Chemical composition of the essential oil of *Artemisia Absinthium* from Romania

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In the present study we have obtained and investigated the chemical composition of the essential oil extracted by hydrodistillation, from dried herb of Artemisia absinthium, cultivated in Romania. The results were compared with those reported by other authors (from other geographical areas), in specialty studies. The obtained essential oils were analyzed using the techniques of Gas Chromatography coupled with Mass Spectrometer (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). A large number of monoterpenes (76.92%) and a small number of sesquiterpenes (13.87%) were identified. In the examined essential oil, from a total of 76.92% monoterpene components, the hydrocarbon monoterpenes represent 45.24%, while the oxygenated monoterpenes represent 31.05%. As monoterpene hydrocarbons, five compounds were identified in essential oil: α and β pinene (24.47%), pseudolimonen (8.95%), geranyl bromide (3.70%), terpinolen (2.74%) and α and β fellandrene (2.38%).

Keywords: Artemisia absinthium, essential oil, composition, GC-MS, FTIR

Artemisia absinthium L., commonly known as "wormwood", is a yellow-flowering perennial plant distributed throughout various parts of Europe, Asia, North America and Africa. It is an aromatic plant of the family Asteraceae, subfamily Asteroideae, tribe Anthemideae [1-3]. Essential oil extracted from *Artemisia absinthium* is used for healing various diseases [4-6]. Anthelmintic, antibacterial, antifungal, insect repellent, narcotic, digestive, tonic and other bioactivities are characteristic of preparations from wormwood plants. The aerial parts are present in many gastric herbal preparations, in dietary supplements, and in alcoholic beverages, for example absinthe products, which are enjoying a resurgence of popularity all over the world [2, 7, 8]. The essential oil of *Artemisia absinthium* showed *antibacterial activity* against common human pathogens (*Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, and acaricidal properties [1, 9, 10]. Belay G. and collaborators [11] showed that *Lysteria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* were the most sensitive bacteria to *Artemisia absinthium* with a minimum inhibitory concentration (MIC) of 0.14, 0.8 and 0.62μL/mL, respectively. The essential oil of *Artemisia absinthium* exhibited strong *fumigant toxicity* against *Rhyzopertha dominica* adults, a stored product pest, with a LC50 value of 18.23 μL/L air and LC90 value of 41.74 μL/L air. The wormwood essential oil showed high fumigant activity against *Spodoptera littoralis*, one of the most dangerous pests of protected crops, with a LC50 value of 10.59 μL/L air and a LC90 value of 17.12μL/L air [12, 13]. Their stimulant property is dependent on bitter substances such as artabsin (sesquiterpene lactone) and absinthin (dimer of sesquiterpene lactones) present in plant extracts [6]. Major essential oil components (>10%) of *Artemisia absinthium* are: camphor, chamazulene, â-myrcene, â-pinene, *trans*-sabinyl acetate, â-thujone etc. [1]. Variation in the composition of essential oils depends on their genetic variations, geography, time of collection, stages of plant growth and seasonal and environmental factors. Variations in the traded herbal composition occurs on account of geo-climatic conditions of their growth, maturity at the time of collection, the variation of species, substitutability on the basis of perceived efficacy and dubious trade practices [14, 15, 16].

The objectives of this study were to evaluate chemical composition of the essential oil of *Artemisia absinthium* from Romania and comparing the results with those reported by other authors, from other geographical areas. Thus, the volatile oil obtained by hydrodistillation of the aerial parts of Artemisia absinthium cultivated in Romania was analyzed by gas chromatography/mass spectrometry (GC/MS) and the Fourier Transform Infrared spectrophotometer (FTIR).

Experimental part

Materials and methods

The aerial parts in dried state of *Artemisia absinthium* L. were purchased from Stef Mar SRL Ramnicu Valcea Romania. Plants were cultivated in specially designed greenhouses located in the Ramnicu Valcea area of Romania and were harvested and dried according to requirements of British Pharmacopoeia. [17].

The essential oil extraction

100 g of dried plant of *Artemisia absinthium* were crushed and transferred in a balloon with a round bottom having the capacity of 2 L and over it 1.5 L of water was added. The balloon was connected to the Clevenger-type apparatus for distillation. The mixture was hydrodistilled for 5 h. The distillate obtained was dried over anhydrous sodium sulphate and stored in air tight amber-coloured bottle at 4°C until it was analyzed.

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The analysis of the essential oil

Gas Chromatography coupled with Mass Spectrometer (GC-MS) analysis

The analysis of the volatile components of the obtained essential oil was carried out on a gas chromatograph coupled with a mass spectrometer of GC-MS Agilent 5975C type. The column used for the separation of the sample components was a DB5-MS capillary column (30 m length × 0.32 mm internal diameter, film thickness 0.25 μm). Hydrogen (99.999% high purity) was used as the carrier gas (inlet pressure 82 737 Pa at a flow rate of 1 .6667 × 10^{-8} m^3 s^{-1} (1 mL min^{-1}) (splitting ratio 40:1). Oven temperature was initially 40°C and then was raised progressively to 80°C with a rate of 3° C/min, to 180°C at a rate of 5° C/min and finally was raised to 280°C at a rate of 8°C/min and maintained there for 20 min. 1.0μL of each sample, dissolved in hexane were injected. The injector temperature was 280°C. The mass spectrometry conditions were as follows: ionization voltage of 70 eV, emission current of 35 mA, scan rate of 2.8scan/s, mass range of 10–350 Da and ion source temperature of 200°C. The percentage composition (area percent) of the oils was computed by the normalization method from the GC peak areas, by means of three injections from each sample, without using correction factors. The constituents of the volatile oils were identified by two methods: by comparing their calculated GC retention indices with literature data and by library searching.A mixture of aliphatic hydrocarbons (C8–C24) in hexane was injected as under the above-mentioned temperature programme and to calculate the retention indices the generalized equation of Van den Dool and Kratz (1963) was used.

Kovats retention indices are calculated using the formula:

$$K = 100 \times \left[n + \frac{\log t_{Rx} - \log t_{Rn}}{\log t_{Rn+1} - \log t_{Rn}} \right]$$

where:

- *x* is the test compound;
- *n* is the alkane with n carbon atoms into the molecule, whose peak is placed on the left side of the analyzed peak from the chromatogram;
- *n+1* is the alkane with n carbon atoms into the molecule, whose peak is placed on the right side of the analyzed peak from the chromatogram.

Fourier Transform Infrared Spectroscopy (FTIR)

The IR fingerprint of the essential oil was highlighted using a FTIR spectrophotometer – Thermo Scientific Nicolet 6700 Model 912A063, by ATR measurement method. The spectra of the samples are the averages of 32 scans, in the range of 4000 – 800 cm^{-1}, with a resolution of 4 cm^{-1}.

Results and discussions

The volatile oil obtained from *Artemisia absinthium* was green and extremely clear. The hydrodistillation technique applied to this plant led to the obtaining of 1.00% (v/w) essential oil. The oil was analyzed using the technique of Gas Chromatography coupled with Mass Spectrometer (GC-MS), in order to determine the existing compounds and their percentage. The results are summarized in table 1.

A total of twenty-five components were identified. As can be seen from figure 1 the volatile oil of *Artemisia absinthium* was characterized by a small number of sesquiterpenes (6.87%) and a large numbers of monoterpenes (76.29%). In the obtained essential oil, from the total of 76.29% monoterpene components, the monoterpene hydrocarbons represent 45.24%, while the oxygenated monoterpenes represent 31.05%.

As monoterpene hydrocarbons, five compounds were identified in essential oil: pinene (α and β), pseudolimonen, geranyl bromide, fellandrene (α and β) and terpinolen and as oxygenated monoterpene, the representative compounds found, are: isopulegol, ethanol,2-(3,3-dimethylcyclohexylidene), 4-terpineol, eucalyptol and terpenol. The level of these compounds is shown in figure 2.

Our results obtained in the present work are different from those reported by other authors. The first difference is the obtaining yield of essential oil which was of 1.0% (v/w). This amount is smaller than or comparable to others reported: Wani H. [18] have reported 0.15% (v/w), Baykan-Erel S. [19], 1.10% (v/w), and Rezaeinodehi A. [20], 1.30% (v/w), respectively. The second one, very important also, is related to the composition of the extracted essential oil.

In Table 2 are shown, by comparison, the results of five studies regarding the composition of five types of essential oil obtained from species of *Artemisia absinthium* reared in five different geographical areas. The results presented in the last column of table 2 are the results of this work.

From table 2 it can be seen that five of the four main components of *Artemisia absinthium* essential oil are present in the samples obtained and analyzed in our study, as follows: monoterpene hydrocarbons (45.2%), oxygenated monoterpenes (31.0%), sesquiterpene hydrocarbons (3.9%) and oxygenated sesquiterpenes (2.9%). The results of our study show that the essential oil is dominated by the monoterpene hydrocarbons as in studies from India, Turkey and Iran. Thus, in our study it was found that β-pinene (27.47%) is the major component, followed by pseudolimonen (8.94%) and geranyl bromide (3.69%). Wani H. [18] in their investigation regarding the composition of essential oil of *Artemisia absinthium* from India, from the total of 12 compounds which have been determined, β-pinene (42.15%) and chrysanthenyl acetate (49.15%) were found to be major constituents. Rezaeinodehi A. [20] have also found β-pinene (27.6%) as a major component, followed by β-thujone (19.5%) and sabiene (8.9%). These compounds were the major components of essential oil obtained from Artemisia absinthium from Iran. Baykan-Erel S. [19] have obtained and analyzed essential oil of *Artemisia absinthium* from Turkey (Anatolia). The representative compounds of the essential oil have been sabinene (17.56%), myrcene (10.96%) and chrysanthenyl acetate (10.97%). In their study made also on *Artemisia absinthium* from Lithuania this time, Judţentienë A. [4] have reported that trans-sabinyl acetate (20.0%), thujone (10.2%), β-pinene (6.0%) and 1.8 cineole (3.7%) are the major constituents of essential oil. The registered differences can be attributed to the ecological factors or to some unknown genetic modifications. Depending on the destination, the presence in a large amount or the absence of one of the components may be advantages for the quality of products which are based on essential oil (fragrance, cream, drug, oil for aromatherapy etc.).

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy is one of the most widely employed techniques for functional groups identification. Figure 3 and table 3 show the infrared spectra and the characteristic bands observed in Artemisia absinthium essential oil in the range of 4000-800 cm^{-1}.

The broad band centered between 3450 3350 cm^{-1} corresponds to O-H stretching of hydroxyl groups (alcohols, phenols and carboxylic acids) [21]. The characteristic bands of the saturated aliphatic sp^3 C–H bonds are observed

RT(min.)	Name	Formula	RC (%)	K (x10^3)
7.051	α-Fellandrene	$C_{10}H_{16}$	0.627	488.034
8.916	L-β-pinene	$C_{10}H_{16}$	7.008	697.584
9.861	L-β-pinene	$C_{10}H_{16}$	20.465	717.722
10.263	α-Fellandrene	$C_{10}H_{16}$	1.753	725.709
10.837	Terpinolen	$C_{10}H_{16}$	1.021	$1.047x10^3$
11.206	o-Cymol	$C_{10}H_{14}$	6.112	$1.056x10^3$
11.364	Pseudolimonen	$C_{10}H_{16}$	0.789	$1.06x10^3$
11.460	Eucalyptol (Cineole)	$C_{10}H_{18}O$	1.277	$1.062x10^3$
12.839	Terpinolen	$C_{10}H_{16}$	1.720	$1.093x10^3$
14.930	Isopulego	$C_{10}H_{18}O$	12.213	$1.133x10^3$
15.514	Sabinol	$C_{10}H_{16}O$	0.981	$1.513x10^3$
18.007	Ethanol, 2-(3,3-dimethylcyclohexylidene)	$C_{10}H_{18}O$	5.159	$1.571x10^3$
18.227	4-Terpineol	$C_{10}H_{18}O$	5.081	$1.576x10^3$
18.828	Terpenol	$C_{10}H_{18}O$	0.775	$1.589x10^3$
20.053	Ethanol, 2-(3,3-dimethylcyclohexylidene)	$C_{10}H_{18}O$	0.808	$1. 958x10^3$
20.355	Ethanol, 2-(3,3-dimethylcyclohexylidene)	$C_{10}H_{18}O$	3.533	$1. 968x10^3$
20.445	Citronellyl formate	$C_{11}H_{20}O_2$	0.878	$1. 97x10^3$
21.339	Ethanol, 2-(3,3-dimethylcyclohexylidene)	$C_{10}H_{18}O$	1.219	$1. 996x10^3$
22.243	Estragole	$C_{10}H_{12}O$	0.907	$2. 022x10^3$
22.564	2,6-Dimethyl-1,3,5,7-Octatetraene, E,E	$C_{10}H_{14}$	1.346	$2. 03x10^3$
26.168	Caryophyllene	$C_{15}H_{24}$	1.032	$2. 593x10^3$
26.467	Bicyclo[3.1.1]heptane-2-methanol, 6,6-dimethyl-, acetate	$C_{12}H_{20}O_2$	0.967	$2. 604x10^3$
27.872	α-Longipinene	$C_{15}H_{24}$	0.702	$2. 657x10^3$
27.968	Curcumene	$C_{15}H_{22}$	2.229	$2. 66x10^3$
28.233	Pseudolimonen	$C_{10}H_{16}$	1.735	$2. 67x10^3$
28.734	Geranyl n-propionate	$C_{13}H_{22}O_2$	2.802	$2. 857x10^3$
28.770	Lavandulol, acetate	$C_{12}H_{20}O_2$	2.950	$2. 858x10^3$
30.284	.Spathulenol	$C_{15}H_{24}O$	0.690	$2. 92x10^3$
30.378	Pseudolimonen	$C_{10}H_{16}$	6.421	$2. 924x10^3$
30.546	Geranyl bromide	$C_{10}H_{17}Br$	3.699	$2. 93x10^3$
30.999	Geranyl propionate	$C_{13}H_{22}O_2$	0.875	$2. 948x10^3$
31.136	Linalyl iso-valerate	$C_{15}H_{26}O_2$	0.396	$3. 101x10^3$
39.199	E-Nuciferol	$C_{15}H_{22}O$	0.466	-
39.304	E-Nuciferol	$C_{15}H_{22}O$	1.362	-

Table 1
THE RETENTION TIME, RELATIVE CONCENTRATION (PERCENTAGE OF AREA) AND KOVATS RETENTION INDICES OF ARTEMISIA ABSINTHIUM OIL

Note: RT - Retention time, RC - Relative concentration (percentage of area, %), K - Kovats retention indices

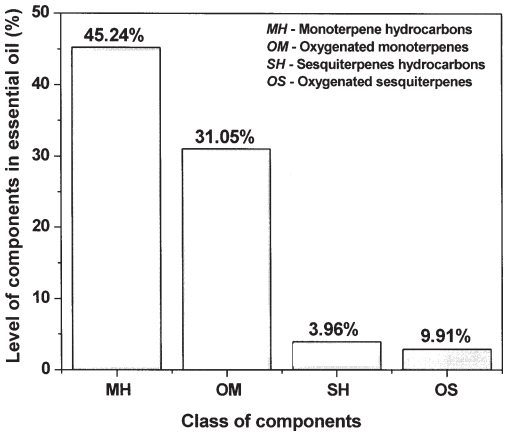

Fig. 1. The levels of monoterpenes and sesquiterpenes in the essential oil of *Artemisia absinthium*

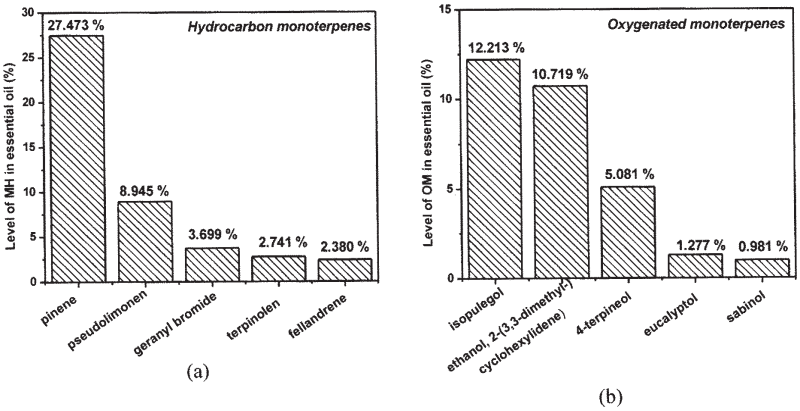

Fig. 2. The levels of representative monoterpenes (a) hydrocarbon and (b) oxygenated in the essential oil of *Artemisia absinthium*

in the range of 2950-2850 cm^{-1} which are assigned to ν_{as} (CH_3), ν_{as} (CH_2), and ν_s (CH_2) respectively (the bands situated at 2926.17 cm^{-1} and 2871.99 cm^{-1} correspond to methylene C-H asymmetric and symmetric stretching vibrations respectively, and the band situated at 2961.65 cm^{-1} is due to methyl C-H asymmetric and symmetric stretching vibrations) [21]. Any band structures observed between 3150 and 3000 cm^{-1} are almost exclusively an indication of the unsaturation (C = C- H) and/or a presence of aromatic rings but their absence in the obtained IR spectrogram is an indicative of the absence of aromatic compounds [22]. The band located between 1750-1710 cm^{-1} was assigned to the C=O vibration of bonded

Composition of essential oil of *Artemisia absinthium (%)*	Lithuania	Turkey	India	Iran	Romania (present work)
Monoterpene hydrocarbons	11.5	40.7	44.5	47.8	45.2
Oxygenated monoterpenes	47.8	15.5	54.3	27.6	31.0
Sesquiterpenes hydrocarbons	9.9	0.3	1.2	9.1	3.9
Oxygenated sesquiterpenes	9.3	0.4	-	8.8	2.9

Table 2
THE COMPOSITION OF SOME ESSENTIAL OILS OBTAINED FROM SPECIES OF *ARTEMISIA ABSINTHIUM* REARED IN DIFFERENT GEOGRAPHICAL AREAS AND ROMANIA

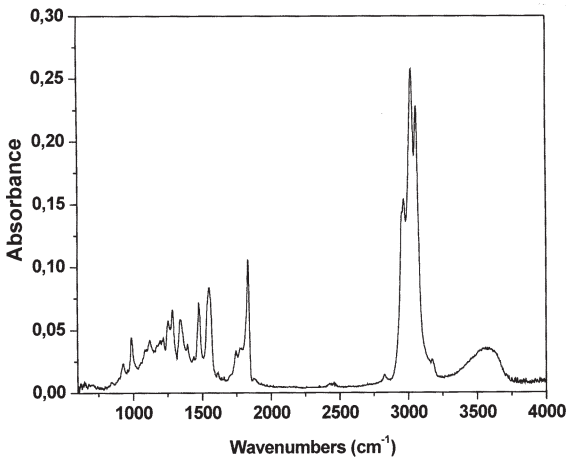

Fig. 3. FTIR spectra of *Artemisia absinthium* essential oil in the range of 4000-600 cm^{-1}

Band position (cm^{-1})	Class of Compounds
3462.00	O-H stretching vibrations of hydroxyl groups (alcohols, phenols and carboxylic acids)
2961.65	—CH_3 asymmetric and symmetric stretching vibrations
2926.17 and 2871.99	—CH_2—, symmetric and symmetric stretching vibrations
1735.52	C=O stretch in non-conjugated ketones, carbonyls and in ester groups
1646.01	C=C vibration of aromatic structures C=O stretching of carboxylic acids
1451.96	C-H asymmetric and symmetric bend and OH bend (phenol or tertiary alcohol)
1376.40	C-H asymmetric and symmetric bend; OH bend (phenol or tertiary alcohol)
1293.55	CH_2=C-H alkanes bending vibration
1240.80	C-O-C stretching bands (aromatic acid ester), C-OH (from phenols) stretching vibrations
1185-1020	C-O stretching vibration (primary, secondary and tertiary alcohol) or C-O-C stretching bends (alkyl-substituted ether),
900-800	=C-H out of plane bending vibration from aromatics
800-750	C-H out of plane bending vibration from aromatics, (—CH_2—)$_n$ rocking (n≥3); skeletal C—C vibrations

Table 3
THE INFRARED CHARACTERISTIC BANDS OBSERVED IN *ARTEMISIA ABSINTHIUM* OIL

conjugated ketones, aldehydes and esters [21, 23, 24]. The band in the range of 1630-1685 cm^{-1} was attributed to the C=C vibration of aromatic structures, C=O stretching of carboxylic acids [21]. In addition, the bands observed between 1450-1370 cm^{-1} can be attributed to C-H groups or OH bend (phenol or tertiary alcohol). The band at 1450 cm^{-1} is very characteristic also for an alcohol C-OH within the bending vibration absorption [24]. The band around 1300 cm^{-1} is attributed to the CH_2 alkanes that face the swing and the aromatic ring =C-H for the in-plane bending absorption and the band at 1240 cm^{-1} corresponds to the aromatic acid ester C-O-C symmetric expansion and the stretching vibration of the phenolic C-OH groups, which displays the characteristic absorptions of esters in volatile oil [24]. The bands located in the range of 1185 – 1020 cm^{-1} can be attributed to C-O stretch (primary, secondary or tertiary alcohol) or C-O-C stretch (alkyl-substituted ether) [21, 24]. The bands in the range of 900-800 cm^{-1} represent the out of plane bending vibration from aromatics =C-H and at 800-750 cm^{-1} out-of-plane due to the aromatic C-H bending, methylene $(CH_2)_n$ rocking (n≥3) or skeletal C-C vibrations [23, 25].

Conclusions

The chemical composition of the essential oil extracted by hydrodistillation, from dried herb of Artemisia absinthium cultivated in Romania was analyzed using the techniques of Gas Chromatography coupled with Mass Spectrometer (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). A large number of monoterpenes (76.92%) and a small number of sesquiterpenes (13.87%) were identified. In the examined essential oil, from a total of 76.92% monoterpene components, the hydrocarbon monoterpenes represent 45.24%, while the oxygenated monoterpenes represent 31.05%. As monoterpene hydrocarbons, five compounds were identified in essential oil:α and β pinene (24.47%), pseudolimonen (8.95%), geranyl bromide (3.70%), terpinolen (2.74%) and α and β fellandrene (2.38%). The results were compared with those reported by other authors (from other geographical areas), in specialty studies.

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