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*RESEARCH ARTICLE*

# Comparison of the *Alchemilla* L. Samples from Turkish Herbal Market with the European Pharmacopoeia 8.0

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***Comparison of the Alchemilla L. Samples from Turkish Herbal Market with the European Pharmacopoeia 8.0***

***SUMMARY***

*The aims of this study are; to investigate the morphological properties of Alchemilla samples which are purchased from Turkish herbal market and to examine the suitability to the pharmacopoeia, according to the monograph of A. vulgaris which is registered as official species in European Pharmacopoeia. 13 samples were collected from different herbal market in Turkey, afterwards morphological analyses were carried out. Foreign matter and loss on drying analyses, total ash quantities and TLC analyses were performed according to the monograph in European Pharmacopoeia 8.0. Zones which were obtained with the chlorogenic acid and cafeic acid solutions compared with the zones of sample extracts in the TLC chromatograms. It was determined that the samples examined belong to different species from A. vulgaris. Also volatile organic compounds of the samples were determined by a solid phase micro extraction (SPME) method coupled with gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Aldehydes were found as the major group of compounds in all of the samples in the range of 49.7% - 93.4% ratio. The major volatile constituents of the samples were found to be hexanal (in the range of 15.2%-29.0%) excluding sample 9 of which the major compound was furfural (18.8%). It was determined that none of the investigated samples meet the requirements of the European Pharmacopoeia 8.0 monograph.*

***Key words:*** *Alchemilla, GC-MS, monograph, pharmacopoeia, Rosaceae, herbal market*

***Türkiye'deki Aktarlardan Alınan Alchemilla L. Örneklerinin Avrupa Farmakopesi 8.0 ile Karşılaştırılması***

***ÖZET***

*Bu çalışmada Türkiye'deki aktarlardan alınan Alchemilla numunelerinin morfolojik özelliklerini araştırmak ve farmakopeye uygunluklarını Avrupa Farmakopesi'nde ofisinal tür olarak kayıtlı olan A. vulgaris monografı ile kıyaslayarak incelemek amaçlanmıştır. Türkiye'deki değişik aktarlardan 13 örnek alınmış, sonrasında morfolojik analizler yapılmış, Avrupa Farmakopesi 8.0 monografına göre yabancı madde, kurutmada kayıp, kül miktar tayini ve İTK analizleri gerçekleştirilmiştir. İTK kromatogramında klorojenik asit ve kafeik asit çözeltileriyle elde edilen zonlar numune ekstrelerine ait zonlarla karşılaştırılmıştır. İncelenen numunelerin A. vulgaris'ten farklı türlere ait olduğu belirlenmiştir. Ayrıca numunelerin uçucu organik bileşikleri, katı faz mikroekstraksiyon yöntemi ve devamında gerçekleştirilen gaz kromatografisi kütle spektroskopisi analiziyle belirlenmiştir. Aldehitlerin tüm örneklerdeki major bileşik grubu olduğu (%49.7' den %93.4'e değişen oranlarda) belirlenmiştir. Hexanal %15.2 ve %29.0 arasında değişen oranlarla, 9 numaralı örnek hariç tüm örneklerde ana bileşik olarak bulunmuştur ve 9 numaralı örnekte en fazla furfural (%18.8) bileşiği olduğu tespit edilmiştir. İncelenen numunelerin hiçbirinin Avrupa Farmakopesi 8.0 monografında belirtilen gereksinimleri karşılamadığı tespit edilmiştir.*

***Anahtar kelimeler:*** *Alchemilla, GK-KS, monograf, farmakope, Rosaceae, aktar*

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## INTRODUCTION

*Alchemilla* L. genus (Rosaceae) is represented by more than 1000 species all around the World and 77 species in Turkey (Pawlowski and Walters, 1972; Hayırlıoğlu and Beyazoğlu, 2002; Hayırlıoğlu and İnceer, 2009). *Alchemilla* species have been used against acute diarrhoea, dysmenorrhoea and menorrhagia in Bulgarian folk medicine, as well as they have been used as antiinflammatory and wound healer in Sweden (Tunon et al., 1995). Aerial parts of some *Alchemilla* species are used as diuretic, constipant, tonic, emmenagogue, menstrual regulator, wound healer and for bronchitis, rheumatoid arthritis and menstrual pain in Turkish folk medicine (Altındağ and Öztürk, 2011; Kaval et al., 2014; Güzel et al., 2015).

*A. vulgaris* which is known as lady's mantle, described with a monograph in European Pharmacopoeia (Özüdoğru et al., 2011; Council of Europe, 2013). Its oral use in mild and nonspecific diarrhea and gastrointestinal disorders is also approved by Commission E (Gruenwald et al., 2000). *Alchemilla* species are rich in flavonoids, phenolic acids and tannins which are proved to be responsible for the some of the pharmacologic activities (Akkol et al., 2015; Fraisse et al., 2000; Trendafilova et al., 2012). Quercetin and kaempferol glycosides were reported to be characteristic for *Alchemilla* species up to date (Lamaison et al., 1991; Trendafilova et al., 2012).

The aims of this study were: to investigate the morphological characters of the samples supplied from herbal market, to analyse the samples according to European Pharmacopoeia 8.0 Monograph, to compare the results with *A. vulgaris* sample, and to evaluate the volatile compounds of the samples with SPME-GC-FID/MS.

## MATERIALS AND METHODS

### Plant material of *Alchemilla* species

13 Samples of *Alchemilla* were obtained from different herbal market in May to September, 2015 (Table 1) mainly from Northern parts of Turkey because of the distribution of the species in Turkish flora. Since the reference sample, *A. vulgaris* was not a native species for Turkey, it was purchased from USA (UPC: 767963112460). All samples were received as dried raw material.

### Morphological analysis of *Alchemilla* species

Dried aerial parts (flowers and leaves) of samples were photographed. Leaves and flowers in different sizes were chosen to make a detailed morphological comparison. Morphological characters such as shapes and dimensions of the lamina, petioles and flowers; hair properties of leaves and hypanthium were examined.

### Foreign matter of *Alchemilla* species

10 g of each sample were weighed and spread on a thin layer. Impurities examined with naked eye were collected, then weighed and the percentages of foreign matters were calculated according to European Pharmacopoeia 8.0 (Council of Europe, 2013).

### Loss on drying of *Alchemilla* species

5 g of each sample were weighed and placed in bottles that were previously dried. Samples were dried at 105 °C for 2 hours to constant mass. Percentages of weight losses were calculated accrording to European Pharmacopoeia 8.0 (Council of Europe, 2013).

### Total ash of *Alchemilla* species

A platin crucible was heated to redness for 30 minutes, then cooled in a desiccator and weighed. 1 gram of each sample were burned at 600 °C and allowed to cool in a desiccator. Then, weighed and the percentage of ash content was calculated (Council of Europe, 2013).

### Thin layer chromatography (TLC) analysis of the *Alchemilla* extracts

TLC analysis were performed according to the "*Alchemilla* herba" monograph in the European Pharmacopoeia 8.0 (Council of Europe, 2013). Caffeic acid and chlorogenic acid were used as references. Development was made on silica gel TLC plates (Merck,

**Table 1.** Location of the market samples of *Alchemilla* species*.

| No | City | No | City |
|---|---|---|---|
| 1 | Ankara | 7 | Samsun |
| 2 | Ankara | 8 | Mersin |
| 3 | Ankara | 9 | Mersin |
| 4 | Trabzon | 10 | Balıkesir |
| 5 | Trabzon | 11 | İstanbul |
| 6 | Trabzon | 12 | İstanbul |
| 7 | Samsun | 13 | Ordu |

* Reference sample (No:14) were purchased from USA

Silicagel 60$F_{254}$). A mixture of anhydrous formic acid, water, ethyl acetate (8:8:84) was used as mobile phase. Detection was made with spraying solution of diphenylboric acid aminoethyl ester in methanol, then subsequently spraying solution of macrogol 400 in methanol and by examining the plate in UV 365 nm.

**SPME analysis of *Alchemilla* species**

All samples were separately analyzed by a SPME device (Supelco, USA). Each sample (1.00 g, each) was grounded and placed in a 10 mL vial sealed with a silicone-rubber septum cap. GC-FID and GC-MS analyses were done as described previously (Yaylı et al., 2014). The volatile compounds were identified by comparison of their retention indices (relative to $C_7$-$C_{30}$ alkane standards) with the mass spectra of the two libraries (FFNSC1.2 and W9N11) and also confirmed by comparing the retention indices with the data published in the literature (Adams, 2001).

## RESULTS

**Morphological properties of *Alchemilla* species**

*Alchemilla* species have palmately lobed leaves and inflorescences of these species form a compound cyme, epicalyx present while petals are absent. None of the leaves of examined samples were divided to the base. The number of the leaf lobes with the angle called sinus which occurs between the basal lobes of the leaves, are the important characteristics in determination of *Alchemilla* species. Consequently, the number of lobes and the dimensions of leaves were compared within the species (Table 2). Also the incision developed between the lobes was observed at all of the samples. Pictures of the flowers of were given in Figure 1.

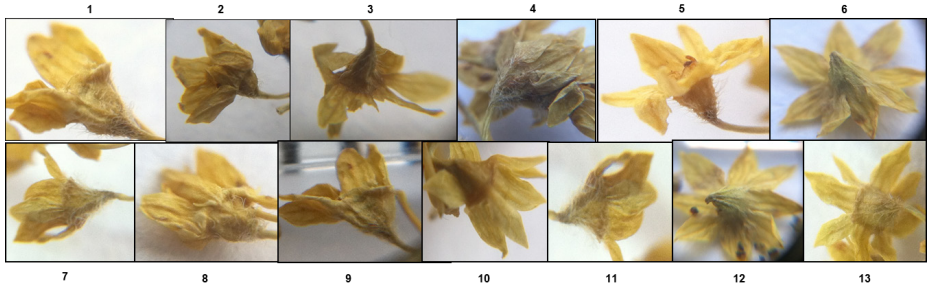


**Figure 1.** Flowers of *Alchemilla* samples.

1-13: The number of *Alchemilla* species as 1-13 explained in Table 1.

**Table 2.** Comparison of the morphological properties of leaves belong to samples of *Alchemilla* species.

| No | Hairs on stem | Hairs on petioles | Hairs on leaves | Sinus | Length of leaves (cm) | The number of the lobes |
|---|---|---|---|---|---|---|
| 1 | Dense patent | Pubescent | Both of the faces dense hairy | Closed | 4-4.5 | 10-11 |
| 2 | Patent | Pubescent | Lower surface dense hairy upper surface glabrous | Closed | 9-10 | 7-8 |
| 3 | Patent | Pubescent | Lower surface dense hairy upper surface glabrous | Closed | 10 | 9-10 |
| 4 | Pubescent | Pubescent | Lower surface dense hairy upper surface glabrous | Wide | 3.5-4.5 | 8-9 |
| 5 | Pubescent | Pubescent | Lower surface dense hairy upper surface pubescent | Closed | 7-9 | 9-10 |
| 6 | Dense hairy | Dense hairy | Lower surface dense hairy upper surface sparsely hairy | Narrow | 6.5-7 | 9-11 |
| 7 | Pubescent | Pubescent | Lower surface dense hairy upper surface glabrous | Wide | 9-10 | 11-9 |
| 8 | Sparsely hairy | Pubescent | Lower surface hairy upper surface dense hairy | Wide | 8.7-11.1 | 11-10 |
| 9 | Sparsely hairy | Pubescent | Lower surface hairy upper surface sparsely hairy | Wide | 6-8 | 8-9 |
| 10 | Dense patent | Dense hairy | Lower surface sparsely hairy upper surface glabrous | Closed | 9,1-10 | 10-11 |
| 11 | Dense hairy | Dense hairy | Both of the faces pubescent | Closed | 8.1-7.9 | 10-11 |
| 12 | Pubescent | Pubescent | Lower surface dense hairy upper surface sparsely hairy | Wide | 7.3-7 | 8-9 |
| 13 | Pubescent | Pubescent | Lower surface dense hairy upper surface pubescent | Wide | 7.5-8.1 | 8-9 |

Dimensions of hypanthium and sepal with hair plays an important role in distinguishing species (Table 3). All of the sepals of the examined samples were glabrous. Morphological characters of the samples were compared with monographs of *Alchemilla* species (Pawlowski and Walters, 1972; Hayırlıoğlu and Beyazoğlu, 2002; Hayırlıoğlu and İnceer, 2009). The description of the reference sample was confirmed from the Flora of North America and European Pharmacopoeia 8.0 monograph (Torrey and Gray, 1838; Council of Europe, 2013).

**Table 3.** Morphological properties of the flowers belong to samples of *Alchemilla* species.

| No | Length of flowers (mm) | Pedicel | Hypanthium | Length of sepals (LS) and length of hypanthium (LH) |
|---|---|---|---|---|
| 1 | 2.4-2.6 | Glabrous | Dense hairy | LS > LH |
| 2 | 4-4.5 | Glabrous | Sparsely hairy | LS > LH |
| 3 | 4.2-4.5 | Glabrous | Sparsely hairy | LS > LH |
| 4 | 3-3.5 | Sparsely hairy | Hairy | Almost equal |
| 5 | 3.6-5 | Patent | Hairy | LS > LH |
| 6 | 3.5-5 | Erecto patent | Sparsely hairy | LS > LH |
| 7 | 3.5-4.4 | Glabrous | Patent | LS > LH |
| 8 | 3.5-5.2 | Glabrous | Hairy | LS > LH |
| 9 | 3-3.5 | Glabrous | Dense hairy | Almost equal |
| 10 | 4.1-4.3 | Glabrous | Hairy | Equal |
| 11 | 3.5-3.7 | Sparsely hairy | Hairy | Equal |
| 12 | 2.4-2.5 | Glabrous | Sparsely hairy | Equal |
| 13 | 3.5-3,7 | Dense hairy | Sparsely hairy | LS > LH |

**Foreign matter of *Alchemilla* species**

In the "Herbal drugs" monograph of the European Pharmacopoeia 8.0, content of foreign matter is stated to be not more than 2 per cent m/m unless otherwise prescribed or justified and authorized (Council of Europe, 2013). There isn't any specification in "*Alchemilla* herba" monograph in the European Pharmacopoeia 8.0. But, in "*Alchemilla* herba" monograph in the European Pharmacopoeia 5.0, a maximum of 2% is allowed (Council of Europe, 2005; Council of Europe, 2013). Percentages of foreign matter were calculated (Table 4). It was detected that the reference sample does not contain any foreign matter. Amounts of foreign matters in samples 1, 3, 5, 6, 7, 8, and 12 were found to be above the specified value in pharmacopoeia. Especially sample 6 was found beyond the criteria with the foreign matter value of 11.90%. The lowest amount of foreign matter was found to belong to the sample 4 with the ratio of 0.09% (Table 4).

**Loss on drying of *Alchemilla* species**

In the "*Alchemilla* herba" monograph, loss of weight after drying is stated to be max 10% (Council of Europe, 2013). In the loss of weight determinations, the highest amount of weight loss belongs to sample 13 (13.70%) and the lowest amount to weight loss belongs to sample 10 (10.14%) (Table 4). None of the samples except for the reference example meet the max 10.0% limit specified in the pharmacopoeia (Table 4).

**Total ash of *Alchemilla* species**

In the "*Alchemilla* herba" monograph, total ash is stated to be max 12% (Council of Europe, 2013). The highest amount of ash belongs to sample 8 (7.84%) and the lowest sample 1 (3.95%). All samples are found to be below the specified limit (Table 4).

**Table 4.** Results of Pharmacopoeia analysis of the market samples.

| No | Foreign Matter (%) | Loss on Drying (%)* | Total Ash (%)* |
|---|---|---|---|
| 1 | 2.97 | 11.99±0.21 | 3.95±0.50 |
| 2 | 1.35 | 11.28±0.18 | 4.81±0.04 |
| 3 | 3.02 | 10.38±0.15 | 4.50±0.32 |
| 4 | 0.09 | 11.29±0.02 | 4.71±0.24 |
| 5 | 3.71 | 11.30±0.60 | 5.29±0.40 |
| 6 | 11.90 | 10.95±0.00 | 7.01±0.18 |
| 7 | 4.78 | 11.97±0.05 | 6.66±0.45 |
| 8 | 8.78 | 11.76±0.18 | 7.84±0.09 |
| 9 | 1.53 | 10.33±0.03 | 6.94±0.48 |
| 10 | 1.69 | 10.14±0.12 | 6.46±0.60 |
| 11 | 1.62 | 13.84±0.50 | 6.34±0.13 |
| 12 | 2.46 | 13.81±0.24 | 6.07±0.01 |
| 13 | 1.69 | 13.70±0.10 | 6.44±0.68 |
| Ref | 0 | 9.38±0.01 | 4.69±0.08 |
| European Pharmacopeia Criterion | ≤2 | ≤10 | ≤12 |

* Experiments were done in triplicate and results were expresses as mean ±S.D.

**Statistical Analysis**

The entire pharmacopoeia analysis were done in triplicates. The mean of three results were calculated, standart deviation and standart error were given in table 4.

**TLC analysis of *Alchemilla* species**

Extracts, reference sample and pure substances were subjected to TLC analysis in the conditions specified in the pharmacopoeia. Chlorogenic acid standart showed a light blue fluorescent zone below the half middle part of the TLC plate and caffeic acid showed a light blue fluorescent zone at the top of the TLC plate. In our samples, only reference sample showed both of the zones at same $R_f$ values. The zone of chlorogenic acid was not seen at any of the market samples. A non-intense light blue zone was seen at all of the market samples at the same $R_f$ value (Figure 2). "1 or 2 intense green or greenish yellow fluorescent zones" at the above half part of the plate and "one or several intense green or greenish yellow fluorescent zones" at the middle of the plate as described in the Pharmacopoeia are also present in the chromatogram.

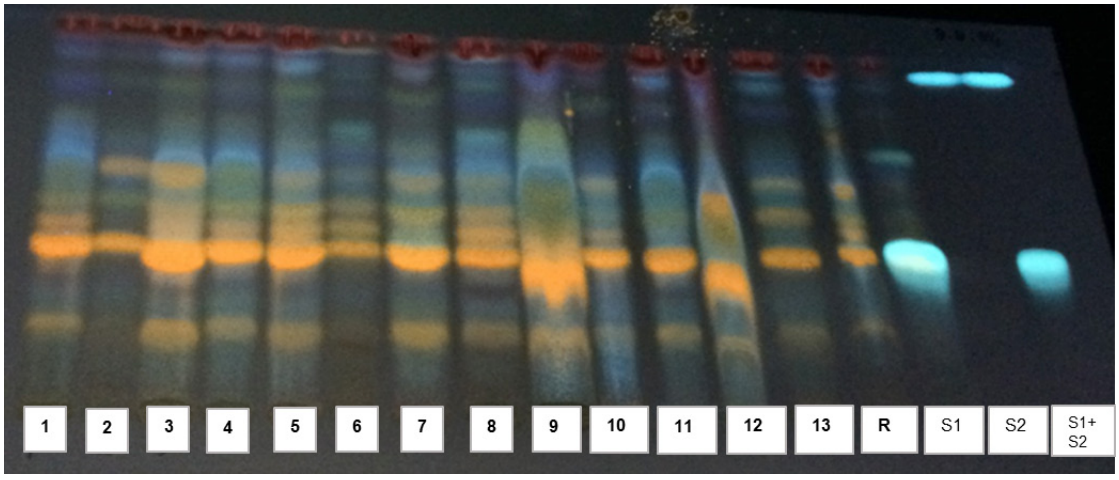


**Figure 2.** Chromatograms of the extracts prepared from market samples of *Alchemilla* species, reference sample and standarts.

**Table 5.** Identified volatile organic compounds of *Alchemilla* species.

| Compound | Ref. | Lit. RI | RI[p] | RT | 1 (%)[r] | 2 (%)[r] | 3 (%)[r] | 4 (%)[r] | 5 (%)[r] | 6 (%)[r] | 7 (%)[r] | 8 (%)[r] | 9 (%)[r] | 10 (%)[r] | 11 (%)[r] | 12 (%)[r] | 13 (%)[r] | 14 (%)[r] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Monoterpene hydrocarbons | | | | | | | | | | | | | | | | | | |
| *p*-Cymene | a | 1025 | 1026 | 16.796 | 1.5 | - | - | - | - | - | - | 0.4 | 2.6 | 0.5 | - | - | - | 3.6 |
| Limonene | b | 1030 | 1031 | 16.982 | 2.3 | - | - | 1.6 | 4.4 | 5.6 | 3.1 | 2.5 | 2.5 | 2.3 | 0.6 | 0.5 | - | 12.1 |
| Allo-ocimene | c | 1128 | 1130 | 21.709 | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Oxygenated monoterpenes | | | | | | | | | | | | | | | | | | |
| *Z*-Linalool oxide | b | 1074 | 1073 | 18.838 | 0.8 | - | 1.4 | 0.3 | 0.1 | - | 0.1 | 0.1 | 0.1 | - | 0.6 | 0.1 | - | 1.2 |
| *E*-Linalool oxide | b | 1088 | 1089 | 19.534 | - | - | 0.2 | 0.5 | 0.1 | - | 0.5 | - | 0.1 | 0.4 | 0.1 | 0.3 | 0.2 | 0.2 |
| Linalool | c | 1098 | 1098 | 19.874 | 1.6 | 1.8 | 5.6 | - | 2.9 | 1.0 | - | 2.2 | 0.7 | 1.4 | 0.1 | - | 2.3 | 10.1 |
| Terpinen-4-ol | d | 1179 | 1180 | 23.444 | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.4 |
| α-Terpineol | d | 1192 | 1193 | 23.995 | 1.7 | 1.9 | 2.8 | - | 2.6 | 4.0 | 2.8 | - | - | 1.0 | 2.3 | - | 1.8 | 4.5 |
| *Z*-Isopulegone | c | 1233 | 1233 | 26.122 | 1.3 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Carvone | d | 1246 | 1247 | 26.268 | 3.0 | 1.1 | - | - | 0.4 | 0.1 | - | 0.3 | - | - | - | - | - | 1.9 |
| Anethole | e | 1283 | 1281 | 27.930 | - | - | - | - | 4.0 | 3.4 | - | 4.7 | - | - | - | - | - | - |
| Thymol | c | 1290 | 1290 | 28.067 | 1.3 | - | - | 0.2 | 0.1 | - | - | 1.5 | 0.3 | - | 0.6 | - | - | - |
| Carvacrol | f | 1299 | 1300 | 28.493 | - | 0.1 | - | - | - | 0.1 | - | - | 0.1 | 0.1 | - | - | - | - |
| α-Terpinyl acetate | g | 1350 | 1350 | 30.488 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.6 |
| Sesquiterpene hydrocarbons | | | | | | | | | | | | | | | | | | |
| α-Copaene | b | 1370 | 1371 | 31.724 | - | - | - | - | 0.2 | - | - | - | - | - | - | - | - | 0.9 |
| *E*-Caryophyllene | h | 1418 | 1417 | 33.546 | 1.7 | 2.0 | 0.5 | - | 6.2 | 4.0 | 5.2 | 2.7 | 0.7 | 2.0 | 2.5 | - | 1.6 | 1.7 |
| α-(E,E)-Farnesene | b | 1508 | 1504 | 36.486 | - | - | - | - | 1.6 | - | - | - | - | - | - | - | - | - |
| α-Amorphene | i | 1507 | 1505 | 36.506 | - | - | - | - | - | - | - | - | - | - | - | - | 0.4 | - |
| Aliphatic hydrocarbons | | | | | | | | | | | | | | | | | | |
| Decane | h | 1000 | 997 | 15.532 | - | - | - | - | - | - | - | - | - | - | - | - | - | 2.5 |
| Undecane | c | 1100 | 1095 | 19.774 | - | - | - | - | - | - | - | - | - | - | - | - | - | 1.6 |
| Dodecane | h | 1198 | 1194 | 24.041 | - | - | - | - | - | - | - | - | - | - | - | - | - | 2.9 |
| Tetradecane | h | 1400 | 1404 | 32.243 | 0.1 | 0.4 | 0.7 | 0.2 | 1.7 | 0.7 | 0.4 | 0.2 | 0.8 | 0.5 | - | 0.3 | 0.5 | 0.2 |
| Aldehydes | | | | | | | | | | | | | | | | | | |
| 2-Methylbutanal | j | 668 | 670 | 6.062 | 15.0 | 3.3 | 3.7 | 5.0 | 8.2 | 7.8 | 12.8 | 9.1 | 13.9 | 5.2 | 1.8 | 4.4 | 1.5 | 1.1 |
| Pentanal | j | 715 | 721 | 6.497 | - | 2.8 | - | 4.3 | 5.5 | 4.7 | 2.5 | 3.1 | 2.4 | 3.9 | 2.5 | 2.2 | 3.3 | 2.0 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2*E*-Pentenal | a | 754 | 756 | 7.589 | 4.3 | 5.8 | 3.4 | 5.8 | 4.2 | 4.8 | 5.6 | 6.7 | - | 8.4 | 5.2 | - | 4.3 | 2.0 |
| Hexanal | j | 803 | 803 | 8.610 | 22.2 | 24.1 | 20.3 | 24.2 | 17.1 | 19.7 | 19.5 | 15.2 | 16.3 | 18.6 | 18.7 | 22.4 | 29.0 | 15.8 |
| Furfural | k | 834 | 833 | 9.632 | 3.1 | 7.1 | 6.0 | 9.8 | - | 4.7 | 7.6 | 3.7 | 18.8 | 6.0 | 7.0 | 23.8 | 5.5 | 9.9 |
| 2*E*-Hexenal | d | 852 | 849 | 10.183 | 12.0 | 18.4 | 16.1 | 15.9 | 10.4 | 7.0 | 10.7 | 11.8 | 13.2 | 16.7 | 15.1 | 15.9 | 11.7 | 7.2 |
| Heptanal | k | 904 | 905 | 11.782 | 2.0 | 2.0 | 1.4 | 1.5 | 1.0 | - | 2.9 | - | - | - | 0.5 | - | 1.4 | 1.5 |
| (2*E*,4*E*)-Hexadienal | j | 907 | 907 | 12.142 | 2.5 | 1.8 | 1.6 | 3.0 | 0.1 | - | - | 1.6 | 0.6 | 2.5 | 3.5 | 3.5 | 2.0 | 0.7 |
| 2*E*-Heptenal | l | 959 | 957 | 13.890 | 6.5 | 5.1 | 7.6 | 6.1 | 3.4 | 4.3 | 4.2 | 4.0 | 5.2 | 5.9 | 5.5 | 5.0 | 4.4 | 0.9 |
| Benzaldehyde | k | 968 | 965 | 14.235 | 1.7 | - | 3.2 | 2.9 | 3.4 | 4.0 | 3.3 | 2.6 | 4.4 | 1.6 | 1.0 | 1.6 | 0.7 | 1.1 |
| Octanal | c | 1003 | 1002 | 15.726 | 1.1 | 1.9 | - | 3.6 | 1.5 | - | - | 1.6 | - | - | 3.5 | 1.5 | 2.7 | - |
| (2*E*,4*E*)-Heptandienal | h | 1013 | 1011 | 16.125 | 3.6 | 3.4 | 3.7 | 4.6 | 3.5 | 3.4 | 2.6 | 5.4 | 3.0 | 4.2 | 3.0 | 3.0 | 2.3 | 2.1 |
| Benzene acetaldehyde | h | 1045 | 1046 | 17.634 | 0.8 | 0.1 | 1.0 | 0.8 | 1.9 | 2.7 | 2.5 | 1.8 | 1.6 | 0.8 | - | 1.1 | 0.9 | 0.1 |
| 2*E*-Octenal | g | 1044 | 1047 | 18.107 | 1.2 | 0.4 | 1.6 | 0.3 | 0.2 | - | - | - | - | - | 0.8 | 0.6 | 0.3 | - |
| Nonanal | h | 1101 | 1102 | 20.045 | 4.7 | 5.8 | 4.7 | 4.4 | 4.4 | 4.6 | 3.7 | 7.0 | 4.5 | 5.4 | 9.1 | 5.2 | 6.3 | 5.3 |
| (2*E*,6*Z*)-Nonadienal | m | 1151 | 1151 | 22,205 | 0.1 | 0.2 | 0.1 | 0.4 | 0.1 | 0.3 | - | - | 0.1 | 0.1 | 0.1 | 0.3 | - | - |
| 2*E*-Nonenal | m | 1158 | 1157 | 22.457 | - | - | 0.2 | - | - | - | - | 0.1 | 0.2 | - | - | - | - | - |
| Decanal | h | 1202 | 1202 | 24.400 | 1.1 | - | 0.7 | 0.8 | 4.2 | 3.1 | 0.1 | 1.5 | - | 0.1 | 3.0 | 2.3 | 2.3 | - |
| *E*-Cinnamaldeyhde | m | 1277 | 1274 | 27.384 | - | - | - | - | - | - | - | - | - | - | - | - | 8.2 | - |
| Ketones | | | | | | | | | | | | | | | | | | |
| 2-Heptanone | n | 892 | 895 | 11.394 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.2 |
| 6-Methyl-5-hepten-2-one | e | 986 | 986 | 15.084 | - | 3.2 | 3.6 | 0.8 | 4.1 | 2.1 | 3.7 | 3.2 | 2.8 | 4.7 | 5.8 | - | 1.9 | 2.5 |
| *β*-Ionone | n | 1486 | 1489 | 35.910 | - | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - |
| Other compounds | | | | | | | | | | | | | | | | | | |
| 2-Ethyl-furan | o | 728 | 724 | 6.577 | 2.3 | 6.8 | 2.3 | 1.8 | 1.8 | 7.6 | 6.0 | 6.6 | 4.9 | 7.5 | 6.5 | 5.8 | 4.3 | 1.3 |
| Butanoic acid | o | 793 | 789 | 7.889 | - | 0.1 | 7.1 | 0.3 | - | - | - | | - | - | - | - | - | - |
| Hexanoic acid | o | 981 | 983 | 14.553 | 0.1 | 0.1 | 0.2 | - | - | 0.2 | 0.1 | 0.2 | 0.1 | - | 0.2 | - | - | - |
| (3*Z*)-Hexen-1-yl acetate | o | 991 | 994 | 15.835 | - | - | - | - | - | - | - | 0.1 | - | - | - | - | - | - |
| Caryophylene oxide | h | 1583 | 1584 | 39.744 | 0.1 | - | 0.1 | - | - | - | - | - | - | - | - | - | - | - |
| Total | | | | | 99.8 | 99.8 | 99.8 | 99.1 | 99.3 | 99.9 | 99.9 | 99.9 | 99.9 | 99.8 | 99.6 | 99.8 | 99.8 | 99.1 |

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1-13: Samples described at Table1. R: Reference sample (*A. vulgaris*) S1: Cafeic acid S2: Chlorogenic acid S1+S2: the mixture of cafeic acid and chlorogenic acid

**Volatile organic compounds of *Alchemilla* species**

There is not any specification given on volatile organic compounds of *Alchemilla* species at "*Alchemilla* herba" monograph in the European Pharmacopoeia 8.0. Therefore, all of the samples were analyzed by a SPME-GC–FID/MS method and identifications were made on the basis of comparison of GC Kovats retention indexes (RIs) with the data published in the literature moreover reference to a homologous series of *n*-alkanes and results were given in table 5.

A total of 49 compounds were identified and aldehydes were found as the major group of compounds in all of the samples in the range of 49.7% to 93.4%. The smallest ratio of aldehydes were at the reference sample (*A. vulgaris*) (49.7%). The major volatile constituents of the reference sample were found to be hexanal (15.8%) and limonene (12.1%). Similarly, the major compound of the samples were hexanal (in the range of 15.2% to 29.0%) excluding sample 9 of which the major compound was furfural (18.8%). Terpinen-4-ol (1.4%), α-terpinyl acetate (0.6%), decane (2.5%), undecane (1.6%), dodecane (2.9%), and 2-heptanone (0.2%) were present only in the the reference sample (*A. vulgaris*). But, 2-methyl-butanal, hexanal, 2*E*-hexenal, 2*E*-heptenal, (2*E,4E*)-heptandienal, nonanal, and 2-ethyl-furan were identified in all of the *Alchemilla* species which studied (Table 4).


**Figure 3. Packages of the market samples of *Alchemilla* species.**

1-13: The number of *Alchemilla* species as 1-13 explained in Table 1.

## DISCUSSION

There have been previous studies conducted on the comparison of monographs in European pharmacopoeia with market samples of *Mentha* L., *Hibiscus* L., *Eucalyptus* (L'Hér.), and *Melissa* L. in Turkey (Şaşkara et al., 2010; Özdoğan et al., 2011; Tombul et al., 2012; Demirez et al., 2014). *A. vulgaris* which is used as reference plant has a monograph in European Pharmacopoeia 8.0 and has patented preparations sold in herbal market (Mazurkova et al., 2016; Shchetinin et al., 2016). This is the first comparative study on *Alchemilla* species sold in supermarket and the reference sample in European Pharmacopoeia.

Although some morphological studies on *Alchemilla* species naturally growing in Turkey, has been conducted up to date, the identification key has not been updated to include newly added species in the flora (Pawlowski and Walters, 1972). Ungrounded leafy samples were taken during the supply of the samples. But the identification of the species were not made due to the fact that the distinguishing characters according to the present identification key could not be observed in many samples and the mixed results of many species were obtained from the samples because the samples examined were not homogeneous.

It can be seen in the photos of the samples that, "*Alchemilla vulgaris*" was written as content information on the package of sample 3 and "*Alchemilla alpina*" on samples 1, 2, 4, 11 and 12 (Figure 3) despite no sample was identified as *A. alpina* or *A. vulgaris*. The results showed that the content information was written without identification. Most of the samples were not found to be suitable to the officinal drug standarts in European Pharmacopoeia in terms of foreign matter and loss on drying. TLC results also couldn't exactly match the specifications. TLC profile of reference sample was similar to the other samples whereas chlorogenic acid and caffeic acid zones were only seen together in the reference sample. Due to the large

number of substances present in the main extract, TLC analysis does not allow us to reach a clear conclusion regarding the presence or absence of these two compounds. Hyperoside and isoquercetin which were previously identified in the aerial parts of A. mollis can be determined as standard compounds and pharmacopoeial analysis can be done by detecting these substances with advanced methods such as HPLC (Akkol et al., 2015).

All could be related with inappropriate collection and storage conditions resulting from the collection by uneducated people.

According to the results of the SPME analysis, the composition of the essential oils from the samples obtained from Turkey, were similar to the reference sample by the preponderance of aldehydes, mainly hexanal. In contrast to the oil from reference sample, in samples obtained from Turkey, monoterpene hydrocarbones and oxygenated monoterpenes was present in low percentage. The differences in the quantity and quality of the volatile organic compounds will cause changes in the effects that may occur due to the use of the plant.

Volatile constituents of nine *Alchemilla* samples from Turkey are defined by this study for the first time. Although, the study of the volatile organic compound in the essential oil of *Alchemilla* species are limited, different chemical compositions have been reported. Alcohols, mainly cis-3-hexenol (11.2%) were found as the main compounds of *A. xanthochlora* Rothm. which is growing in Alpine pastures (Falchero, 2009). The essential oil composition of *A. alpina* L. em Buser from Western Alpine pastures were investigated and terpenes were identified as the major compounds (36.9 %) (Falchero, 2008). Alkanes were reported as the major components of the essential oil of *A. percisa* Rothm. growing in Iran (Heshmati, 2005). It appears therefore imperative to prepare standarts and monographs of our own plant resources.

The results suggest that, none of the specimens examined were in pharmacopoeia standards and the use of the samples in terms of public health is not appropriate. Plants which are sold to the public in the herbal market for medicinal usage, should be cultivated in accordance with good agricultural practices and made available to the patients under the supervision of pharmacists. Additionally, progressive pharmacognostic studies are required to prepare a monograph for one of the *Alchemilla* species that grow widespread in Turkey.

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