Alpha and Beta Pinene in Medicinal Plants of Albanian Origin: A Study and Identification

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Abstract

The category of monoterpenes includes the well-known compounds alpha and beta pinenes, which are present in the essential oils of many plants. Several pharmacological activities have been identified, such as modification of antibiotic resistance, anticoagulant, anticancer, antibacterial, antimalarial, antioxidant, anti-inflammatory, and analgesic properties.The existence of alpha and beta pinene in the plant extract of several Albanian species, including Salvia Rosmarines, Hypericum Perforatum, Cistus Incanus, and Teucrium polium, has been investigated in an Albanian study using modern essential oil extraction techniques such as Supercritical Fluids with CO_2, where CO_2 is the solvent used. While using GC-FID, accurate profiling of these analyzed plants was performed, evidencing the presence of alpha and beta pinenes in varying amounts. Significant amounts of these monoterpenes have been identified in Hypericum Perforatum (19.75% alpha and 3.93% beta-pinene) and Teucrium Polium (6.46% alpha and 18.75% beta-pinene), Salvia Rosmarines (10, 41% alpha-pinene and 3.41% beta-pinene) and Citrus Incanus (respectively with the highest amount of 21.56% alpha-pinene and less than 0% beta-pinene). These plants are widely known for their antioxidant, anticancer, anti-inflammatory, hypoglycemic, hepatoprotective, hypolipidemic, and antibacterial properties. and antifungal, properties which are also attributed to the presence of these monoterpenes. After, the essential oil was subjected to several chemical analyses, including heavy metal testing and microbial analysis, which were found to conform to the standards set by the WHO for plants. The recommendations that can be considered, after the investigations, are medicinal plants that are usable by consumers in their natural state, after collection they should always be provided with the necessary labelling and an accompanying sheet of profile and physicochemical analysis.

Keywords: alpha and beta-pinene, SC- CO_2 extraction, GC-FID profile, heavy metals.

1. Introduction

Pinene is an organic substance with the chemical formula $C_{10}H_{16}$. It is a monoterpene molecule with two cycles. Both the alpha and beta-pinene structural isomers exist. These two isomer types are crucial components of pine resin. These isomers are also present in the resins of numerous other conifers and non-coniferous plants. Several insects can make use of these pinene isomers for their chemical communication systems. Furthermore, alpha- and beta-pinene are essential components in turpentine. Pinenes are found in pines, as the name implies. The terebinth or turpentine tree, Pistacia terebinthus, produces a resin that is high in pinene. Pinene is a compound found in pine nuts. [1]. Many different plants, including conifers, contain alpha-pinene. [1] The essential oils of Salvia spp. and Sideritis spp. (ironwort) both contain a significant amount of pinene (sage). [2] Furthermore, alpha- and beta-pinene are found in cannabis[1]. [3].

Traditional medicine has used ***H. perforatum*** for millennia to treat a variety of conditions, including mild to moderate depression, anxiety, and small burns. It uses as an Antiseptic, diuretic, anti-inflammatory, antidepressant, astringent, and antiviral/Bacterial Agent.

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Figure 1. Hypericum perforatum (St John's wort)

It has been shown that H. perforatum extracts and a number of its key chemical parts can defend against toxic assaults both directly and indirectly through antioxidant capabilities and neuroprotective processes. Hence, H. perforatum has the potential to develop into a strong treatment for neuroprotection. Very extensive research has been done on H. perfpratum's ability to treat depression, and the underlying mechanisms are well recognized. [4] [5] [6] [7].

Many biologically active substances are produced by Hypericum perforatum, but two of them—hypericin, a naphthodianthrone, and hyperforin, a lipophilic phloroglucinol—have the strongest therapeutic effects. Many other substances, such as the flavonoids rutin, quercetin, and kaempferol, also seem to have therapeutic effects.

Traditionally, ***Teucrium*** **polium** has been used to treat a variety of pathological illnesses, including rheumatism, diabetes, inflammation, and gastrointestinal issues.

Figure 2. Teucrium polium, (felty germander).

The tea of T. polium is used in traditional Iranian medicine (TIM) to cure a variety of ailments, including type 2 diabetes, indigestion, stomach pain, and common colds. Based on this background, numerous investigations have been conducted to confirm the aforesaid features scientifically. Recent research has demonstrated T. podium's antioxidant capacity. It was thought that the flavone B-ortho-dihydroxy ring's substitution was what gave this herb its antioxidant properties. [8] Many biological processes and medicinal traits have been identified by several studies: the healing of a stomach ulcer, a substantial drop in the level of blood sugar, sluggishness, jaundice, increased liver enzymes, a drop in blood sugar within 8 days, hepatic lobules undergoing generative alterations, lower triglycerides and cholesterol painkilling effect, lower levels of blood sugar in diabetic mice, etc.

Salvia Rosmarinius is a herb that has stimulating, antibacterial, and tonic characteristics in addition to its tonic and anticonvulsive effects. Intestinal infections, diarrhea, colitis, flatulence, liver diseases and jaundice, influenza, colds, rheumatism, indigestion, and oral cavity wounds are among the conditions it is used to treat. The herbal extract stimulates the circulatory system, which also improves vessel blood flow. [9]

Figure 3. Salvia Rosmarinus

One of the Lamiaceae plants with significant antioxidant properties is rosemary. Phenolic diterpenes including carnosol, rosmanol, carnosic acid, and methyl carnosate, as well as phenolic acids like rosmarinic and caffeic acid, are the main compounds linked to antioxidant action. The most prevalent antioxidant compound found in rosemary extracts is known to be carnosic acid. [10]

Cistus incanus contains antioxidants as well as antibacterial, anti-inflammatory, anticancer, and antifungal properties. It is used in the prevention and/or treatment of influenza. Rock rose is thought to be an active inhibitor of prostate enlargement. [11] [12].

Figure 4. Cistus incanus.

Cistus incanus extracts are used to make a medicine for the prevention and/or treatment of the flu. [13]. The composition of Cistus incanus lends itself to use as multifunctional active components in cosmetics that protect the skin from hazardous external elements. According to research, its extract has strong antioxidant qualities, tyrosinase inhibitory action, and UV-protective potential. [14].

2.Materials and Methods

2.1 Materials

- 4 different plants,
- SC – CO_2 apparatus,
- Hexane,

Four distinct plants from the Administrative Unit of th e Elbasan Municipality that are almost identical in ori gin are subjected to analysis. The plant's various sections, including the aerial portion (T. polium), flower, and twig (H.perforaturm) were examined to determine the presence of alpha and beta–pinene. The table below presents the plants used for this analysis.

Table 1. Plants analyzed, origin, and partly used.

	PLANT	ORIGIN	PART OF THE PLANT USED	HARVESTERS
1	***H. perforatum***	Librazhd	Flower and twig	late June
2	***T. polium***	Librazhd	Aerial part	August
3	***Salvia Rosmarinius***	Librazhd	Leaf	June to September
4	***Cistus incanus***	Gjinar	Flower	April and May

The essential oil of each of these plants is extracted using the supercritical CO_2 extraction method.

Plant selection and milling take place in a controlled, dry, and ventilated environment. A humidity monitor and a microscope are used to assess the plant's integrity and health. In terms of the current state of production: According to SCFE (supercritical fluid extraction) technology criteria, the plant particle size used is 0.3 mm with a maximum humidity of 6%. Solids extraction from ground materials (or pellets, granulates) is frequently done in batch mode with food carbon dioxide as a solvent. The apparatus includes two separators that remove CO_2 from the extract before recycling it, as well as two or more extractors that work above the critical pressure of CO_2. As the first step in the supercritical CO2 extraction procedure, the temperature and pressure of the carbon dioxide gas are increased until they reach the supercritical state. Carbon dioxide reaches a supercritical state at 31.1 °C and a pressure of 1071 psi (72.87723 atm). A heater and a high-pressure pump are used to accomplish this. The extractors through which the supercritical CO_2 passes contain a significant amount of organic raw.

The supercritical CO_2 removes the oils from the plant and passes through a series of pressure-regulating valves before entering the separators, depending on the elements present in the raw materials and the pressure-temperature conditions. To separate the various extract components, the separator's two pressures are independently adjusted.

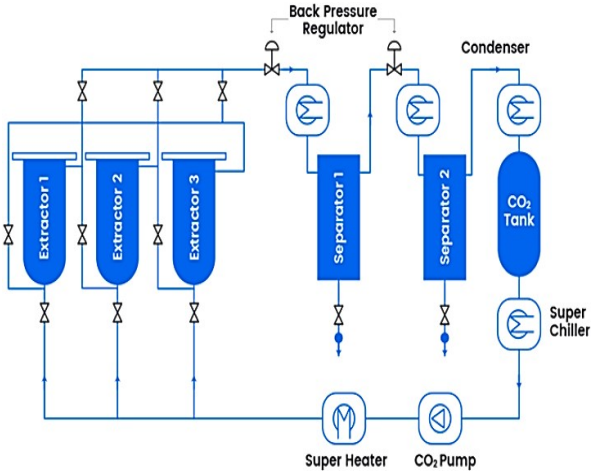

Figure 5. The Supercritical CO_2 extraction apparatus.

After the oil is removed and separated from the CO_2 that is released as a gas, the CO_2 is recycled by condensing and storing it as a liquid in the tank. After that, the oil is collected in collection containers. The cycle is then repeated by incorporating this CO_2 throughout the batch. After obtaining the entire CO_2 extract, it is diluted in a 1:2 ratio with n-hexane before being used for analysis.

Figure 6. The Supercritical CO_2 extraction apparatus while performing in the laboratory.

2. Results and discussion

After CO_2 extraction, the main components were found using the GC-FID method performed by

Essential's in-house laboratory. These data are shown in Table 2. The GC - FID method is faster, more sensitive, reproducible, and precise.

Table 2. The GC- FID analysis for the presence of alpha and beta-pinene.

Name of the plant	Alpha-pinene (%)	Beta-pinene (%)
Salvia Rosmarinus	10.41	7.83
Hypericum Perfotatum	19.75	3.93
Cistus Incanus	21.56	0
Teucrium Polium	6.46	18.75

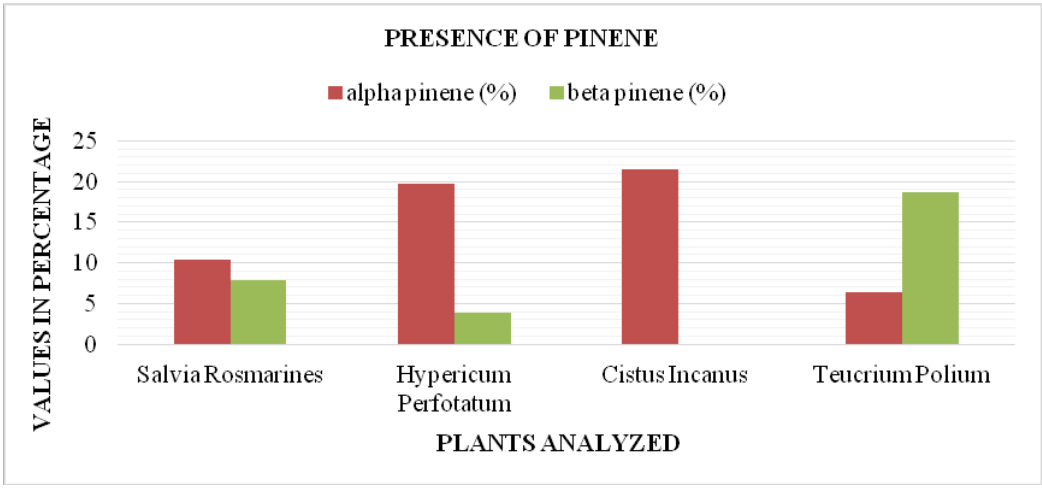

Figure 7. The graphical presentation of the alpha and beta-pinene.

The presence of Heavy Metals (lead, cadmium, arsenic and mercury) was detected using ISO 11212 Spectrophotometry, the result obtained are shown in the table below :

Name of the plant / Heavy metals	Salvia Rosmarinus	Hypericum Perfotatum	Cistus Incanus	Teucrium polium
Lead (Pb)	0.3 ppm	0.27 ppm	0.27 ppm	0.21 ppm
Arsenic (As)	0.12 ppm	0.03 ppm	0.03 ppm	0.06 ppm
Cadmium (Cd)	0.07 ppm	0.02 ppm	0.02 ppm	0.11 ppm
Mercury (Hg)	<0.06 ppm	<0.05 ppm	< 0.05 ppm	< 0.02 ppm

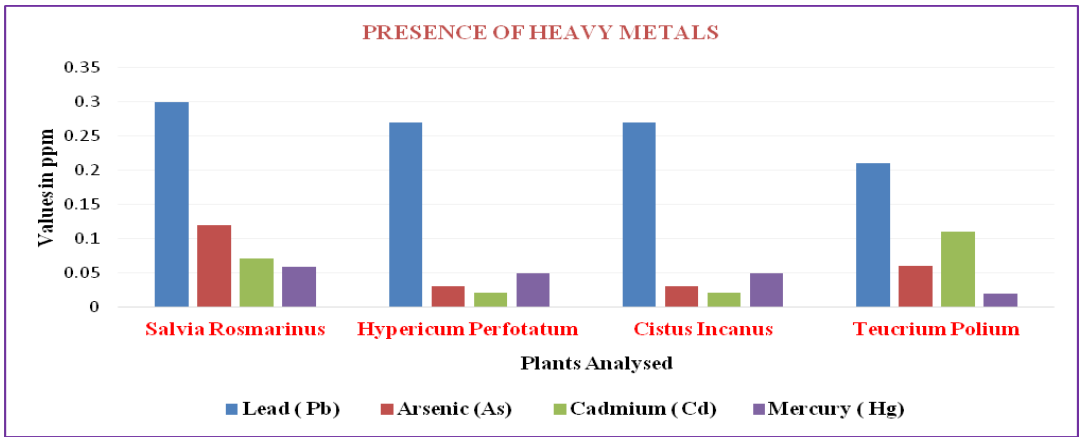

Figure 8. The graphical presentation of heavy metals.

3.1 Results

Following CO_2 extraction, the main components were identified using the GC-FID method by Essential's in-house laboratory. According to the results, the plant with the highest concentration of *alpha-pinene* is Cistus Incanus (21.56%), and the plant with the lowest concentration is Teucrium Polium (6.46%).

And for *beta-pinene*, Teucrium Polium has a high presence of 18.75%, while Cistus Incanus has a low presence of less than 0%.

After the chemical composition, the extract was used to determine the presence of heavy metals. From the analysis obtained, for all 4th plants analysed, the most abundant metal found is Lead (Pb), found in Salvia Rosmarinus in values of 0.3 ppm, and its presence is lower (but still most abundant compared to the other metals) in Teucrium Polium, Pb is found in 0.21 ppm.

The Heavy metal less present is Mercury (Hg), its presence is 'higher' in Rosmarinus <0.06 ppm and 'lower' in Teucrium Polium, < 0.02 ppm. All the values conform to the standards provided by WHO.

3.2 *Discussions*

It is worth emphasizing that CO_2 extraction is a very good method for obtaining extracts (essential oils) that are quite pure and have a fairly accurate profile of the plant.

What remains to be investigated, perhaps in future work, is whether we would have values of beta pinenes in the Cistus Incanus plant by comparing two different extraction methods, as well as whether, apart from the extraction method, only the presence of alpha pinenes to this plant to attribute all the properties it

exhibits or even the totality of secondary compounds, are what create the correct profile of this plant.

4.Conclusions

According to the results of the GC-FID analyses performed on the essential oils of the plants analyzed, we can confirm that the Teucrium polium plant has the highest presence of pinenes (25.21%). (of alpha and beta pinenes).

Salvia Rosmarinius has the lowest presence of pinenes at 18.24%. (sum of alpha and beta pinenes).

In conclusion, Teucrium polium is a plant with numerous beneficial properties when used as a tea or as an essential oil.

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