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COMPARING THE EFFECT OF UTILIZATION OF ETHYL HEPTANOATE AS CO-SOLVENT DURING EXTRACTION OF ESSENTIAL OIL OF *Inula helenium* IN A HYDRODISTILLATION PROCESS

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Inula helenium L. (elecampane) is a perennial plant found in damp meadows and shade areas of Central Europe [1]. Elecampane oil is mainly biosynthesized in the roots, which contain 0.5–2% oil. At room temperature elecampane oil is a solid containing a mixture of white crystals of sesquiterpenoid lactones and a liquid yellow phase attributed to alantol [2].

Hydrodistillation at atmospheric pressure is the most frequently used method for essential oil isolation. Hexane is the most used solvent in extraction processes for both practical and economical reasons. In this study, the concentration and composition of essential oils of elecampane from different extraction process (hydrodistillation and co-hydrodistillation) were compared. The hydrodistillation process was used in essential oil extraction and compared with the co-hydrodistillation process. Ethyl heptanoate was used as co-solvent in the co-hydrodistillation process. The aim of this work was to evaluate the variations in the yield and chemical composition of the essential oil of *Inula helenium* L. root obtained by hydrodistillation and co-hydrodistillation; the obtained essential oils were analyzed by GC and GC/MS.

Yield of essential oils obtained by hydrodistillation was 1.1%. This value compared well with that given by [3, 4] for *Inula helenium* L. root. However, in the co-hydrodistillation process the yield obtained at different concentrations increased. The results obtained in hydrodistillation and co-hydrodistillation process are shown in Table 1. Identification of the components of essential oil obtained by hydrodistillation and co-hydrodistillation were carried out by comparison of their mass spectra and retention indices with those of reference standards. Table 1 summarizes the obtained essential oil compounds and their chemical compositions. Only compounds having more than 0.1% content were considered in this study. In these conditions 70 major compounds were identified in the essential oils obtained by hydrodistillation and 20 compounds by co-hydrodistillation, where 10 mL of ethyl heptanoate was added. In this table, it can be seen that these oils were characterized by the presence of monoterpenes, hydrocarbons, and oxygenated monoterpenes and sesquiterpenes, but quantitative differences were observed in the contents of these components. The main components of the volatiles obtained by hydrodistillation and co-hydrodistillation were sesquiterpene lactones: alantolactone 56.6% in hydrodistillation and 49.5% in co-hydrodistillation and isoalantolactone 37.3% and 32.2% respectively. The lactones fraction represents 92% of the essential oil obtained by hydrodistillation. Similar results were obtained by [5] in the quantification of the main compounds in the hydrodistillation process of *Inula helenium* root. Nevertheless, the results suggest that with the utilization of ethyl heptanoate at different concentrations in the hydrodistillation process (called co-hydrodistillation), a modification in chemical composition is produced. This modification is evidenced by the presence of compounds not found in the essential oil obtained by hydrodistillation. Fatty acid ethyl esters are molecules with lower melting points and larger hydrophobicity [6, 7]. These characteristics are responsible for the dissolution of compounds contained in *Inula helenium* L. root that are not extracted in the hydrodistillation process utilizing water as solvent. Volatile compounds identified in the essential oil obtained by co-hydrodistillation (representing 10%) that were not present in the essential oil obtained by hydrodistillation were: α-pinene, camphene, β-pinene, ethyl hexanoate, camphor, eremophilene, calarene, *trans*-acorenone, α-amorphene, α-asarone, and α-selinene. The respective percentages of these constituents are reported in Table 1.

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TABLE 1. Yield (in %) and Composition of Essential Oils from Hydrodistillation and co-Hydrodistillation of *Inula helenium*

Compound*	RI	Hydrodistillation essential oils	Co-hydrodistillation**		
			+5 mL ethyl heptanoate	+10 mL ethyl heptanoate	+20 mL ethyl heptanoate
α-Pinene	6.65	Nd.	Nd.	0.20	Nd.
Camphene	7.16	Nd.	0.92	0.30	0.93
β-Pinene	8.02	Nd.	Nd.	0.23	Nd.
Ethyl hexanoate	8.57	Nd.	Nd.	0.33	2.24
Ethyl heptanoate	12.74	Nd.	c*	c*	c*
Camphor	14.58	Nd.	Nd.	0.28	Nd.
β-Elemene	24.89	1.10	Nd.	0.48	Nd.
Aristolene	26.09	Nd.	1.18	0.65	0.86
Calarene	26.59	Tr.	3.06	1.62	2.61
Octyl heptanoate	28.80	Nd.	Nd.	0.31	Nd.
Eremophilene	28.92	Nd.	Nd.	1.08	1.04
α-Selinene	29.26	0.33	Nd.	0.77	Nd.
α-Calacorene	31.04	Nd.	Nd.	0.16	Nd.
α-Asarone	33.81	Nd.	Nd.	1.20	Nd.
Junipene	35.61	0.40	Nd.	0.47	Nd.
trans-Acorenone	36.59	Nd.	Nd.	2.18	3.24
1,4-Pentadien	41.50	0.27	Nd.	Nd.	Nd.
Alantolactone	44.29	56.64	53.90	49.50	51.90
Isoalantolactone	45.75	37.31	35.59	32.21	35.52
α-Amorphene	45.82	1.88	1.14	1.83	1.65
Yield, %		1.14	1.30	1.41	1.28

*Main compound detected in GC-MS library NIST Standard Reference Database [9]; **ethyl heptanoate added in co-hydrodistillation process.

Nd.: not detected.

The co-hydrodistillation process using ethyl heptanoate as co-solvent can increase the extraction of new essential oil compounds. This method of essential oil extraction with ethyl heptanoate ensures the isolation of monoterpene, sesquiterpene, and diterpene compounds. Co-hydrodistillation can be performed with 10 mL of ethyl heptanoate. The order of exit of the components is dictated by their polarity; it is thus probable that the phenomena of diffusion and polarity due to the action of ethyl heptanoate intervene simultaneously during the co-hydrodistillation process.

Materials. Elecampane (*Inula helenium* L.) was supplied by the Association Interregional Recherche Experimentation Legumiere (AIREL), cultivated and collected in the south of France. Ethyl heptanoate (99.97% pure) was obtained by an esterification process in the Laboratoire de Chimie Agro-industrielle (LCA-CATAR, Toulouse, France) using 90% pure heptanoic acid (Fulka, France) with 95% pure ethanol (Prolabo, France) and 98% pure sulfuric acid (Prolabo, France) as catalyst according to Hernandez-Ochoa [7].

Hydrodistillation and co-Hydrodistillation Process. A modified Schilcher apparatus was utilized in hydrodistillation and co-hydrodistillation. The flow rate was 10 to 15 mL/min. Cooling water was kept at 19°C to prevent the higher- molecular-weight components from being stuck in the cooler. In the hydrodistillation process, 400 g of vegetable matter and 5 L of water were introduced in a boiling flask. The system was heated to 100°C during 5 h. For the co-hydrodistillation process, ethyl heptanoate is added as co-solvent (at different concentrations: 5, 10 and 20 mL) and immersed with the vegetable matter and water in the boiling flask. The operation conditions depended on the vegetable material used, according to Hernandez-Ochoa [8]. The homogeneous mixture obtained in this process, composed of essential oil and ethyl heptanoate, was called extract. The essential oil and extract were collected and diluted with hexane in a volumetric flask; the obtained solutions were analyzed by GC-FID (gas chromatography coupled with flame ionization detector) and GC-MS (gas chromatography–mass spectrometry).

Analysis Conditions. GC-FID was used to determine the content and number of compounds of the essential oils; similarly, GC-MS analysis was performed to identify the main components.

The analysis of all essential oils was performed using a gas chromatographic system (Hewlett-Packard Inc., Palo Alto, CA, USA) equipped with a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a mass spectrometer (Hewlett-Packard 5971 A) as detector. The carrier gas was helium, at a flow rate of 1 mL/min. For the GC-FID analysis, the temperature

was increased from 70°C to 180°C at 4°C/min. The injector and detector temperature were set at 220°C. The GC/MS system was equipped with a BPx5 Capillary column (50 m × 0.25 mm i.d., 0.25 µm film thickness). The temperature was increased from 60°C to 220°C at 3°C/min. For detection, an electron ionization system at 70 eV was used.

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