Comparative Study of α and β- pinene Content in Volatile Oils of *Abies alba, Pinus sylvestris, Juniperus communis, Rosmarinus officinalis, Salvia officinalis* and *Coriandrum sativum*

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The study describes the composition of volatile oils obtained by steam distillation of water fir (Abies alba), pine (Pinus sylvestris), juniper (Juniperus communis), rosemary (Rosmarinus officinalis), sage (Salvia officinalis), and coriander (Coriandrum sativum) using the gas chromatography technique coupled with mass spectrometry (GC-MS). Chromatographic profile revealed the presence of specific components for each studied volatile oil. It noted the presence of α and β- pinene in all volatile oils studied at different concentrations. Volatile oils studied, from green sources and rich in α, β-pinene will be used as raw materials for creating products with anti-inflammatory, antiseptic, bronchodilators properties.

Keywords: volatile oils, pinene, steam distillation, GC-MS

Natural substances with flavouring character and bioactive properties, volatile oils, also called essential oils or fragrances, are being obtained from various plant parts (flowers, leaves, roots, stems). It uses different extraction procedures (maceration, solvent extraction, cold pressing, extraction with supercritical fluids, steam distillation, rectification, enfleurage, solid phase extraction etc.), particularized for the type of raw material, or for product aimed to be obtained [1-4].

Phyto-extracts are being used to prevent, ameliorate, and even cure some illnesses and health disorders, this kind of therapy being known as phyto-therapy. In phyto-therapy, different medicinal plants are being combined in various forms (vegetable powders, cold macerates, hydro-alcoholic extracts, syrups, lotions, natural ointments) in order to restore the organism's balance and to naturally eliminate the factors that have caused the disease [1, 5-7]. Phyto-therapeutic products are the plant-derived products whose pharmacologic activity is well known, but for which their bio-compounds spectrum is unknown, or at most partially-known, while phyto-pharmaceutical products are the ones containing pure bioactive substances, well defined from the physical-chemical point of view, and in a precise therapeutic dosage. Paracelsus (c.1493-c.1541), a Swiss-German doctor and alchemist, had predicted the differentiation of plant therapeutic activity in respect to the plant part that is being used, and to the moment in plant's life. Paracelsus supported the idea of using the full plant's extract as a quintessence of its beneficial properties, notion that is of high actuality and that is known today as phyto-complex [8-10].

Essential oils in different formulations have a large field of applications, from antibacterial [11-13], antifungal [14-17], and natural insecticide [18, 19], to perfumes, cosmetics, aromatherapy, product. Plant oil is of highest importance for human beings, to prevent, ameliorate, or cure certain health disorders like infections [20], inflammations [21, 22], scars [23, 24], hormonal imbalances [25-27], mental illnesses [9, 28-30], diabetes [30, 31], and even some types of cancer [30, 32, 33].

There are known different extraction methods to obtain volatile oils from plant parts. The most used being steam extraction, solvent extraction, and maceration, while less used, but which involve an intensification of the extraction process, are microwave-assisted extraction, ultrasound-assisted extraction, and hypercritical CO_2 extraction [3, 34-36]. Maceration is being used since ancient times, our ancestors performing hydro-alcoholic maceration by leaving plant parts for a number of hours or days in wine or water-wine mixtures [37]. But as extraction method, maceration is a time-consuming method that requires also additional separation steps, respectively filtration of liquid phase and centrifugation of the water-oil emulsion. Solvent extraction has as disadvantages the necessity of solvent recovery together with the required equipment, and the traces of solvent in the volatile oils that might be undesirable for some types of end products like food supplements and cosmetics. However, solvent extraction method is necessary to extract thermally-labile bio-compounds, and hexane is preferred to methanol, ethanol, or petroleum ether due to its high efficiency and high recovery yields, the traces in the volatile oils being of ppm order. Microwave-assisted extraction, ultrasound-assisted extraction, and supercritical CO_2 extraction are quite expensive extraction methods that require advanced equipments or expensive extraction agents (supercritical CO_2).

Experimental part

Volatile oils obtained by steam distillation from water fir, pine, juniper, sage, rosemary, and coriander were analysed in order to determine their composition in terpene hydrocarbons (with a focus on α - and β - pinene). The steam distillation method consisted in subjecting 50 g of each plant in a 700 mL Clevenger-type extractor for 3 h. As the vegetable product is brought to a more advanced degree of crunching, the larger the contact surface is, and the extraction is deeper, with more compounds and higher yields.

Volatile oils thus obtained were characterized by gas chromatography coupled with mass spectrometry (GC-MS) using a Thermo Electron Corporation equipment, type Focus GS with a DSQ II mass spectrometer detector and a chromatographic column of fused silica, 30 m in length, 0.25 mm internal diameter, having a Macrogol 20.000 R as

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stationary phase at 0.25 μm film thickness. The chemical compounds present in the volatile oils were identified and quantified using a NIST spectra library.

Results and discussions

Water fir

From fir branches (twigs with green needles), species *Abies alba*, is being obtained a volatile oil rich in terpene compounds (especially pinene) that shows various therapeutic properties like antiseptic, sedative, diuretic, expectorant, revulsive, tonic, or stimulant for mucous secretions [38].

Pine

Similar to other fragrances from conifers (rich in alpha and beta pinene), the volatile oil of pine (*Pinus sylvestris*) possess excellent therapeutics properties, like antiinflammatory, antiseptic for respiratory and urinary tract, antibiotic, and anodyne properties [11].

Juniper

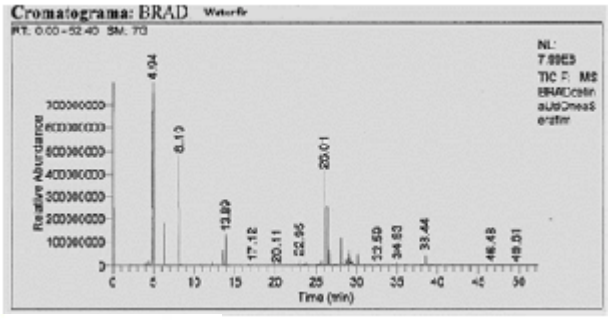

Fig. 1. GC-MS evaluation of bio-compounds found in volatile oil of water fir

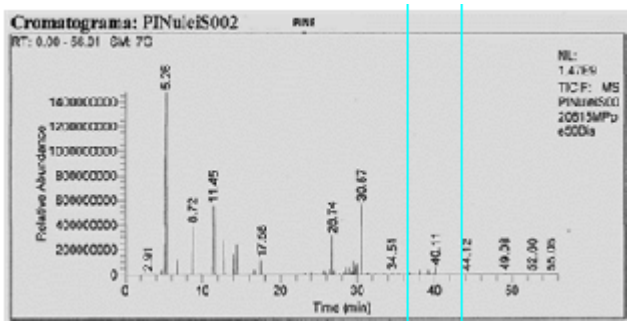

Fig. 2. GC-MS evaluation of bio-compounds found in volatile oil of pine

Table 1

BIO-COMPOUNDS FOUND BY GC-MS IN WATER FIR VOLATILE OIL, AND THEIR RELATIVE ABUNDANCE

Compound name	RT, min	A, %
Pinene α	**4.93**	**28.70**
Camphene	6.30	7.30
Pinene β	**8.18**	**27.10**
Myrcene	12.20	0.83
Limonene	13.54	2.44
Phellandrene β	13.89	5.02
Bornil acetate	26.00	10.34
Cariophilene β	26.37	6.75
Himachalene β	26.55	1.45
Cariophilene α	28.13	2.89
Terpineol α	28.91	0.84
Germacrene	29.08	1.70

Table 2

BIO-COMPOUNDS FOUND BY GC-MS IN PINE VOLATILE OIL, AND THEIR RELATIVE ABUNDANCE

Compound name	RT, min	A, %
Pinene α	**5.25**	**37.00**
Camphene	6.72	2.54
Pinene β	**8.72**	**11.10**
3-Carene	11.45	15.09
Myrcene β	12.78	5.72
D-Limonene	14.05	3.14
Phellandrene β	14.41	4.50
Cariophilene β	26.74	4.18
Longifolene	26.98	0.80
Cariophilene α	28.50	0.86
Germacrene	29.45	1.41
Eudesmadiene	29.65	0.76

The volatile oil of juniper fruits (*Juniperus communis*) is a good general antiseptic, tonic for the nervous system, beneficial for lungs, digestive tract, urinary tract, blood, has depurative, antirheumatic, scar healing, and diaphoretic properties, and favours the elimination of toxins and a psychological and mental balance [39].

Rosemary

Of rosemary (*Rosmarinus officinalis*) it can be extracted a volatile oil with purifying action on the organism, refreshing, expectorant, antispasmodic, scar healing, fungicidal, tonic for muscular and nervous systems, and with proved action in decreasing the amplitude of cardiac contractions [7].

Sage

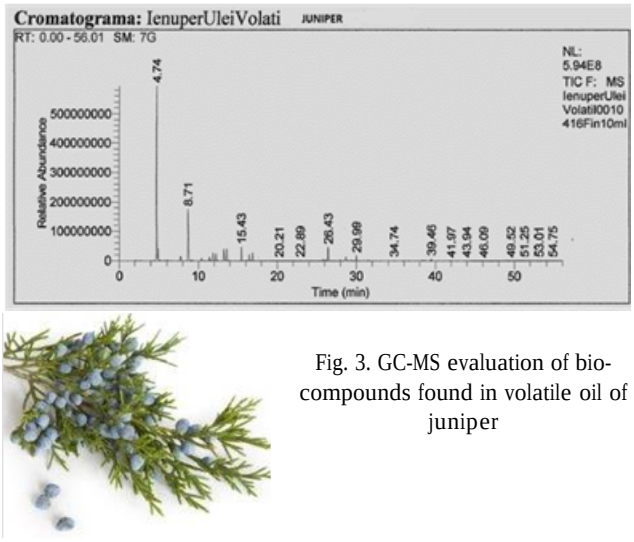

Fig. 3. GC-MS evaluation of bio-compounds found in volatile oil of juniper

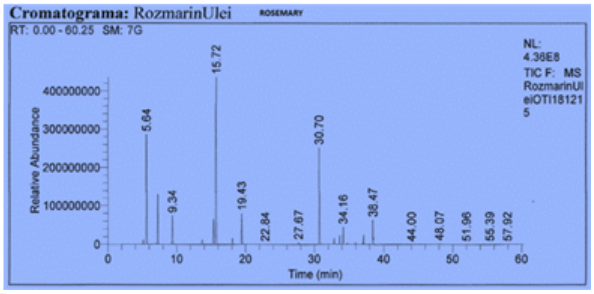

Fig. 4. GC-MS evaluation of bio-compounds found in volatile oil of rosemary

Table 3

BIO-COMPOUNDS FOUND BY GC-MS IN JUNIPER VOLATILE OIL, AND THEIR RELATIVE ABUNDANCE

Compound name	RT, min	A, %
Pinene α	**4.74**	**43.90**
Sabinene	4.91	3.25
Pinene β	**7.75**	**1.60**
Phelandrene α	8.70	20.04
3-Carene	10.40	1.23
Phellandrene β	11.43	1.45
Myrcene	11.86	2.99
Terpinene	12.24	2.75
Limonene	13.26	4.38
Phellandrene γ	13.60	4.39
Terpinene γ	15.43	4.24
Terpinene 4-ol	26.43	3.06

Table 4

BIO-COMPOUNDS FOUND BY GC-MS IN ROSEMARY VOLATILE OIL, AND THEIR RELATIVE ABUNDANCE

Compound name	RT, min	A, %
Pinene α	**5.64**	**14.70**
Camphene	7.24	8.27
Pinene β	**9.34**	**5.80**
Myrcene	13.70	0.77
Limonene	15.35	4.58
Cineol	15.71	34.49
o-Cimene	19.43	4.73
Camphor	30.70	13.93
Linalol β	32.83	0.70
Bornil acetate	33.64	1.16
Borneol	37.14	1.27
Terpineol α	38.47	4.89

With many uses in different health conditions, both internally and externally, the volatile oil of sage (*Salvia officinalis*) has been showing antiseptic, astringent, anti-infections, sedative, digestive tonic, diuretic, and scar healing effects, being able also to regulate the activity of the endocrine glands, to increase organism resistance, and to reduce pain and menstrual bleeding [2].

Coriander

The volatile oil of coriander (*Coriandrum sativum*) obtained from the fruit of the plant has antiseptic, tonic, anti-parasitic, carminative, aphrodisiac, and soothing effects, and helps memory, being used in various diseases, both internally and externally [40].

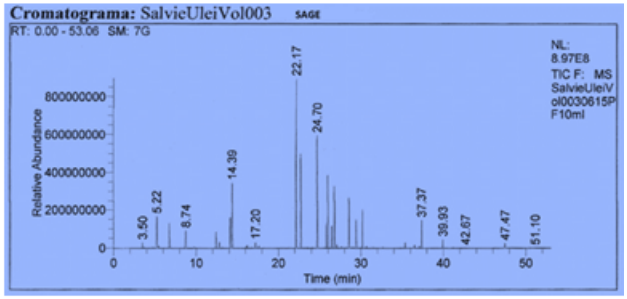

Fig. 5. GC-MS evaluation of bio-compounds found in volatile oil of sage

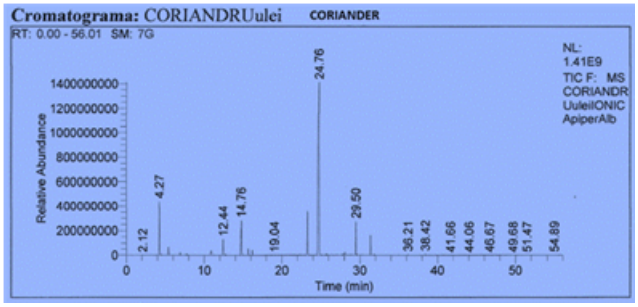

Fig. 6. GC-MS evaluation of bio-compounds found in volatile oil of coriander

Table 5

BIO-COMPOUNDS FOUND BY GC-MS IN SAGE VOLATILE OIL, AND THEIR RELATIVE ABUNDANCE

Compound name	RT, min	A, %
Pinene α	**4.55**	**4.90**
Camphene	5.87	4.05
Pinene β	**7.59**	**2.00**
Myrcene	11.74	0.92
Cineol	13.13	1.45
Limonene	13.42	10.48
Thujona trans	21.43	34.63
Thujona cis	21.98	13.10
Camphor	23.93	16.02
Cariophilene β	27.89	1.62
Viridifloral	36.70	4.51
Epimanool	46.77	2.42

Table 6

BIO-COMPOUNDS FOUND BY GC-MS IN CORIANDER VOLATILE OIL, AND THEIR RELATIVE ABUNDANCE

Compound name	RT, min	A, %
Pinene α	**4.26**	**8.10**
Camphene	5.38	1.43
Pinene β	**6.92**	**0.60**
Phelandrene α	7.73	0.34
Myrcene	10.89	1.08
Limonene	12.44	3.48
Terpinene γ	14.76	6.73
o-Cymene	15.67	1.21
Camphor	23.27	6.24
Coriandrol	24.75	63.50
Geraniol trans	29.50	3.70
Epimanool	31.38	2.00

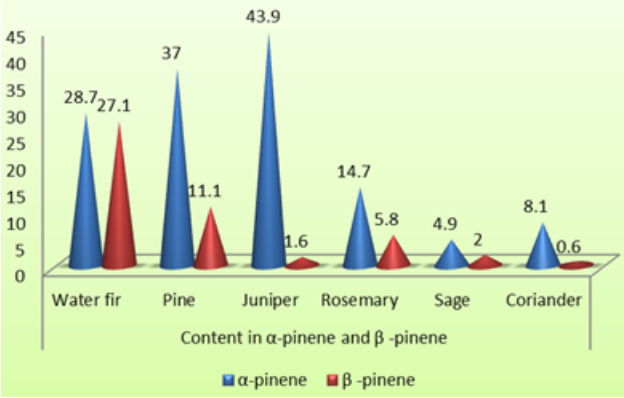

Fig. 7. The variation in α and β - pinene in the studied volatile oils

Conclusions

Six plants, water fir (*Abies alba*), pine (*Pinus sylvestris*), juniper (*Juniperus communis*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and coriander (*Coriandrum sativum*) were submitted to steam distillation in order to obtain the corresponding essential oils. GC-MS investigations showed that the chemical compounds present in the studied essential oils are consistent with the provisions of the European Pharmacopoeia, actual edition. It was found that the volatile oils obtained from conifers (pine, fir, and juniper) are richer in α - pinene than rosemary, sage, and coriander, and that juniper is the richest in α - pinene. Also, it was determined that the largest amount of β - pinene is found in pine and fir. Based on this research on the composition of volatile oils in α and β -pinene, and after studying the vast documentation of traditional and modern medicine, we will be able to develop new food supplements and cosmetics with anti-inflammatory, antiseptic properties and high bioavailability.

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