**Essential Oil Composition of two *Lallemantia* (Lamiaceae)**

 **species from Bingol -Turkey**

 **Ömer KILIÇ\***, **Şinasi YILDIRIMLI**\*\* **& Alaattin Selçuk ERTEKİN\*\*\***

\* Bingöl Technical Science, Vocational College, Bingöl, Turkey.

\*\* Hacettepe University, Science Faculty, Department of Biology, Ankara-Turkey.

\*\*\* Dicle University, Science Faculty, Department of Biology, Diyarbakır-Turkey.(Corresponding author’s e-mail: omerkilic77@gmail.com)

**Abstract**

The essential oil of the dried flowering aerial parts of *Lallemantia iberica* (M. Bieb.) Fisch. & C.A.Mey. and *Lallemantia peltata* Fisch. & C.A.Mey. were analyzed by means of HS-SPME/GC-MS. As a result thirty one and thirty two components were identified representing 95.1% and 94.6% of the oil of *L. iberica* and *L. peltata*, respectively. The main constituents of *L. iberica* were *p*-cymene (25.6%),-caryophyllene (12.3%) and caryophyllene oxide (16.5%); whereas -caryophyllene (15.6%), *p*-cymene (14.8%), caryophyllene oxide (11.9%) and germacrene D (10.4%) were the major constituents of *L. peltata.* With this study, chemotypes of *L. iberica* and *L. peltata* were detected *p*-cymene,-caryophyllene and caryophyllene oxide. In addition essential oil structure of studied samples have detected and studied plant samples were found to be rich in respect to essential oils. The results discussed natural product, renewable resources, chemotaxonomy and for uses purposes of plants.

**Key Words:** *Lallemantia*, Essential oil, HS-SPME/GC-MS.

 **Bingöl’de (Türkiye) Bulunan iki *Lallemantia* (Lamiaceae)**

**Türünün Uçucu yağ Kompozisyonu**

**Özet**

Bu çalışmada*Lallemantia iberica* (M. Bieb.) Fisch. & C.A.Mey. ve *Lallemantia peltata* Fisch.& C.A.Mey. türlerinin toprak üstü kısımlarının uçucu yağ içerikleri HS-SPME/GC-MS ile analiz edildi.Sonuçta *L. iberica* and *L. peltata* türlerine ait %95.1 ve %94.6’ lık toplam yağ miktarından sırasıyla otuzbir ve otuziki bileşen tespit edildi. *L. iberica*’ nın ana bileşenleri *p*-simen (%25.6),-karyofillen (%12.3) ve karyofillen oksit (%16.5) olarak tespit edilirken; -karyofillen (%15.6), *p*-simen (%14.8), karyofillen oksit (%11.9), germakren D (%10.4) ise *L. peltata*’ nın ana bileşenleri olarak bulundu. Bu çalışma ile *L. iberica* ve *L. peltata* ‘nın kemotipleri *p*-simen,-karyofillen ve karyofillen oksit olarak tespit edildi. Buna ek olarak çalışılan bitkilerin uçucu yağ içerikleri belirlendi ve bu bitkilerin uçucu yağ içeriği bakımından zengin olduğu bulundu. Sonuçlar doğal ürünler, yenilenebilir kaynaklar ve bitkilerin kemotaksonomileri ile kullanım alanları açılarından tartışıldı.

**Anahtar Kelimeler:** *Lallemantia*, Uçucuyağ, HS-SPME/GC-MS.

**1. INTRODUCTION**

Essential oils, also named volatile odoriferous oil, are aromatic oily liquids extracted from various parts of plants, for instance; flowers, leaves, peels, barks, buds, seeds, and so on. Essential oils can be extracted from plant materials by several methods, among all methods steam distillation has been widely used, especially for commercial scale production (1). Essential oils have been widely used as food flavors and found in many different plants, especially the aromatic plant families (Lamiaceae, Myrtaceae, Apiaceae, etc.), vary in odor and flavor; the amount of essential oil from different plants is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments conduce to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods. Essential oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products (2). Because of the increasing attention in natural additives, essential oils of plants have been used more widely, thus, essential oils can serve as the alternative additives or processing aid as green technology, phytoteraphy and aromateraphy (3). Plant essential oils are assumed to be one of the potential sources for the screening of anticancer, antimicrobial, antioxidant, etc., and free radical scavenging agents (4). It is now broadly accepted that certain classes of plant-based compounds play preventive role against the incidence of some common diseases like cancer, cardiovascular and neurodegenerative disorders, inflammations as well as the ageing process (5).

*Lallemantia* Fisch. & Mey genus is in the family Lamiaceae family; Lamiaceae consists of about 252 genera and more than 7000 species, that grow on wide area extending from the Asia minor to the Caucasian countries, the Middle East, and the Central and the Southern Europe and blooms during April to June each year (6-8). Some of Lamiaceae taxa are frequently used in cooked dishes, medicinal uses, ethnobotany, alternative medicine and are recognized as important preventive factor of many diseases (9). Essential oils and extracts of these plants are known to possess antiseptic, anti-inflammatory, antimicrobial activities, and so on (10). *Lallemantia* species are underutilized of Lamiaceae family; even though some reports on the use of plant species as a natural antioxidants are available (5), in the literature no such study on the essential oils of *Lallemantia* species native to Bingol from Turkey has been carried out. *Lallemantia*, is distributed in various regions of Asia and Europe; in the Turkish flora *Lallemantia* includes three species (*L. canescens* (L.) Fisch. & Mey, *L. iberica*, *L. peltata*); *Lallemantia* species are annual and perennial herbs, verticillasters subtended by floral leaves, forming an elongate oblong inflorescence; calyx tubular; corolla 2-lipped; stamens 4; style with 2 unequal lobes; nutlets oblobg (11).

*Lallemantia* species are beautiful ornamental plants that grows over large area in Turkey and they are economically important and has high ornamental, medicinal value, as it is used in arid landscaping and urban horticulture at different places in Turkey. *Lallemantia* taxa are also grown for use in food, lighting, pharmaceutical and other purposes (12), and traditional medicinal systems (13) as stimulant, diuretic, expectorant and uses in ethnobotany (14). Traditionally, it is a very common practice that local people use indigenous plants or plant pruducts to cure infectious diseases; or those are the part of food as dietary components are termed as ethnomedicine (15). Inspite of, there are very few researches on mechanism of action and phytochemistry of *Lallemnatia* species based phytomedicines. But traditional knowledge report that aromatic and medicinal plants and ethnomedicines have been receiving considerable attention by scientist and pharmaceutical research industries with the aim to examine for more effective substitute (16). *L. iberica* originated from Caucasian region, that has been found in Asia, but it now the crop also appears in central and southern Europe; unfortunate ventures were made to introduction the plant species in Germany, Austria and Canada, seed production, were found to be low and unstable, possibly due to relatively wet climate condition (17). *L. peltata* is an anuual; stem erect, simple or branched, 15-40 cm., lower leaves ovate or oblong, petiolate, upper leaves lanceolate to linear, calyx cylindrical, corolla violet-blue t opale bluish; *L. iberica* is similar to *L. peltata* but lowest leaves ovate, lamina to 18x10 mm, crenate; corolla violat-blue pale blue or white; flowering times between 4.-6. months; distribution and growing areas are generally roadsides, slopes, fallow fields, weed of cultivated land, an altitude of 500-2150 m (11).

Essential oil composition of *L. iberica* and *L. peltata* are no investigated before, Bingol from Turkey. Therefore, the aim of this study is to provide essential oil composition of these species, that might be helpful in potential usefulness, biological activities, chemotaxonomy and other studies with *Lallemantia* taxa.

**2. MATERIALS AND METHODS**

**2.1. Plant Materials**

*Lallemantia iberica* was collected from south of Yelesen village, steppe, stony areas Bingol / Turkey, on 20.05.2013, at an altidude of 1600-1650 m., by O. Kilic, collect no: 4624. *Lallemantia peltata* was collected east of Dikme village, moisty and stony areas, Bingol / Turkey, on 20.05.2013, at an altidude of 1550-1600 m., by O. Kilic, collect no: 4590. Plant materials were identified with volume 7 of Flora of Turkey and East Aegean Islands (11). Voucher specimens were deposited in the Bingol University, Department of Park and Garden Plants, Yildirimli and Hacettepe University herbariums.

**2.2. HS-SPME Procedure**

Dried aerial part powder of five grams plant samples were carried out by a (HS-SPME) head space solid phase microextraction method using a divinyl benzene / carboxen / polydimethylsiloxane fiber, with 50/30 um film thickness; before the analysis the fiber was conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of plant samples, previously homogenized, were weighed into a 40 ml vial; the vial was equipped with a ‘‘mininert’’ valve. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibratefor 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analytes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al., (18).

**2.3. GC-MS Analysis**

 A Varian 3800 gas chromatograph directly inter faced with a Varian 2000 ion trap mass spectrometer was used with injector temperature, 260°C; injection mode, splitless;column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 lm film thickness. The oven temperature was programmed as follows: 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C. at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisit ion range, 40 to 200 m/z; scan rate, 1 us-1. The compounds were identified using the NIST library, mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz (19). The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of studied species are listed in Table 1.

 **Table 1.** Essential oil composition of *Lallemantia* species.

|  |  |  |  |
| --- | --- | --- | --- |
| **Compounds** | **RRI\*** |  ***L. iberica*** (%) |  ***L. peltata*** (%) |
| -Thujene | 932 | 0.8 | 1.2 |
| -Pinene | 935 | 1.2 | 0.3 |
| Sabinene | 965 | 0.9 | 1.6 |
| -Pinene | 975 | 1.6 | 2.3 |
| 3-Octanone | 981 | - | 0.4 |
| -Myrcene | 985 | 0.2 | 1.9 |
| **3-Octanol** | 992 | 3.6 | 2.4 |
| -Terpinene | 1010 | 0.3 | - |
| ***p*-Cymene** | 1023 | **25.6** | **14.8** |
| Limonene | 1028 | 0.6 | 0.6 |
| 1,8-Cineole | 1032 | 1.5 | 1.1 |
| -Terpinene | 1062 | - | 0.6 |
| Terpinolene | 1082 | 0.8 | 1.6 |
| Linalool | 1085 | 0.4 | - |
| Terpinen-4-ol | 1175 | 1.1 | 2.1 |
| Verbenone | 1195 | 4.7 | 4.9 |
| n-Decanal | 1205 | 0.3 | - |
| Trans-Anethol | 1280 | 0.9 | 1.4 |
| -Copaene | 1352 | - | 0.4 |
| -Ylangene | 1372 | 0.1 | - |
| -Bourbonene | 1382 | - | 0.2 |
| -Elemene | 1395 | 2.4 | 3.7 |
| -Cubebene | 1402 | 3.6 | 3.4 |
| **-Caryophyllene** | 1412 | **12.3** | **15.6** |
| -Humulene | 1450 | 1.6 | 1.3 |
| **Germacrene D** | 1476 | 5.8 | **10.4** |
| Bicyclogermacrene | 1495 | 0.7 | 1.4 |
| -Farnesene | 1504 | 1.6 | 0.7 |
| -Bisabolene | 1505 | 1.3 | 2.4 |
| -Farnesene | 1508 | 1.7 | 2.7 |
| -Cadinene | 1512 | 0.8 | 0.9 |
| -Cadinene | 1522 | - | 0.1 |
| Spathulenol | 1572 | 0.2 | - |
| Isospathulenol | 1575 | - | 0.1 |
| **Caryophyllene oxide** | 1578 | **16.5** | **11.9** |
| Cadinol | 1635 | 1.7 | 2.1 |
| -Muurolol | 1645 | 0.3 | - |
| Isophytol | 1942 | - | 0.1 |
|  | **Total** | **95.1** | **94.6** |

 **RRI\*:** Relative Retention Index

**3. RESULTS AND DISCUSSION**

The essential oil composition of dried aerial parts of *L. iberica* and *L. peltata* were analyzed by HS-SPME/GC-MS. 31 and 32 compounds were identified in *L. iberica* and *L. peltata* accounting from 95.1% and 94.6% of the whole oil, respectively. The main compounds of *L. iberica* were *p*-cymene (25.6%), caryophyllene oxide (16.5%) and -caryophyllene (12.3%); whereas in the *L. peltata* oil -caryophyllene (15.6%), *p*-cymene (14.8%), caryophyllene oxide (11.9%) and germacrene D (10.4%) were found to be the main compounds (Table 1). *L. iberica* and *L. peltata* included high concentrations of *p*-cymene (25.6%-14.8%), -caryophyllene (12.3% -15.6%) and caryophyllene oxide (16.5%-11.9%), respectively. Among the monoterpenes, *p*-cymene was found principal constituents of *L. iberica* (25.6%), this compound also principal constituents of *L. peltata* (14.8%) (Table 1).

In a study the volatile constituents analysis of *Lallemantia iberica* from Iran, observed that, its oil has 33.7% germacrene, 19.0% 3-carene, 12.8% isocaryophyllene and sabinene, 6.5% (20). In our study *L. iberica* has different chemical properties from cited study, producing high concentration of *p*-cymene (25.6%) and caryophyllene oxide (16.5%); followed by low percentages of germacrene D (5.8%), sabinene (0.9%) and no percentages of 3-carene and isocaryophyllene (Table 1). In another research, the water-distilled essential oil from of aerial parts of *L. peltata* were collected at full flowering stage, from Hamedan and West Azerbaijan of Iran was analyzed by GC and GC/MS and the major compounds were found to be germacrene D (42.5% and 49.9%),-caryophyllene (20.6% and 26.0%) and germacrene B (5.6% and 1.6%), respectively (21). In this study dried aerial parts of *L. peltata* was analyzed by HS-SPME/GC-MS and the main constituents were determined -caryophyllene (15.6%), *p*-cymene (14.8%), caryophyllene oxide (11.9%) and germacrene D (10.4%); it is noteworthy that germacrene B was not detected in this research (Table 1). Detailed of essential oil composition of the *L. iberica* and *L. peltata* oils can be seen in Table 1. Comparison of the oil composition from two species showed, the amounts of caryophyllene oxide and *p*-cymene were higher in the oil of sample *L. iberica* than *L. peltata*. Another difference is the low amount of germacrene D (5.8%) in *L. iberica* and than *L. peltata*. The percentage of some minor components in both oils, were near together, but there are some differences between the percentages of some other components. For example, -thujene, -pinene, sabinene, -myrcene,-elemene, -humulene and so on, presents in the oil of sample *L. iberica* at lower percentage. Instead of, the percentage of 3-octanol, verbenone and so on are higher in the oil of *L. iberica* (Table 1). -terpinene, linalool, spathulenol and -muurol were not found in the oil of *L. peltata*. These differences could be resulted from different ecological situation and might have been derived from local, climatic factors of two plant localities. Of course *L. iberica* and *L. peltata* oils were very similar in the minor components, especially in monoterpenoid compounds (Table 1). The essential oil of aerial parts of *Lallemantia royleana* (Benth. in Wall.) Benth. from Iran rich in verbenone (16.4%) and *trans*-carveol (9.8%) (22); similarly in our study *L. iberica* and *L. peltata* rich in verbenone (4.7% and 4.9%, respectively); whereas no percentange of *trans*-carveol (Table 1).

In this study many of the compounds were present in very low or no amounts. Contrary to the earlier report that germacrene-D and geijerene were predominantly present in the oil of another species of *Lallemantia*,we did not find geijerene in the present study; however, some other identified compounds in our essential oil were *p*-cymene,-caryophyllene, caryophyllene oxide and germacrene D, these components were also present in the essential oil of *L. iberica* aerial parts reported previously (23). The volatile constituents of the aerial parts of *L. iberica* growing wild in Iran have been examined by GC-FID and GC-MS; the oil of *L. iberica* consisted mainly of germacrene-D (33.7%), -3-carene (19.0%), iso-caryophyllene (12.8%) and sabinene (11.1%) (24); like this study, in our research germacrene-D were the main compounds aerial parts of two *Lalemantia* species; hovewer -3-carene and iso-caryophyllene were not detected in our study and sabinene was determined low amounts in both oils (Table 1). Previously reported results about the essential oil analysis of *L. royleana* from Iran have determined that verbenone (16.4%), *trans*-carveol (9.8%) and -cubebene (8.9%) major constituents of the obtained oil (22); these results showed that the major component of *L. royleana* was verbenone (16.4%); verbenone detected in the *L. iberica* and *L. peltata* low amounts, collected form Dikme and Yelesen villages (in Bingol province, Turkey) (Table 1). The second major difference is the presence of trans-carveol (9.8%) in the *L. royleana*, which is not detected in the *L. iberica* and *L. peltata*, (Table 1). These differences could be an effective factor causing different biological activities in these plants. In another research the essential oil of wild-growing *Lallemantia iberica* from Turkey was obtained by hydrodistillation and analyzed by GC and GC-MS; in the oil of the *L. iberica* 40 components were characterized representing 90.1 % of the total oil, with germacrene-D (36.0 %), -caryophyllene (18.3 %) and bicyclogermacrene (9.7%) as the major compounds (25); germacrene-D and -caryophyllene were also detected the main compounds in this research; whereas bicyclogermacrene was determined very low amounts both oils (Table 1). The comparison between two speciesevidenced a similarity, at least with reference to the presence of the main constituents: in fact *p*-cymene, -caryophyllene and caryophyllene oxide were among the principal one in both species; the percentages of other compounds were comparable (Table 1).

 In conclusion, this study demonstrates the occurrence of *p*-cymene, -caryophyllene, germacrene D and caryophyllene oxide chemotypes of *L. iberica* and *L. peltata* in Eastern Anatolian region of Turkey. The essential oil results have given some clues on the chemotaxonomy of the genus patterns and usability of *L. iberica* and *L. peltata* as natural product. According to these results, studied plants were found to be rich in respect to essential oils. So these plants can be used different purposes in industry, ethnobotany and can be cultivated to richened natural products. In addition, many plant species are threatened due to overharvesting for medicinal or other use, so there is great need to protect plant diversity. There is also a need to develop more sustainable ways of obtaining industrial products from renewable resources. The cultivation of medicinal and aromatic plants for industrial products can address these issues. Furthermore, the results showed that the analysis of essential oil composition will add some contributions on the usability of this plant as a crop and renewable resources and chemotaxonomy of the genus patterns.

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