Türkiye Tarımsal Araştırmalar Dergisi
dergipark.org.tr/tutad

Research Article

Turk J Agric Res
2025, 12(1): 1-8
© TÜTAD
ISSN: 2148-2306
e-ISSN: 2528-858X
doi: 10.19159/tutad.1577667

Effect of Morphogenetic Variability on Volatile Oil Composition of Immortal Flower (*Helichrysum arenarium* subsp. *rubicundum*)

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Received: 01.11.2024 **Accepted:** 28.02.2025

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Abstract: In this study aimed to determine the effects of morphogenetic variability on the essential oil composition of immortal flower (*Helichrysum arenarium* subsp. *rubicundum*) plants collected from Subaşı village of Köse district of Gümüşhane province, Türkiye. The aromatic components in the essential oil of the plant were determined using Gas Chromatography Mass Spectrometry device attached with solid phase microextraction. Totally 69 different components were identified. The main components of the volatile oil were methyl p-tert-butylphenylacetate (17.57%), β-caryophyllene (13.42%), undecly alcohol (10.33%), phtyone (9.01%), δ-cadinene (8.89%), ɣ-cadinene (7.74%) and tetradecane (5.64%). Alloaromadendrene (3.24%), α-humulene (3.07%) and caryophyllene oxide (1.79%) were found only in the flower part, acetyl tributyl citrate (3.52%) and α-pinene (1.89%) only in the leaf part and phthyol acetate (4.70%) and tridecylaldehyde (1.26%) only in the stem part. The main reasons for these differences include genetic and ecological factors. Factors such as the use of different organs of the plant (morphogenetic variability) also play an important role. Similarly, the differences in the essential oil ratios and essential oil components of plants are also thought to be due to these factors.

Keywords: Gümüşhane, β-caryophyllene, α-pinene, aromatic plants, *Helichrysum arenarium*

1. Introduction

Throughout history, people have used plants for their health and the health of animals. Today, the active ingredients of many medicines are derived from medicinal and aromatic plants. The materials of these plants are used in processed or unprocessed form to produce medicines. Medicinal and aromatic plants form the basis of natural treatment methods and have an important place in modern medicine.

The components derived from these plants are effectively used in the treatment of various diseases and the maintenance of health (Bayram et al., 2010). The important effects of plants on human health have been the subject of extensive research in Türkiye and worldwide since 1926. These studies aimed to reveal the medicinal properties of plants and their health benefits (Segler, 1992). Today, scientists are investigating how the active ingredients in plants can be used to treat diseases and support health. In this way, knowledge about herbal treatment methods and natural medicines is constantly growing and developing. According to the World Health Organization (WHO), the number of plant species used for medicinal purposes today is around 20000. Of these plants, 4000 are widely used and about 2000 medicinal plants are traded worldwide. In Western Europe, the number of medicinal plants traded is around 500 (Yurteri, 2023).

The flora of Türkiye, with over 10.000 plant species, has a plant diversity close to that of the whole of Europe. Approximately one-third of these plants have aromatic properties and about 3.000 plant species are endemic. The number of herbs sold by herbalists in Türkiye is approximately 300 and 70-100 herbs are exported (Kalaycıoğlu and Öner, 1994; Başer, 1997, 1998; Güner and Aslan, 2012). In Türkiye, the number of plants used for therapeutic purposes is thought to be around 500 (Baytop, 1999). People living in rural areas in

Türkiye show great interest in natural plants. These plants are used in various fields such as cosmetics, medicine, food, spices and dyestuffs (Özkan, 2002).

Essential oils are found in different parts of plants such as leaves, flowers, roots, and stems, and are stored in the plant's special oil cells and oil passages (Sangwan et al., 2001). The characteristic odors of many plants are due to the volatile oil they contain. It has been reported that many of the volatile oil components are affected by internal (plant genetics, origin, etc.) and external factors (such as climate, growing conditions, drying methods and extractions, distillation time, and analysis conditions) (Moghaddam and Mehdizadeh, 2017). The content of bioactive substances formed from secondary metabolites shows morphogenetic variability according to the organs of the plant, ontogenetic change according to the life cycle of the plant and the harvest/collection time of the plant (Ramakrishna and Ravishankar, 2011; Baydar, 2013; Saha et al., 2016).

Morphogenetic variability is the difference in the active ingredient found in medicinal plants according to the plant organs within the same harvest. The active ingredients vary in quality according to the underground and aboveground organs of the plant (Asal, 2022).

One of these medicinal plants is the immortal flower known as "*Helichrysum arenarium* subsp. *rubicundum*", which is the matter of the present study. The genus *Helichrysum* belongs to the Asteraceae family and is derived from the Greek words "helios" (sun) and "chrysos" (gold). Plants belonging to the Asteraceae family can be annual, biennial or perennial in terms of their life span. These plants are herbaceous, shrubby, climbing or rarely woody. These plants, which are very flexible in terms of habitat diversity, are widespread on all continents except Antarctica. Worldwide, there are approximately 23.000 species of the Asteraceae family (Güner et al., 2000). *Helichrysum* genera are divided into three sub-varieties, Virginea, *Helichrysum* and *Xerochlaena*. There are approximately 600 species of *Helichrysum* worldwide, of which 250 species, including the vast majority, are distributed in South Africa. It is also found in Southern Europe, South-West Asia, South India, Sri Lanka, Australia and regions with Mediterranean climate (Anderberg, 1991).

The immortal flower plant got this name because of its golden yellow color. It is a fragrant herbaceous plant with yellow flowers that can grow up to 50-60 cm in length. It is also known as golden flower, immortal flower, sun flower, bee flower among the people. Immortal flower (*H. arenarium* subsp. *rubicundum*) is a valuable plant because of its medicinally important components. Compounds such as ethereal oils, flavones and flavone glycosides, sterins, bitter substances, tannins, dyes, resin, carotene, vitamin C, vitamin P and coumarin are used in various fields (Şen and Kalaycı, 2016).

In Europe and Türkiye, there are about 25 *Helichrysum* species including subspecies. In Türkiye, these species are generally distributed in Central and Eastern Black Sea, Eastern and Southeastern Anatolia, Central Anatolia and Mediterranean regions. *Helichrysum italicum*, which is under the *Helichrysum* sub-section of the *Helichrysum* genus, is the most cultivated species in the world and consists of 6 subspecies (Galbany-Casals et al., 2006). The flowers of *H. arenarium* exhibit various biological activities. These activities include antibacterial, antiviral, antifungal, anti-inflammatory, antiproliferative, antimicrobial, antiallergic, antioxidant, antiradical, cholinergic, hepatoprotective and detoxification properties (Tepe et al., 2005; Liu et al., 2019a). It is popularly used in the treatment of various ailments such as liver and gallbladder disorders, lumbago, stomach pain, asthma, arthritis, cystitis, jaundice, skin infections, respiratory and digestive system disorders, kidney stones and uro-genital problems. It has also been preferred in the cosmetics industry for many years due to its pleasant odor (Liu et al., 2019b; Umaz and Umaz, 2020). The effects of morphogenetic variability on the essential oil composition of *H. arenarium* subsp. *rubicundum* plants collected from Subaşı village of Köse district of Gümüşhane (Türkiye) province were investigated in the present study.

2. Materials and Methods

Stems, leaves and flower parts of *H. arenarium* subs. *rubicundum* collected from a natural plant population of the Subasi village of Köse district of Gümüşhane province (40° 26.232' N, 39° 61.732' E) at an altitude of 1750 m during the full flowering period was used as plant material. Ten plants representing detected population in the Köse village distrubuted in a limited area were collected using simple sampling method.

The collected plants were dried to constant weight in the shade and stored at +4 °C for analysis. The leaves, stems and flowers of the plant were ground separately in a coffee grinder, weighed 0.2 g before analysis, placed in GC (Gas Chromatography) tubes and read in the device (Figure 1).

The used equipment [Shimadzu model 2010 Plus GC/MS (Gas Chromatography-Mass Spectrometry)] and modified analysis method (Yurteri et al., 2021) was as follows. In the GC

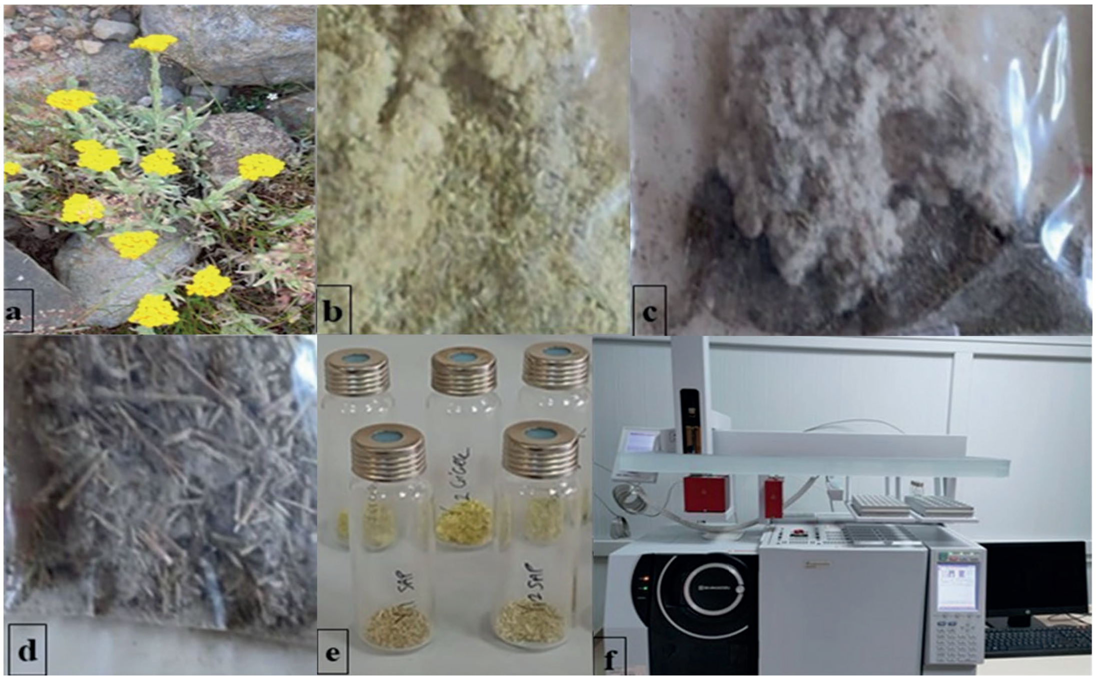

Figure 1. *H. arenarium* subs. *rubicundum* collection of plants samples (a), ground flowers (b), ground stems (c), ground leafs (d), 0.2 g dry plants GC-MS tubes (e) and GC-MS analayses (f)

injector, the SPME (Solid Phase Microextraction) fibers were conditioned for five minutes at 250 °C. A Shimadzu GC/MS instrument equipped with HP-WAX and HP-5 capillary column equipment (30 mx0.25 mm, 0.25 mm film thickness) was used to conduct GC studies. The alkane series has a length of C8-C24. The GC/MS equipment was used to examine volatile oil. A Shimadzu GC/MS-QP 2010 GC/MS system running in electro spray ionization mode, with a CP 5MS (30 m x 0.25 mm i.d., film thickness 0.25 mm), was used to perform the GC/MS studies. Helium (1 mL min^{-1}) was used as the carrier gas.

3. Results and Discussion

A total of 69 different components were detected in stem, leaf and flower parts of *H. arenarium subs. rubinucum* collected from Subaşı village of Köse district of Gümüşhane province. Components displayed with the highest ratios were methyl p-tert-butylphenylacetate (17.57%), β-caryophyllene (13.42%), undecly alcohol (10.33%), phytone (9.01%), δ-cadinene (8.89%), ɣ-cadinene (7.74%) and tetradecane (5.64%) (Table 1).

As can be seen in Figure 2, in all plant parts (stem, leaf and flower) methyl p-tert-butylphenylacetate (17.57%, 10.64%, 9.74%), undecly alcohol (10.33%, 5.66%, 6.11%), phtyone (6.91%, 9.01%, 2.10%), tetradecane (5.64%, 4.16%, 3.25%), hexadecane (4.94%, 3.52%, 2.48%), heptadecane (4.94%, 2.31%, 1.90%), tridecyl alcohol (4.12%, 2.06%, 2.22%), ɣ-cadinene (2.36%, 4.68%, 7.74%), aromadendrene (1.91%, 4.66%, 4.35%), δ-cadinene (1.27%, 2.48%, 8.89%) and β-caryophyllene (1.21%, 1.63%, 13.42%) were detected in highest amounts.

While methyl p-tert-butylphenylacetate was found to be high in the stem and leaf parts of the plant, β-caryophyllene was found to be high in the flower of the plant. The components found only in the flower of the plant were alloaromadendrene (3.24%), α-humulene (3.07%), α-amorphene (2.08%), viridiflorene (2.01%), caryophyllene oxide (1.79%), α-acorenol (1.19%), β-selinene (0.75%), thujopsene <cis-> (0.69%), methyl dihydrojasmonate (0.62%), ɣ-udecalactone (0.48%) and lauric aldehyde (0.45%) (Table 1).

Unlike other parts of the plant, 18 components were found in the leaves. These were; acetyl tributyl citrate (3.52%), isoborneol (3.32%), α-pinene (1.89%), fenchyl alcohol (1.25%), β-sesquiphellandrene (1.23%), furan (1.21%), ligustral (1.08%), myristic alcohol (1.06%), nonyl acetate (1.03%), bornly acetate (0.98%), epi-gamma-eudesmol (0.87%), hexadec-6-enoic acid -<16-hydroxy - > omega lactone (0.87%), trans-pinocarveol (0.82%), pelargonaldehyde (0.74%), α-bisabolol oxide-B (0.71%), germancrene-B (0.57%), limonene (0.56%) and (e)-citronellyl tiglate (0.45%) (Table 1).

In a study conducted on the *H. arenarium* subsp. *aucheri* plant collected from the Feke village of

Table 1. Volatile component composition of *H. arenarium* subs. *rubicundum* (%)

Component name	Kovats retention index	Flowers	Leaf	Stem
α-Pinene	929	-	1.89	-
Limonene	1023	-	0.56	-
Pelargonaldehyde	1100	-	0.74	-
Fenchyl alcohol	1107	-	1.25	-
Ligustral	1120	-	1.08	-
Trans-Pinocarveol	1133	-	0.82	-
Isoborneol	1160	-	3.32	-
Dihydrocitronellol	1186	2.39	3.14	3.68
Dodecane	1195	0.59	-	0.87
Pentyallyl butyrate	1275	0.67	-	1.15
Bornly acetate	1280	-	0.98	-
Tridecane	1293	1.38	1.47	2.27
Propanoate <octyl->	1321	0.41	-	0.66
Nonyl acetate	1329	-	1.03	-
α-Copaene	1371	2.78	0.97	-
Undecly alcohol	1385	6.11	5.66	10.33
Tetradecane	1393	3.25	4.16	5.64
α-Gurjunene	1406	1.01	-	0.86
Lauric aldehyde	1404	0.45	-	-
β–Caryophyllene	1415	13.42	1.63	1.21
Aromadendrene	1435	4.35	4.66	1.91
Thujopsene <cis->	1440	0.69	-	-
Geranyl acetone	1447	-	2.94	1
α-Humulene	1450	3.07	-	-
γ-Declactone	1455	-	1.66	0.83
Alloaromadendrene	1458	3.24	-	-
Germancrene-D	1472	-	1.01	0.63
α-Amorphene	1473	2.08	-	-
Viridiflorene	1492	2.01	-	-
β–Selinene	1483	0.75	-	-
(E)-β-Lonone	1482	-	1.21	-
Pentadecane	1492	1.74	1.83	2.86
α-Muurolene	1495	2.46	0.98	-
Tridecylaldeyhyde	1535	-	-	1.26
Methyl p-tert-butylphenylacetate	1506	9.74	10.64	17.57
ɣ-Cadinene	1510	7.74	4.68	2.36
δ-Cadinene	1518	8.89	2.48	1.27
β-Sesquiphellandrene	1533	-	1.23	-
Germancrene-B	1549	-	0.57	-
Nonaonate <isoamyl->	1540	0.47	-	0.4
Citronellyl butyrate	1527	0.45	0.82	0.76
Nerolidol	1556	0.86	0.81	-
α-Undecalactone	1564	0.48	-	-
Viridiflorol	1581	0.45	2.24	1.09
Caryophyllene oxide	1582	1.79	-	-
Tridecyl alcohol	1584	2.22	2.06	4.12
Hexadecane	1591	2.48	3.52	4.94
Tetradecanal	1606	0.81	-	0.89
epi-gamma-Eudesmol	1625	-	0.87	-
α-Acorenol	1625	1.19	-	-
Muurolol <alpha-,epi->	1637	3.31	2.85	1.89
Furan-2- carboxylic acid <octyl-> ester	1656	-	-	0.91
α-Bisabolol oxide-B	1655	-	0.71	-
(E)-Citronelly tiglate	1647	-	0.45	-
methyl dihydrojasmonate	1656	0.62	-	-
Myristic alcohol	1666	-	1.06	-
Heptadecane	1706	1.90	2.31	4.94
α-Bisabolol oxide-A	1742	-	0.86	0.94
Farnesal	1749	0.55	-	0.68
Caprylate <-octyl->	1763	-	-	0.64
Octadecane	1790	1.12	1.64	3.12
Phtyone	1836	2.10	9.01	6.91

Table 1. (Continued)

Component name	Kovats retention index	Flowers	Leaf	Stem
Nonadecane	1890	-	-	1.65
Hexadec-6-enoic acid - <16-hydroxy - > omega lactone	1910	-	0.87	-
Geranly benzoate	1961	-	3.83	3.90
Civetone	2011	-	-	1.13
Phtyol acetate	2214	-	-	4.70
Acetyl tributyl citrate	2255	-	3.52	-

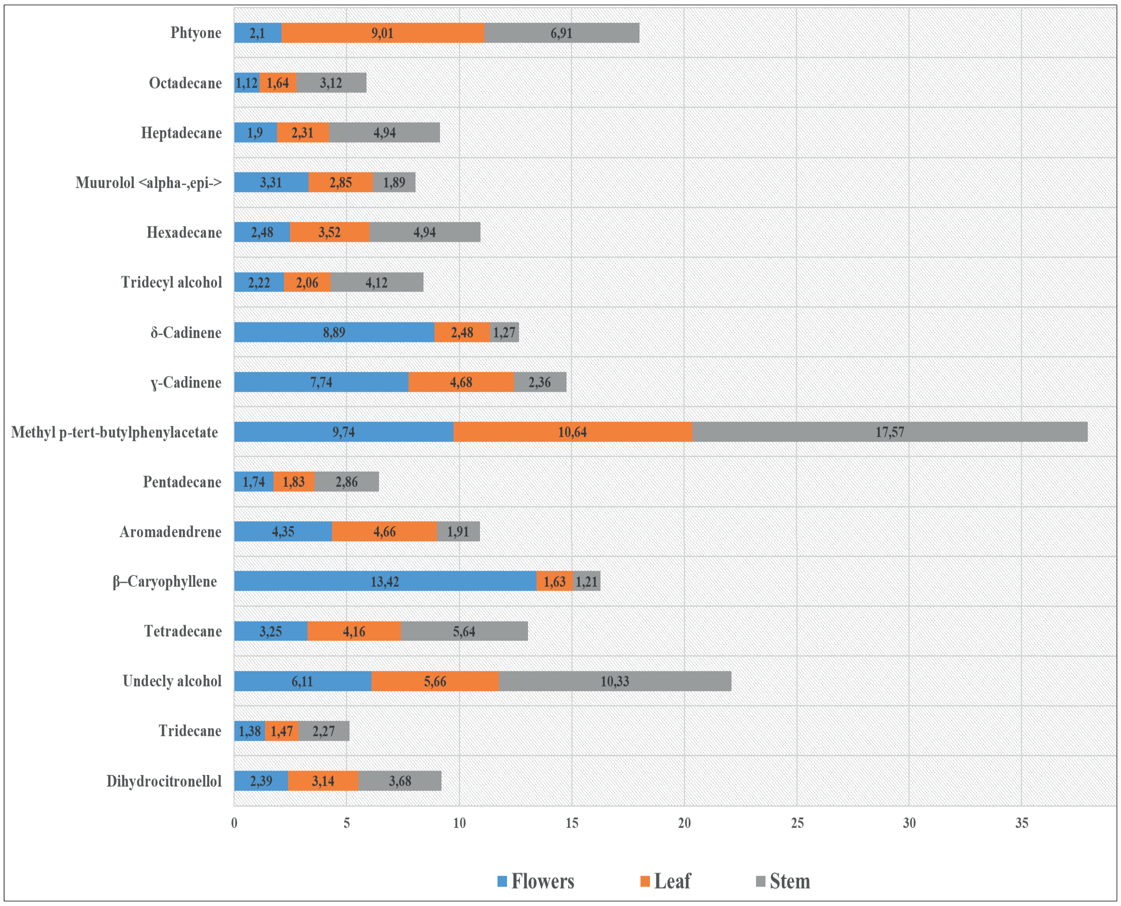

Figure 2. Composition of volatile compounds prominent in different parts of *H. arenarium* subs. *rubicundum* (%)

Adana, it was reported that the essential oil of the plant contained a total of 86 components, the main components being caryophyllene (24.57%), α-pinene (22.5%), β-pinene (8.98%) and limonene (8.18%) (Tığlı Kaytanlıoğlu, et al., 2021). Another study reported that more than 60 components were identified, 24 of which were identified as the main components of the *H. arenarium* plant. The important components found were: linalool (1.7%), anethole (3.2%), carvacrol (3.6%) and α-muurolol (1.3%) (Czinner et al., 2000). In a study conducted with samples taken from Bitlis Nemrut Crater Lake and the mountainous areas of Giresun Şebinkarahisar District, a total of 21 volatile compounds were detected in the immortal flower sample taken around Bitlis Nemrut Crater Lake, while a total of 33 volatile compounds were detected in the immortal flower sample in the mountainous areas of Giresun Şebinkarahisar District. While α-sedrene (26.65%), α-pinene (14.97%), α-humulene (10.65%), aromadendrene (6.79%), α-curcumene (6.31%), germancrene-B (4.43%), α-cadinene (3.82%), eucalyptol (3.57%), α-dupresianen (3.57%) and limonene (2.69%) were detected as the main volatile components in the immortal flower sample around Bitlis Nemrut Crater Lake, α-pinene (47.63%), α-himakhalene (17.01%), α-humulene (5.21%), δ-cadinene (4.98%), γ-cadinene (4.01%) and eucalyptol (3.46%) were detected as the main volatile components in the immortal flower sample from the mountainous areas of Giresun Şebinkarahisar District (Umaz and Umaz, 2020).

In another study on the determination of the components found in different parts of the *H. arenarium* plant, the main components found in the flower and leaf parts of the plant were determined as β-caryophyllene, β-pinene, 1,8-cineole, pentadecanoic acid, 6,10,14-trimethyl-2-pentadecanone and bis-(2-methylpropylester)-1,2-benzenedicarboxylic acid (Reidel et al., 2017). In a study conducted in Lithuania to determine the content of the components in the flower part of *H. arenarium*, the main components detected were α.-humulene and γ-cadinene (Radušienė and Judžentienė, 2008). In another study, more than 80 components were identified and 68 of them were identified. The 68 identified components constituted 73.8-90.7% of the total oil content, and the main components were found to be; β-caryophyllene (in three inflorescences and one leaf oil), δ-cadinene (in two leaf oils), octadecane (in one leaf oil) and heneicosan (in one inflorescence sample) (Judzentiene and Butkiene, 2006). The results obtained in our study are generally similar to the findings obtained by different researchers. Since volatile components may vary due to many factors such as different locations, collected part, collected time, etc., components that we found in our study but were not found in the studies conducted separately from the studies conducted were also detected.

When other studies investigating the effects of morphogenetic variability on the essential oil composition of medicinal and aromatic plants are examined; Ayanoğlu et al. (2016) investigated the effects of morphogenetic and ontogenetic variability on the essential oil content of rosemary (*Rosmarinus officinalis* L.), and determined that the highest essential oil was in the leaves (0.78%). In a study conducted on the lavandin plant (*Lavandula x intermedia* Emeric ex Loisel.), the essential oil ratio and components showed differences according to the plant organs that make up the herb. In the study, it was determined that the main components of the essential oil were 1.8-cineole in the stem and leaf, camphor in the flower axis and linalool in the flower (Özel, 2023).

González-Burgos et al. (2011) reported that there were differences in quality and quantity between the essential oil components of the same species. They revealed that the differences in both essential oil ratio and essential oil components could be due to genetic and ecological factors as well as morphogenetic, ontogenetic and diurnal variations. In *Thymus vulgaris*, the total phenolic, flavonoid contents and antioxidant activities of the leaves vary depending on where they are located in the plant, and the highest antioxidant activity is found in the upper leaves (Rahimi et al., 2018). Özyazıcı and Kevseroğlu (2019) reported that the essential oil content and yield in mint, oregano, lavender and lemon balm plants vary depending on the genetic structure of the plant and the ecological conditions of the environment in which it is grown. In another study, Kevseroğlu and Özkul (1997) found that there was no essential oil in the stems of the lemon balm plant, but the highest essential oil content was found in the leaves. The results obtained in the present study are generally similar to the findings obtained by different researchers. Volatile components may vary depending on many factors such as locations, plant organs, collection time. In this study, components that were not found in studies conducted in the same species were also detected.

4. Conclusions

The diversity of essential oil constituents of *H. arenarium* subsp. *rubicundum* and their contribution to therapeutic properties expands the potential uses of the plant. The influence of regional and environmental factors on the chemical profiles of plants reveals the importance of such studies once again.

In future studies, the therapeutic aspects of these substances, especially the anti-inflammatory, antioxidant and antimicrobial effects of the components (methyl p-tert-butylphenylacetate, β-caryophyllene) should be investigated in more detail. The anti-inflammatory, antioxidant and antimicrobial effects of the components with high ratios (methyl p-tert-butylphenylacetate, β-caryophyllene) should be tested to understand the potential health benefits of these components.

In our study, when we examined the volatile compounds in different parts of the *H. arenarium* subsp. *rubicundum* plant, it was observed that the differences that emerged could be due to a number of reasons. The main reasons for these differences are genetic and ecological factors. However, factors such as different harvest and collection times of the plants (ontogenetic variability) and the use of different organs of the plant (morphogenetic variability) also play an important role.

In this context, the differences observed in the essential oil ratios, components and ratios of components can be explained by variables such as the genetic structure of the plant, the environmental conditions in which it grows, the time of collection and which part of the plant is used. Therefore, all of these factors create significant differences by affecting the chemical composition and ratios of essential oils. The results of this study are a source for studies on morphogenetic variability on *Helischyrsum* species.

Ethical Statement

The author declares that ethical approval is not required for this research.

Funding

This research received no external funding.

Declaration of Conflicts of Interest

No conflict of interest has been declared by the author.

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CITATION: Yurteri, E., 2025. Effect of Morphogenetic Variability on Volatile Oil Composition of Immortal Flower (*Helichrysum arenarium* subsp. *rubicundum*). *Turkish Journal of Agricultural Research*, 12(1): 1-8.