silica gel plate 10x20 cm² and 5x20 cm² of : A- standard cineol (eucalyptol) 30 µl in 20 ml alcohol (ethanol) and B- essential oil obtained by CO₂ extraction.

The constituents present in the essential oil are indicated by arrows in Fig. 1. These constituents are identified based on their R_f values Farmacopea ufficiale della Repubblica Italiana, 1981; British Pharmacopoeia, 2007. Additionally, Fig. 2 displays the composition of oil extracts obtained by liquid CO₂ solvent (A) and by hexane distillation (B).

Figure 2. Chromatographic silica gel plates 10x20 cm² and 5x20 cm² for the extracts obtained by liquid CO₂ (A) and by hexane (B).

It is observed from the chromatograms in Fig. 1 and 2 that extracting solvents, carbon dioxide and hexane are able to extract from the sage leaves numerous constituents. Moreover, the color of the spots indicates the presence of the same components in both extracts. Finally, Fig. 3 displays an overview of the spots observed on the Si plate for the extract obtained by liquid carbon dioxide (A) and fractions (6 different droplets) taken from the oil extract obtained by liquid CO₂. It is obvious from the chromatogram in Fig. 3 that the constituents present in the extract can be separated.

Figure 3. Chromatographic silica gel plate of the extract obtained by liquid CO₂ (A) and 6 droplets (B, C, D, E, F, G) taken from the extract obtained by liquid CO₂ as well (fractions). The eluent used was hexane.

Additionally, the identification of the spots on chromatograms was done by comparing the colored spot of the standard component with the colored spots of the extracts and their R_f respective values. Table 2 indicates the R_f respective values and the colored spots on chromatograms according to BP 2007 (British Pharmacopoeia, 2007)

Table 2. R_f respective values and the colored spots on the silica plates British Pharmacopoeia (2007).

Components	The color of spots	Respective R _f values
1-8 cineole	blue-green	0.68
α- thujone	violet	0.56
camphor	pink -violet	0.46
borneol	green	0.39

Conclusion

In this scientific work, the essential oil of sage leaves was obtained using two complementary methods: liquid CO₂ extraction (soxhlet method) and extraction by reflux distillation using hexane as solvent. Both methods proved to be successful, however the yield of the extract obtained by liquid CO₂ was higher. TLC indicated presence of many components identifying several of them as well. Both methods revealed also the presence of the same components. Separation of the constituents was also possible.

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