

# The Composition of Essential Oils Obtained from *Achillea millefolium* and *Matricaria chamomilla* L., Originary from Romania

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*In the present study we have investigated the chemical composition of the essential oils extracted by the procedure of hydrodistillation, from fresh flowers of Matricaria Chamomilla L. and Achillea Millefolium from Romania, two genres belonging to the same family, Asteraceae. The obtained essential oils were analyzed using the techniques of Gas Chromatography coupled with Mass Spectrometer and Fourier Transform Infrared Spectroscopy. It was found that both essential oils were dominated by the presence of terpenes. Aliphatic components were modest represented in both essential oils. The results were compared with those reported by other authors. Similarities consist in the domination of the same class of compounds and in the same modest representation of others. One of differences consists in the presence of some compounds with significant medicinal role, which were not reported by other authors. Another difference consists in the presence of some compounds in higher concentrations than those reported by other authors.*

**Keywords:** essential oil, chemical composition, *Matricaria Chamomilla* L., *Achillea Millefolium*

Essential oils are volatile, natural, complex compounds characterized by a strong odour and which are formed by aromatic plants as secondary metabolites. As described by Bakkali et al. [1] they were first developed in the Middle Ages by Arabs, are known both for their medicinal properties and their fragrance and in present represent an important part of the traditional pharmacopoeia of many countries. They can be synthesized by all plant organs, i.e. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes. There are several methods for extracting essential oils which may include use of liquid carbon dioxide or microwaves, and mainly low or high pressure distillation employing boiling water or hot steam. The genres *Matricaria Chamomilla* L. and *Achillea Millefolium* belong to the Asteraceae family.

As Pirzad et al. [2] specify, *Matricaria Chamomilla* L. is a herbaceous plant, native from south and east Europe but at present it has spread over nearly all the European continent. The plant can be also found in North Africa, Asia, north and South America and even in New Zealand. It is an annual and spontaneous, reaching a height between 20 and 60 cm, branched, having the flowers grouped in terminals capitule. The edge of each capitule is occupied by white flowers, and in its central region there are numerous golden-yellow tubular flowers. The capitule receptacle is plan at the early blooming and becomes tapered and hollow, fact which allows distinguishing the true chamomile flowers compared to those from related species which do not have therapeutic properties. The properly time for harvesting is when most inflorescences were marginal petals horizontally, because in this stage, the flowers have the highest essential oil content. Harvesting is done only after dew, on sunny, usually once a year, in May and June. Pharmacological properties includes: anti-inflammatory, antiseptic, carminative, healing, sedative and spasmolytic activity.

As Afsharypuor et al. [3] have mentioned *Achillea Millefolium* is also a perennial herbaceous plant, originary

from Europe. Now is commonly found growing wild in the British Isles, Asia, Australia and North America. The generic name of the plant, as Chatzopoulou et al. [4] have pointed out, comes from Achilles, the legendary hero of the Trojan War, who discovered the plant and used it to treat his soldiers' wounds. It is a tall plant up to 80 cm, with two kinds of aerial stems: some thicker, floriferous, branched at the top which ending with capitule made up of small flowers and others sterile wearing only leaves. The leaves are pinnate, more or less hairy, with aromatic odor and astringent taste. The flowers are grouped in ovoid capitule, united in corymb at the end of stem. Each capitule contains 5-6 marginal white flowers, sometimes pink to red. Harvesting is done from July to October, during dry weather, preferably at the midday, when has the maximum concentration of essential oils. Masu et al. [5] and Nadim et al. [6] have noted that *Achillea Millefolium* has been used in traditional medicine for hundreds of years by various cultures, internally as herbal teas for headaches, hepatobiliary disorders, gastrointestinal complaints and as an appetite enhancing drug and externally as lotions and ointments against skin inflammations, wounds, cuts and abrasions.

Depending of the period in which they are collected, of the geographical area in which they grow and many other factors of which Shahram Sharafzadeh [7] mentions the plant part used in extraction, the essential oils obtained from plants belonging to the same genre, shows common constituents in the basic composition, although in different proportions, but also constituents completely different. It was found that the presence or absence as well as larger or smaller percentage of one of the components, are aspects that make authors as Bakkali et al., Silva et al., Hajhashemi et al., and Perry et al. [1, 8-10] to state that essential oils exhibit particular medicinal properties that have been claimed to cure one or another organ dysfunction or systemic disorder.

The purpose of the present study is to analyze the chemical profile of the essential oils obtained from *Achillea*

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*Millefolium* and *Matricaria Chamomilla* L. harvested from Romania. The volatile oils have been obtained separately, by hydrodistillation of flowers of *Achillea Millefolium* and *Matricaria Chamomilla* L. The chemical compositions were investigated using the techniques of Gas Chromatography coupled with Mass Spectrometer (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). The results have been compared with those obtained in other specialty studies.

## Experimental part

### Materials

For the experiments were used fresh flowers of *Achillea Millefolium* and *Matricaria Chamomilla* L., harvested in August from hill area of Romania. Samples were collected at full flowering stage. All other used reagents were of analytical grade and were purchased from Sigma-Aldrich, Bucharest.

### The essential oil extraction

The essential oils of *Achillea Millefolium* and *Matricaria Chamomilla* L. were obtained separately, using the following technique: 200 g of fresh flowers were crushed and transferred in a round bottom flask of 2 L, over which were added 1.5 L of distilled water. The flask was connected to the Clevenger-type apparatus for distillation. Each mixture was hydrodistilled for 3h (*Matricaria Chamomilla* L.) and 4h (*Achillea Millefolium*) respectively, in order to obtain essential oils. The distillates obtained have been dried over anhydrous sodium sulphate and stored in air tight amber-colored bottles at 4°C until were analyzed.

### The analysis of the essential oil

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  $\times$  0.32 mm internal diameter, film thickness 0.25  $\mu$ m). Hydrogen (99.999% high purity) was used as the carrier gas (inlet pressure 82 737 Pa at a flow rate of  $1.6667 \times 10^{-8} \text{ m}^3 \text{ s}^{-1}$  (1 mL min<sup>-1</sup>) (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 with a rate of 5°C/min and finally was raised to 280°C with a rate of 8°C/min and maintained there for 20 min. For GC-MS analysis, 30  $\mu$ L of essential oil were homogenized with 1 mL of hexane. 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 2.8 scan/second, 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 program and to calculate the retention indices the generalized equation of Van den Dool and Kratz was used.

Kovats retention indices are calculated using the formula:

$$K = 100 \times \left[ n + \frac{\log t_{R_x} - \log t_{R_n}}{\log t_{R_{n+1}} - \log t_{R_n}} \right]$$

where:  $x$  is the test compound,  $n$  is the alkane with  $n$  carbon atoms into the molecule, whose peak is placed in the left side of the analyzed peak from the chromatogram, and  $n+1$  is the alkane with  $n$  carbon atoms into the molecule, whose peak is placed in right side of the analyzed peak from the chromatogram.

### Fourier Transform Infrared Spectroscopy (FTIR)

The IR fingerprint of each 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  $\text{cm}^{-1}$ , with a resolution of 4  $\text{cm}^{-1}$ .

## Results and discussions

The volatile oils obtained from both aromatic plants were blue and extremely clear. The hydrodistillation technique applied on fresh flowers of *Matricaria Chamomilla* L. led to the obtaining of 0.25% (v/w) essential oil, while applied on fresh flowers of *Achillea Millefolium* led to the obtaining of only 0.15% (v/w) essential oil. These oils were analyzed using the technique of GC-MS and the results are summarized in tables 1 and 2.

From the analysis of the essential oil obtained from *Achillea Millefolium*, using the mentioned technique, was identified a large number of monoterpenes but a small number of sesquiterpenes and aliphatic components. In the examined essential oil, from the total of 92.89% monoterpenes, the oxygenated monoterpenes represent 88.94%, while monoterpene hydrocarbons represent only 3.95%. The representative oxygenated monoterpenes were the following: Camphor (19.58%), Eucalyptol (19.58%), Borneol (12.18%), Thujone (10.2%), 1,5-Heptadien-4-ol, 3,3,6-trimethyl (8.07%), trans-2,7-Dimethyl-3,6-octadien-2-ol (7.44%) and Artemisia ketone (3.52%). Also, were found but in percentages between 1 and 2%, cis-p-Menth-2-en-7-ol (1.29%), lavandulol (1.95%), 4-Terpineol (1.2%) and Terpineol (1.49%). All the monoterpene hydrocarbons were under 1%, excepting the campherene (1.06%). From the total of 3.91% sesquiterpenes, 3.64% were sesquiterpenes hydrocarbons from which the most representative were  $\gamma$ -Himachalene (1.43%) and  $\gamma$ -Muurolene (1.27%). The only one oxygenated sesquiterpene found in the *Achillea Millefolium* essential oil was Spathulenol (0.27%). The aliphatic components were also modest represented (3.2%), only by the presence of Lavandulol Acetate (2.41%), trans-8-Isopropylbicyclo[4.3.0]non-3-ene (0.4%), trans-Chrysanthenyl Acetate (0.16%), Acetic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester (0.12%) and cis-Carvyl Acetate (0.1%).

The analysis of the volatile obtained from *Matricaria Chamomilla* L. using the mentioned technique, shows the dominance of terpene products, as in the case of *Achillea Millefolium*. The difference is that in this case was identified a large number of sesquiterpenes (91.65%) but a small number of monoterpenes (3.88%). Aliphatic components were also present (0.22%). From the total of 3.88% monoterpene, monoterpene hydrocarbons represent 0.24% and oxygenated monoterpenes represent 3.64%. Also, we can mention the Caprinic acid (2.8%) and limonene (0.23%), Artemisia ketone (0.44%) or Borneol (0.15%) even they were found in small amounts. Sesquiterpenes amounted to 91.65%. 7.48% of these were sesquiterpenes hydrocarbons and 84.17% were oxygenated sesquiterpene. From the first category of sesquiterpenes,  $\beta$ -Farasene is the most representative (6.47%). The second one was dominated by Bisabolol oxide A and B (70.2% and 6.21%

Name	Formula	RT (minutes)	RC (%)	K (x10 <sup>3</sup> )
Santolina triene	C <sub>10</sub> H <sub>16</sub>	6.443	0.494	0.632
trans-8-Isopropylbicyclo[4.3.0]non-3-ene	C <sub>12</sub> H <sub>20</sub>	7.257	0.407	0.656
Camphene	C <sub>10</sub> H <sub>16</sub>	7.790	1.065	0.670
α-pinene	C <sub>10</sub> H <sub>16</sub>	8.975	0.272	0.698
trans-2,7-Dimethyl-3,6-octadien-2-ol	C <sub>10</sub> H <sub>18</sub> O	10.392	7.444	1.036
Terpinolen	C <sub>10</sub> H <sub>16</sub>	10.855	0.165	1.047
o-Cymol	C <sub>10</sub> H <sub>14</sub>	11.234	0.748	1.057
Eucalyptol	C <sub>10</sub> H <sub>18</sub> O	11.472	19.581	1.062
Terpinolen	C <sub>10</sub> H <sub>16</sub>	12.861	0.288	1.093
Artemisia ketone	C <sub>10</sub> H <sub>16</sub> O	13.149	3.520	1.099
1,5-Heptadien-4-ol, 3,3,6-trimethyl	C <sub>10</sub> H <sub>18</sub> O	14.215	8.074	1.120
Thujone	C <sub>10</sub> H <sub>16</sub> O	15.014	9.999	1.500
Sabinol	C <sub>10</sub> H <sub>16</sub> O	15.533	0.672	1.514
Thujone	C <sub>10</sub> H <sub>16</sub> O	15.772	0.339	1.520
Camphor	C <sub>10</sub> H <sub>16</sub> O	16.740	19.583	1.543
Carveol	C <sub>10</sub> H <sub>16</sub> O	16.873	0.251	1.546
Borneol	C <sub>10</sub> H <sub>18</sub> O	17.764	12.183	1.566
cis-p-Menth-2-en-7-ol,	C <sub>10</sub> H <sub>18</sub> O	17.841	1.293	1.568
Lavandulol	C <sub>10</sub> H <sub>18</sub> O	18.044	1.956	1.572
4-Terpineol	C <sub>10</sub> H <sub>18</sub> O	18.262	1.204	1.577
1,5,5-Trimethyl-6-methylene-cyclohexene	C <sub>10</sub> H <sub>16</sub>	18.430	0.650	1.581
Terpineol	C <sub>10</sub> H <sub>18</sub> O	18.844	1.494	1.589
cis-Piperitol	C <sub>10</sub> H <sub>18</sub> O	19.531	0.161	1.942
Carveol	C <sub>10</sub> H <sub>16</sub> O	19.966	0.388	1.956
cis-p-Mentha-2,8-dien-1-ol	C <sub>10</sub> H <sub>16</sub> O	20.381	0.137	1.968
Isogeraniol	C <sub>10</sub> H <sub>18</sub> O	20.590	0.192	1.975
Carvone, (+)-	C <sub>10</sub> H <sub>14</sub> O	20.822	0.242	1.981
trans-Chrysanthenyl Acetate	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	21.502	0.155	2.001
Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	22.287	0.122	2.023
1,5,5-Trimethyl-6-methylene-cyclohexene	C <sub>10</sub> H <sub>16</sub>	22.442	0.272	2.027
Lavandulol, acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	22.617	2.405	2.032
Carvacrol	C <sub>10</sub> H <sub>14</sub> O	22.905	0.227	2.286
cis-Carvyl Acetate	C <sub>12</sub> H <sub>18</sub> O <sub>2</sub>	23.985	0.107	2.323
Cyperene	C <sub>15</sub> H <sub>24</sub>	27.751	0.254	2.653
Curcumene	C <sub>15</sub> H <sub>22</sub>	27.990	0.201	2.661
Spathulenol	C <sub>15</sub> H <sub>24</sub> O	30.318	0.271	2.921
γ-Himachalene	C <sub>15</sub> H <sub>24</sub>	30.437	1.437	2.926
β-Guaiene	C <sub>15</sub> H <sub>24</sub>	31.553	0.188	3.119
γ-Murolene	C <sub>15</sub> H <sub>24</sub>	31.679	1.279	3.125
β-Guaiene	C <sub>15</sub> H <sub>24</sub>	32.114	0.279	3.144

**Table 1**  
THE RETENTION TIME (RT), RELATIVE CONCENTRATION - PERCENTAGES OF AREA (RC) AND KOVATS RETENTION INDICES (K) OF *ACHILLEA MILLEFOLIUM* ESSENTIAL OIL

Name	Formula	RT (minutes)	RC (%)	K (x10 <sup>3</sup> )
Ethyl 2-methyl butyrate	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	4.565	0.099	0.391
5-Caranol	C <sub>10</sub> H <sub>18</sub> O	10.375	0.238	1.035
o-Cymol	C <sub>10</sub> H <sub>14</sub>	11.220	0.222	1.056
Limonene	C <sub>10</sub> H <sub>16</sub>	11.387	0.235	1.060
Artemisia ketone	C <sub>10</sub> H <sub>16</sub> O	13.139	0.445	1.099
Borneol	C <sub>10</sub> H <sub>18</sub> O	17.717	0.153	1.565
Caprylic acid	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	25.123	2.802	2.362
2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	C <sub>14</sub> H <sub>20</sub> O	25.217	0.270	2.365
β-Farnesene	C <sub>15</sub> H <sub>24</sub>	27.333	6.471	2.637
[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl)-amine	C <sub>28</sub> H <sub>34</sub> N <sub>2</sub> O	27.474	0.029	2.642
β-Cubebene	C <sub>15</sub> H <sub>24</sub>	27.855	0.117	2.656

**Table 2**  
THE RETENTION TIME (RT), RELATIVE CONCENTRATION - PERCENTAGES OF AREA (RC) AND KOVATS RETENTION INDICES (K) OF *MATRICARIA CHAMOMILLA* L ESSENTIAL OIL

Methyl 12,15-octadecadiynoate	C <sub>19</sub> H <sub>30</sub> O <sub>2</sub>	30.036	0.239	3.051
Spathulenol	C <sub>15</sub> H <sub>24</sub> O	30.291	1.375	3.063
γ-Himachalene	C <sub>15</sub> H <sub>24</sub>	30.441	0.348	3.069
cis-Lanceol	C <sub>15</sub> H <sub>24</sub> O	31.281	0.133	3.107
trans-Bergamotol, Z-α-trans	C <sub>15</sub> H <sub>24</sub> O	31.598	0.150	3.121
β-Guaiene	C <sub>15</sub> H <sub>24</sub>	31.818	0.546	3.131
α-Bisabolol oxide B	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	32.159	6.217	3.146
11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	C <sub>15</sub> H <sub>26</sub> O <sub>4</sub>	32.266	1.016	3.15
cis-Lanceol	C <sub>15</sub> H <sub>24</sub> O	32.808	5.071	3.173
3-[3(E)-Hydroxyiminomethyl-4-methoxyphenyl]-L-alanine	C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	32.932	0.098	3.179
Bisabolol oxide A	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	34.238	70.208	3.369
Myristic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	34.562	0.668	3.384
Crestomycin	C <sub>23</sub> H <sub>45</sub> N <sub>5</sub> O <sub>14</sub>	34.676	0.085	3.389
1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)-	C <sub>13</sub> H <sub>12</sub> O <sub>2</sub>	37.020	0.263	3.621
1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)-	C <sub>13</sub> H <sub>12</sub> O <sub>2</sub>	37.312	0.864	3.635
Tetracyclo[5.3.1.0(2,6).0(8,11)]undecan-4-ol, 6-methoxy-2-methyl	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	37.372	0.125	3.638
Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	38.490	1.025	3.805
Linoleic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	40.987	0.140	-
Linolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	41.069	0.132	-
Octacosane	C <sub>28</sub> H <sub>58</sub>	45.273	0.216	-

**Table 3**

THE COMPOSITIONS OF THE ESSENTIAL OILS OBTAINED FROM *ACHILLEA MILLEFOLIUM*. AND *MATRICARIA CHAMOMILLA* L. HARVESTED FROM ROMANIA

Class of compounds (%)	The composition of essential oil obtained from <i>Achillea Millefolium</i> (%)	The composition of essential oil obtained from <i>Matricaria Chamomilla</i> L. (%)
Monoterpene Hydrocarbons, MH	3.954	0.235
Oxygenated monoterpenes, OM	88.940	3.638
Sesquiterpenes hydrocarbons, SH	3.638	7.482
Oxygenated sesquiterpenes, OS	0.271	84.170
Aliphatic hydrocarbons, AH	0	0.216
Oxygenated aliphatic, OA	3.196	0
other compounds	0	4.259

respectively) and the remaining compounds were less than 2%. The aliphatic components were modest represented (0.22%), only by the presence of o-Cymol.

The results which were analyzed above, regarding both *Achillea Millefolium* and *Matricaria Chamomilla* L., are summarized in table 3 and show that even they belong to the same family (Asteraceae), the chemical compositions of the essential oils obtained from them are significantly different.

In order to correlate our results with those obtained in similar studies by others authors, we chose to compare them with those obtained using also the hidrodistillation

technique applied on plants harvested in countries from different geographic areas. Thus, we have selected for comparison, regarding *Achillea Millefolium* essential oil, the results reported by Nadim et al. [6] from India, Chatzopoulou et al. [4] from Greece, Mazandarani et al. [11] from Iran, Mockute et al. [12] from Lithuania and Falconieri et al. [13] from Italy and Portugal. The results are shown in table 4. The domination of monoterpenes in the essential oil of *Achillea Millefolium* was reported in all the above studies. Excepting of the results presented by Nadim et al. [6] where oxygenated monoterpenes and monoterpene hydrocarbons have comparable percentages

Class of compounds	Compounds percentages						
	Romania	India	Greece	Iran	Lithuania	Italy	Portugal
MH	3.95	36.57	18.00	27.50	43.70	31.30	23.00
OM	88.94	35.93	76.80	53.70	18.00	1.70	45.20
SH	3.64	2.56	0.70	2.10	15.20	21.80	6.80
OS	0.27	3.70	0.30	6.00	15.70	0	7.10
AH	0	0.30	0	0.60	0	0	0
OA	3.20	7.98	0	2.50	0	42.50	17.30
Chamazulene	0	5.28	0.8	2.30	4.10	1.60	0

Note: MH (monoterpene hydrocarbons), OM (oxygenated monoterpenes), SH (sesquiterpenes hydrocarbons), OS (oxygenated sesquiterpenes), AH (aliphatic hydrocarbons), OA (oxygenated aliphatic)

**Table 4**  
COMPOSITION OF THE ESSENTIAL OIL OBTAINED FROM FRESH FLOWERS OF *ACHILLEA MILLEFOLIUM* FROM DIFFERENT GEOGRAPHICAL AREAS AND ROMANIA

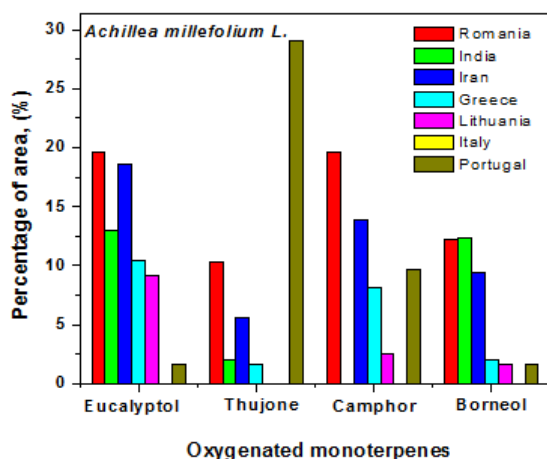


Fig. 1. The most representative classes of compounds from the category of oxygenated monoterpenes found in the essential oil of *Achillea millefolium* obtained from plants harvested from different geographical including Romania

(35.93 and 36.57% respectively), all other authors, Chatzopoulou et al. [4], Mazandarani et al. [11], Mockute et al. [12] and Falconieri et al. [13] respectively, have reported that percentages of oxygenated monoterpenes were much higher than those of monoterpene hydrocarbons (fig. 1).

The total percentage of monoterpenes in the Romanian essential oil (92.89%) was surpassed only by the percentage presented by Chatzopoulou et al. [4] (94.8%). There are eleven components (monoterpenes) in the Romanian essential oil which are completely absent in all others. Out of these, the following components were found in significant percentages: Eucalyptol (19.58%), 1,5-Heptadien-4-ol,3,3,6-trimethyl (8.07%) and Artemisia ketone (3.52%). We mention also, 1,5,5-Trimethyl-6-methylene-cyclohexene (0.9%), for its extremely useful properties in medicine. The percentage of Camphor (19.58%) was the best from all reported: Chatzopoulou et al. [4] have reported 8.1%, Mockute et al. [12] have reported 2.6%, Falconieri et al. [13] have reported 9.7% for Portuguese essential oil, Nadim et al. [6], Mazandarani et al. [11] and Falconieri et al. [13] for Italian essential oil have reported 0%. Some of the components present benefits connected only with flavor and fragrance (Eucalyptol, 1,5-Heptadien-4-ol,3,3,6-trimethyl, Camphor) but all of them present medicinal benefits. Thus, eucalyptol and camphor are well known cough suppressants, o-Cymol protect against intestinal, renal and lung infections, Artemisia ketone has a stronger free radical scavenging effect and a stronger antimicrobial activity than other well known monoterpenes, as Radulovic et al. [14] have mentioned and 1,5,5-Trimethyl-6-methylene-cyclohexene is a strong antibacterial and antineoplastic as Yermakov et al. [15] have mentioned also. The percentages of Borneol (12.18%) and Thujone (10.2%) were surpassed only by

Falconieri et al. [13] for the Portuguese essential oil (29%). Regarding the sesquiterpenes, excepting of the reports made by Nadim et al. [6], Chatzopoulou et al. [4], Mockute et al. [12] and Falconieri et al. [13] for the Portuguese essential oil which present comparable percentages between sesquiterpenes hydrocarbons (2.56, 0.7, 15.2 and 6.8% respectively) and oxygenated sesquiterpenes (3.7, 0.3, 15.7 and 7.1% respectively), Mazandarani et al. [11] and Falconieri et al. [13] for the Italian essential oil, have found some or others as being dominant. There are five types of sesquiterpenes found in the Romanian essential oil which were not found in the others:  $\gamma$ -Himachalene (1.43%),  $\gamma$ -Muuroleone (1.27%),  $\beta$ -Guaiene (0.46%), Cyperene (0.25%) and Curcumene (0.2%). All of them present medicinal benefits as follows: antibacterial, antiviral, antifungal, antimicrobial, analgesic, anti-inflammatory, anticancer effects and antioxidant activity ( $\gamma$ -Himachalene,  $\gamma$ -Muuroleone, Cyperene,  $\beta$ -Guaiene, Curcumene and .... phenolic and flavonoid compounds) [16-20].

Also, we mention that the compound called Spathulenol (well known for its antimicrobial effect as Bahramikia and Yazdanparast [21] have specified), was found only in the Romanian (0.27%) and Lithuanian (1%) essential oils. But it should be mentioned that are compounds in the Lithuanian [12] essential oil which are not found in any other essential oils under discussion so far, including the Romanian one: bourbobene (0.7%), muuroleone (0.2%), humulene epoxide (0.3%), cubenol (0.6%), selin-11-en-ol (1.5%) and 14-hydroxy-9-epi-carbophyllene (0.9%).

The aliphatic were represented in the Romanian essential oil only by oxygenated aliphatic (3.2%). Same results were also reported by Falconieri et al. [13] for Italian (42.5%) and Portuguese (17.3%) essential oils. Excepting of the report made by Falconieri et al. [13] regarding the Italian and Portuguese essential oils, in all others under discussion so far were found modest quantities of aliphatic. More than that, Chatzopoulou et al. [4] and Mockute et al. [12] have reported that they did not identify aliphatic at all. There are three oxygenated aliphatic in the Romanian essential oil which were not found in the others under discussion so far: trans-8-Isopropylbicyclo[4.3.0]non-3-ene (0.4%), Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester (0.12%) and cis-Carvyl Acetate (0.1%).

Chamazulene is an aromatic chemical compound, biosynthesized from sesquiterpene matricin, having anti-inflammatory properties as Rapetto and Liesuy [22] have mentioned. This compound was not found in Romanian and Portuguese Falconieri et al. [13] essential oils from Romania and Portugal, but was found in all others: 5.28% in the Indian as Nadim et al. [6] have mentioned, 0.8% in the Greek as Chatzopoulou et al. [4] have mention, 2.3% in the Iranian as Mazandarani et al. [11] have mention, 4.1% in the Lithuanian as Mockute et al. [12] have mention and 1.6% Falconieri et al. [13] have mentioned.

Class of compounds	Compounds percentages						
	Romania	Morocco	Nepal	Iran	Hungary	Poland	Serbia
MH	0.24	7.84	1.25	0.4	0	1.04	5.20
OM	3.64	17.73	0.41	0.13	0	1.99	0.40
SH	7.48	19.36	59.95	14.43	18.12	6.27	58.00
OS	84.17	6.13	31.96	75.54	56.47	65.43	23.50
AH	0.22	1.74	0	0.18	0	0	0
OA	0	2.90	0.08	0	0	0.1	0
Chamazulene (%)	0	25.21	1.18	2.18	9.31	15.58	5.6

Note: MH (monoterpene hydrocarbons), OM (oxygenated monoterpenes), SH (sesquiterpenes hydrocarbons), OS (oxygenated sesquiterpenes), AH (aliphatic hydrocarbons), OA (oxygenated aliphatic)

Table 5  
COMPOSITION OF THE ESSENTIAL OIL OBTAINED FROM FRESH FLOWERS OF *MATRICARIA CHAMOMILLA L.* FROM DIFFERENT GEOGRAPHICAL AREAS AND ROMANIA

Regarding *Matricaria Chamomilla* L. essential oil, we have selected for comparison the results reported by Hajjaj et al. [23] from Morocco, Heuskin et al. [24] from Nepal, Tolouee et al. [25] from Iran, Szoke et al. [26] from Hungary, Nurzynska-Wierdak [27] from Poland and Sokovic et al. [28] from Serbia. The results are shown in table 5.

In almost all studies that were taken into account for comparison, including ours, is distinguished the domination of the sesquiterpenes detrimental to the monoterpenes in the essential oils of *Matricaria Chamomilla* L. Thus in the Romanian essential oil the percent of sesquiterpenes was 91.95% while the percent of monoterpenes was 3.88%. Heuskin et al. [24] have reported 91.91% sesquiterpenes and 0.53% monoterpenes, Szoke et al. [26] have reported only a percent of 74.59% sesquiterpenes, Nurzynska-Wierdak [27] has reported 71.7% sesquiterpenes and 3.03% monoterpenes and Sokovic et al. [28] have reported 81.5% sesquiterpenes and 5.6% monoterpenes. Only in the report of Hajjaj et al. [23] the percentages of sesquiterpenes and monoterpenes were comparable, being 25.49% and 25.57% respectively. Almost all monoterpenes which were found in the Romanian essential oil of *Matricaria Chamomilla* L. (Borneol - 0.15%, Caprylic Acid - 2.8%, 5-Caranol - 0.23%) were not found in all others, excepting Limonene which was reported as being 0.23% by Heuskin et al. [24] and 0.2% by Sokovic et al. [28] respectively. Also, Artemisia ketone was reported only by Heuskin et al. [24] as being 0.32% and by Nurzynska-Wierdak [27] as being 1.38%. Because the Caprylic Acid is best represented, we mention here its benefits in cholesterol lowering, Crohn's Disease, fighting against bacterial and yeasts infections as Chan-Blanco et al. [29] have mentioned. We cannot finish the discussion about the monoterpenes from *Matricaria Chamomilla* L. essential oil, without notice that Hajjaj et al. [23] have reported high percentages of oxygenated monoterpenes which were not found in other oils in discussion (eucalyptol - 9.1%, camphor - 4.3%, thymol - 3.44%) and once again that Szoke et al. [26] have not reported monoterpenes at all. The total percentage of sesquiterpenes in the Romanian essential oil (91.65%) was surpassed only by the report of Heuskin et al. [24] (91.91%). There are five sesquiterpenes in the Romanian essential oil, both sesquiterpenes hydrocarbons and oxygenated sesquiterpenes, which are completely absent in all others (cis-Lanceol - 5.2%, 11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester- 1.02%,  $\beta$ -Guaiene- 0.55%,  $\gamma$ -Himachalene - 0.35% and trans-Bergamotol, Z- $\alpha$ -trans - 0.15%). From the oxygenated sesquiterpenes (fig. 2), stands out the percentage of Bisabolol oxide A (70.2%) found in the Romanian essential oil which is absent in the Moroccan essential oil but which was found in smaller percentages in all the others. In addition should mention here the presence of  $\beta$ -Farnesene, which was found in the Romanian essential oil in a percent of 64.7%, similar with those reported by Tolouee et al. [25] and Nurzynska-Wierdak [27].

Some of these compounds present benefits connected only with flavor or fragrance (cis-Lanceol, trans-Bergamotol, Z- $\alpha$ -trans) as Misra and Dey [30] have reported and others as  $\beta$ -Guaiene and  $\gamma$ -Himachalene present medicinal benefits as anti-oxidants as Thusoo et al. [19] and Shojaii and Fard [16] have mentioned. Also, Kamatou and Viloen [31] have specified that Bisabolol oxide A is an anti-irritant, anti-inflammatory, anti-microbial or enhancer of the absorption of certain molecules and Gauvin et al. [32] have noted that  $\beta$ -Farnesene is a precious vitamin precursor. We must observe that the Nepalese essential oil from *Matricaria Chamomilla* L. as Heuskin et

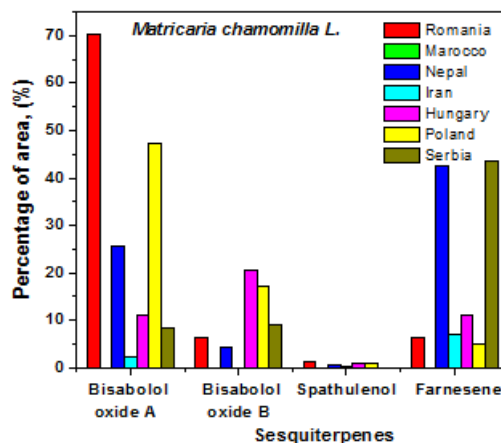


Fig.2. The most representative classes of compounds from the category of sesquiterpenes which were found in the essential oil of *Matricaria chamomilla* L. obtained from plants harvested from different geographical including Romania

al. [24] have reported contains a number of 24 sesquiterpens, while all others contain only 4 to 11 components. The conducted study indicates that the essential oil from *Matricaria Chamomilla* L. is poor in aliphatic components. The Romanian essential oil contains from this category only Octacosane (0.21%), the Moroccan essential oil only Pregnane (0.41%), Triacotane (1.33%) and Lucenin 2 (2.9%) the Iranian essential oil only Methyl Acetate (0.18%) while all other essential oils in discussion did not contain aliphatic at all.

Chamazulene, the aromatic chemical compound biosynthesized from sesquiterpene matricin, having anti-inflammatory properties as Rapetto and Liesuy [22] have mentioned, was not found in the essential oil from Romania but was found in all other under discussion (25.21% in the Moroccan, 1.18% in the Nepalese, 2.18% in the Iranian, 9.31% in the Hungarian, 15.58% in the Polish and 5.6% in the Serbian). We will finish the discussion about the composition of essential oil from *Matricaria Chamomilla* L. with the remark that only the Romanian and the Moroccan essential oils contains Fatty acid in percentages of 1.97 and 1.46% respectively.

As can be seen, there are similarities but also differences between the Romanian essential oil composition of *Achillea Millefolium* and *Matricaria Chamomilla* L. and those previously reported by authors from different countries. Similarities consist in the domination of the same class of compound (monoterpenes in the case of *Achillea Millefolium* and sesquiterpenes in the case of *Matricaria Chamomilla* L.) and in the same modest representation of aliphatic as all reported works in discussion have showed. Some of the differences consist in the presence of some compounds with significant medicinal role which were not reported by other works in discussion (Eucalyptol, 1,5-Heptadien-4-ol, 3,3,6-trimethyl, Artemisia ketone, 1,5,5-Trimethyl-6-methylene-cyclohexene,  $\gamma$ -Himachalene,  $\gamma$ -Muurolene,  $\beta$ -Guaiene, Cyperene and Curcumene - in the essential oil of *Achillea Millefolium* and Borneol, Caprylic acid,  $\gamma$ -Himachalene,  $\beta$ -Guaiene,  $\gamma$ -Muurolene, cis-Lanceol, trans-Bergamotol, Z- $\alpha$  and Octacosane - in the essential oil of *Matricaria Chamomilla* L.) and in the complete absence of others. Another difference consist in the fact that some compounds were found in higher concentrations than those reported in the works in discussion (Camphor, Thujone and Borneol - in the essential oil of *Achillea Millefolium* and Bisabolol oxide A, Spathulenol, Limonene, and  $\beta$ -Farnesene - in the essential oil of *Matricaria Chamomilla* L.). Also, we have to point out that the

Romanian essential oil does not contain Chamazulene at all.

All those similarities and differences might be attributed to environmental conditions, geographic appurtenance, climatic, genetic, chemotypes, plant age, the soil purity, phase of vegetation, anatomical part of plant and harvesting season as Mazandarani et al. [11] have mentioned.

#### Fourier Transform Infrared Spectroscopy (FTIR)

Similar absorption bands in FTIR spectra of *Achillea Millefolium* and *Matricaria Chamomilla L.* were identified. table 6 and figures 3 - 4 shows the infrared spectra and the characteristic bands observed in the range of 4000-600  $\text{cm}^{-1}$ .

The broad band centered between 3450-3350  $\text{cm}^{-1}$  (3447 and 3441  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively) correspond to O-H stretching of hydroxyl groups (alcohols, phenols and carboxylic acids) as Szymczycha-Madeja et al. have described [33]. They also have mentioned that the peaks between 2920 and 2850  $\text{cm}^{-1}$  were due to C-H vibration (stretch) of aliphatic groups. Al-Sheibany [34] has specified that the characteristic bands of the saturated aliphatic  $\text{sp}^3$  C-H bonds are observed in the range of 2959-2855  $\text{cm}^{-1}$  which are assigned to  $\nu$  ( $\text{CH}_3$ ),  $\nu$  ( $\text{CH}_2$ ), and  $\nu$  ( $\text{CH}_2$ ) respectively (the bands situated at 2927.8  $\text{cm}^{-1}$ , 2924.67  $\text{cm}^{-1}$ , 2874.07  $\text{cm}^{-1}$  and 2855.2  $\text{cm}^{-1}$  correspond to methylene C-H asymmetric and symmetric stretching vibrations respectively, and the band situated at 2956.69  $\text{cm}^{-1}$  is due to methyl C-H asymmetric and symmetric stretching vibrations).

Szymczycha-Madeja et al. [33], Al-Sheibany [34] and Li at al. [35] have mentioned that the band at 1750-1710  $\text{cm}^{-1}$  was assigned to the C=O vibration of bonded conjugated

ketones, aldehydes and esters (1742.58 and 1712.55  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively). The band in the range of 1630-1685  $\text{cm}^{-1}$  (1682.12 and 1633.74  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively) was attributed by Szymczycha-Madeja et al. [33] to the C=C vibration of aromatic structures, C=O stretching of carboxylic acids and the bands at 1570  $\text{cm}^{-1}$  and 1595.11  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively) are assigned by Li at al. [35] to the aromatic ring C=C skeleton vibration of an aromatic substance. In addition, the bands observed between 1450-1370  $\text{cm}^{-1}$  can be attributed to C-H groups or OH bend (phenol or tertiary alcohol). The band at 1450  $\text{cm}^{-1}$  is very characteristic also for an alcohol C-OH within the bending vibration absorption as also Li at al. [35] have mentioned.

A band around 1320  $\text{cm}^{-1}$  was attributed to the C=C stretch vibration of aromatic compound. The band around 1300  $\text{cm}^{-1}$  (1306.33 and 1299.16  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively) is attributed by Li at al. [35] to the  $\text{CH}_2$  alkanes that face the swing and the aromatic ring =C-H for the in-plane bending absorption and the band at 1230  $\text{cm}^{-1}$  (1237.84 and 1228.03  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively) 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. The bands located in the range of 1160-1030  $\text{cm}^{-1}$  (1164.91-1020.21 and 1176.84-1028.48  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla L.*, respectively) can be attributed according to Szymczycha-Madeja et al. [33] and Li at al. [35] to C-O stretch (primary, secondary or tertiary alcohol) or C-O-C stretch (alkyl-substituted ether).

Table 6

THE INFRARED CHARACTERISTIC BANDS, OBSERVED IN *ACHILLEA MILLEFOLIUM* AND *MATRICARIA CHAMOMILLA L.* ESSENTIAL OILS

Band position ( $\text{cm}^{-1}$ )		Class of Compounds
<i>Achillea Millefolium</i>	<i>Matricaria Chamomilla L.</i>	
3446.87	3440.25	O-H stretching vibrations of hydroxyl groups (alcohols, phenols and carboxylic acids)
2959.61	-	— $\text{CH}_3$ asymmetric and symmetric stretching vibrations
2927.89 and 2874.07	2924.67 and 2855.21	— $\text{CH}_2$ —, symmetric and symmetric stretching vibrations
1742.58	1712.55	C=O stretching vibration in non-conjugated ketones, aldehydes and esters
1682.12	1633.74	C=C bond stretching vibration and C=O stretching vibration of carboxylic acids
	1595.11	C=C stretching vibration from aromatic ring
1452.98-1375.79	1451.79-1375.29	C-H asymmetric and symmetric bend and OH bend (phenol or tertiary alcohol)
1323.52	-	C=C stretching vibration of aromatic ring
1306.33	1299.16	$\text{CH}_2$ =C-H alkanes bending vibration
1237.84	1228.03	C-O-C stretching bands (aromatic acid ester), C-OH (from phenols) stretching vibrations
1164.91-1020.21	1176.84-1028.48	C-O stretching vibration (primary, secondary and tertiary alcohol) or C-O-C stretching bends (alkyl-substituted ether),
984.64	978.43	— $\text{CH}_2$ — (methylene - cyclohexane ring vibrations), C-H in-plane bend (from aromatics)
900-800 (910.83; 842.48; 808.19)	900-800 (915.91; 858.43; 819.21)	=C-H out of plane bending vibration from aromatics
850-700 (785.31; 749.81)	850-700 (760.02; 722.66)	C-H out of plane bending vibration from aromatics, ( $-\text{CH}_2-$ ) <sub>n</sub> rocking ( $n \geq 3$ ); skeletal C—C vibrations

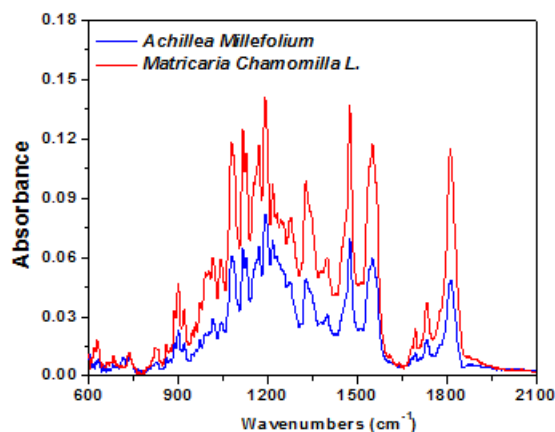


Fig. 3. FTIR spectra of *Achillea millefolium* L. and *Matricaria chamomilla* L. essential oils in the range of 600-2100  $\text{cm}^{-1}$

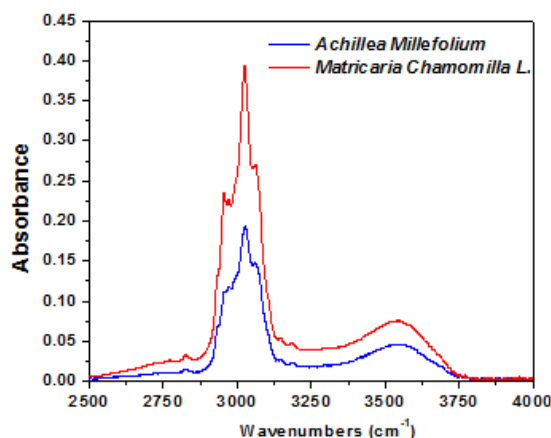


Fig.4. FTIR spectra of *Achillea millefolium* and *Matricaria chamomilla* L. essential oils in the range of 2500-4000  $\text{cm}^{-1}$

The bands located around 980  $\text{cm}^{-1}$  (984.64 and 978.43  $\text{cm}^{-1}$  for *Achillea millefolium* and *Matricaria chamomilla* L., respectively) can be attributed to  $-\text{CH}_2-$  (methylene - cyclohexane ring vibrations) or C-H in-plane bend (aromatic) as Li at al. [35] have mentioned. The bands in the range of 900-800  $\text{cm}^{-1}$  represent according to Al-Sheibany [34] and Heredia-Guerrero et al. [36] the out of plane bending vibration from aromatics,  $=\text{C}-\text{H}$  and at 800-750  $\text{cm}^{-1}$  out-of-plane due to the aromatic C-H bending, methylene  $(\text{CH}_2)_n$  rocking (n3) or skeletal C-C vibrations.

## Conclusions

Two kinds of essential oil obtained by hidrodistillation from fresh flowers of *Achillea Millefolium* and *Matricaria Chamomilla* L. respectively, harvested from hill area of Romania, were obtained and analyzed using the techniques of GC-MS and FTIR. Even though both genres mentioned above belong to the Asteraceae family, the chemical compositions of the essential oils obtained from them are significantly different.

From the analysis of the essential oil obtained from *Achillea Millefolium* was identified a large number of monoterpenes (92.89%) but a small number of sesquiterpenes (3.9%) and aliphatic components (3.19%). The analysis of the volatile obtained from *Matricaria Chamomilla* L. shows the dominance of terpene products, as in the case of *Achillea Millefolium*. The difference is that was identified a large number of sesquiterpenes (91.65%) but a small number of monoterpenes (3.88%). Aliphatic components were also present but in a small amount (0.22%). In order to correlate our results with those obtained

in similar studies by other authors, we chose to compare them with those obtained using the same technique from plants harvested in countries from different geographic areas.

We have highlighted the similarities (the domination of the same class of compounds or the modest representation of others) and the differences (the presence of some compounds with significant medicinal role which were not reported by the other works in discussion or in higher concentrations than those reported) that can be attributed to environmental conditions, geographic appurtenance, climatic, genetic, chemotypes, plant age, the soil purity, phase of vegetation, anatomical part of plant and harvesting season.

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