Bartın Orman Fakültesi Dergisi | Journal of Bartin Faculty of Forestry
2024, 26(1): 13–20 | DOI: 10.24011/barofd.1384784

Analysis of Essential Oil from *Calendula arvensis* L. (Field Marigold Flowers)

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Article History

Received: 01.11.2023
Accepted: 09.02.2024
Published: 29.02.2024

Research Article

Abstract – In this study, the quantity, structure, and classification of the essential oil obtained from the flowers of Field-grown Marigold (*Calendula arvensis* L.) individuals grown in the field environment in Maçka District, Trabzon Province, are presented. The essential oil of the collected and dried flowers was obtained using the Clevenger apparatus through the water distillation method. The components of the essential oil extracted from flowers were determined qualitatively using GC-MS/FID (Gas Chromatography-Mass Spectrometry/Flame Ionization Detector). The components that constitute 99.15% of the essential oil of field-grown Marigold have been identified. According to the results obtained, 74 known components were identified in the extracted essential oil from flowers, while only 2 components remained unidentified. The primary components of essential oil have been identified as %26.34 τ-kadinol, %10.99 δ-kadinol, %16.28 δ-kadinen, %8.79 α-thujen, and %6.04 α-pinen, respectively, in the order of their highest concentration. When considering the chemical classification of components identified in the flowers of *Calendula arvensis* L, sesquiterpenoids were found to be present at the highest proportion, amounting to 42.48%. When terpenes, terpenoids, or their derivatives were evaluated as components, it was found that they constituted 53 compounds and were present at an extremely high level of 93.32% in the essential oil of *Calendula arvensis* L.

Keywords – *Clevenger, Cadinol, Terpen, volatile oil, Thujen, Sesquiterpenoid*

Calendula arvensis L. (Portakal Nergisi) Çiçek Uçucu Yağının Analizi

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Makale Tarihçesi

Gönderim: 01.11.2023
Kabul: 09.02.2024
Yayım: 29.02.2024

Araştırma Makalesi

Öz − Bu çalışma kapsamında, Trabzon İli, Maçka İlçesi'nde tarla ortamında yetiştirilen Portakal nergisi (*Calendula arvensis* L.) bireylerinden toplanan çiçeklerden elde edilen uçucu yağının miktarı yapısı ve bileşenlerin sınıflandırılmasına yer verilmiştir. Toplanan ve kurutulan çiçeklerin uçucu yağı Clevenger aparatı kullanılarak su destilasyonu metodu ile elde edilmiştir. Çiçeklerden alınan uçucu yağın bileşenleri kalitatif olarak GC-MS/FID (gaz kromatografisi-kütle spektrometresi) ile belirlenmiştir. Portakal nergisi bitki çiçeklerinin uçucu yağını oluşturan bileşiklerin %99,15'i tanımlanmıştır. Belirlenen sonuçlara göre çiçeklerden elde edilen uçucu yağda 74 bilinen bileşen tespit edilirken 2 bileşen ise belirlenememiştir. Uçucu yağın ana bileşenleri miktarları sırasıyla %26,34 τ-kadinol, %10,99 δ-kadinol, %16,28 δ-kadinen, %8,79 *α*-thujen ve %6,04 *α*-pinen olarak belirlenmiştir. Çiçek uçucu yağında belirlenen bileşenlerinin kimyasal sınıflandırılmaları dikkate alındığında %42,48 ile seskiterpenoidler en yüksek oranda tespit edilmiştir. Terpen, terpenoid veya terpen benzeri sınıfı bileşikler olarak değerlendirildiğinde 53 adet bileşik ve %93,32 ile oldukça yüksek oranda Portakal nergisi çiçeklerinin uçucu yağında bulunmuştur.

Anahtar Kelimeler − *Clevenger, Kadinol, Terpen, Thujen, Seskiterpenoid, Uçucu yağ*

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1. Introduction

Calendula, a member of the Asteraceae family, comprises three indigenous species in Türkiye: *Calendula officinalis* L., *Calendula arvensis* L., and *Calendula suffruticosa* Wahl. *Calendula officinalis* L. (Asteraceae), which is widely cultivated and known for its aromatic yellow to orange flowers, is also referred to as "pot marigold" due to its historical usage in soups and stews to combat diseases. This herb is of significant importance in traditional medicine (Ak et al., 2021). Calendula is cultivated in a wide range of regions around the world, including Central, South, and Eastern Europe, West Asia, Germany, and the United States. It is known by different names in various countries, such as ringerblume, souci des jardins, and pot marigold. In Turkey, it is referred to as an ointment, gold, velvet, orange, thyme, dead flower, velvet flower, medical nergiz, and okgegözü (Şahingil, 2019). *C. arvensis* exhibits a range of therapeutic properties, including sedative, antibacterial, analgesic, lymphagogue, demulcent, choleretic, antitumor, anti-inflammatory, antioxidant, antiparasitic, antiviral, and antiseptic activities. *C. officinalis* has been found to possess a range of beneficial effects, including immunostimulant activity, protection for the liver, anti-HIV properties, antitumor activity, anti-inflammatory properties, the ability to cause muscle spasms, and the ability to relieve muscle spasms. *C. suffruticosa* is known for its antioxidant and antifungal activities and its ethanolic extract is particularly noteworthy (Servi et al., 2020).

Since the beginning of the 21st century, the composition of *C. officinalis* essential oil has been extensively studied. (Raal et al., 2016). The essential oil derived from the flowers of the Calendula genus possesses a characteristic scent and taste, which is attributed to the presence of mono and sesquiterpenes. This has been the reason for their widespread use in traditional medicine (Yoshikawa et al., 2021). Typically, the essential oil contains of Calendula significant amounts of γ-cadinene, δ-cadinene, and α-cadinol. These compounds were identified as the major constituents of the oil (Raal et al. 2016). According to recent findings, cadinane and muurolane were reportedly present in significant amounts within the essential oils extracted from *C. officinalis* (Paolini et al., 2010). Recently, several initiatives have been launched to deepen our comprehension of Calendula species therapeutic attributes and improve the output of their advantageous compounds in their essential oils (Khalid and Silva, 2010). According to research conducted by Gazim and colleagues, the essential oil extracted from the flowers of *C. officinalis* demonstrated in vitro antifungal activity against various clinical strains of fungi (Gazim et al., 2008b). The research conducted by Mishra et al. has demonstrated that applying Calendula essential oil to the skin can prevent UV-B-induced changes in antioxidant levels in skin tissue (Mishra et al., 2012).

Due to the economic significance of C. officinalis as a medicinal herb and its widespread application in the production of cosmetics, perfumes, pharmaceutical preparations, and food items, there has been a growing interest in the potential health benefits of this plant species (Gazim et al., 2008a). The use of marigold flowers is prevalent in the creation of perfumes, where their distinct aroma is attributed to the presence of sesquiterpenes in the volatile fraction. Specifically, the δ-cadinene present in this fraction provides notes of green, sweet, and refreshing aroma, while the α-cadinol contributes to a woody notes (Şahingil, 2019).

This study aims to contribute to the literature by determining the amount and structure of essential oil obtained from *Calendula arvensis* L. (Field Marigold) flowers grown in a field environment and by classifying the detected compounds.

2. Material and Method

2.1. Material

Calendula arvensis L. (Field Marigold) flower samples were collected from plants grown in a field environment at an altitude of 1033 meters in Gürgen Ağaç locality of Maçka District, Trabzon Province, in August 2019. The collected samples were dried in the shade and stored in a dry and cool environment until analysis.

2.2. Extraction of Essential oils

Homogenized dried flower samples weighing 25 g were placed in a 2 L round-bottom flask of a Clevenger apparatus, to which 1000 mL of pure water was added. n-Hexane (2 mL) was introduced to the collection part of the Clevenger apparatus. The cooler temperature was set to +4.0 °C. The essential oils were extracted by boiling at low temperature for 4 h. The percentage yield of essential oils was calculated for flowers based on their weight (Küçük et al., 2006).

2.3. Analysis of Essential oil Components Using GC-MS/FID

The essential oils obtained by hydrodistillation in the Clevenger system were dissolved in hexane, filtered, and transferred to dark-colored vials that were placed in the autosampler section. The GC analysis duration was 67 min, during which the components were separated. The essential oils were analyzed using Gas Chromatography with Flame Ionization Detection (FID). GC-MS analyses were carried out on an Agilent-5975 model instrument, and an HP-5MS model non-polar capillary column (30 m x 0.32 mm, film thickness 0.25 µm) was used for the analysis. Helium was used as the carrier gas at a flow rate of 1 mL/min, and the injections were applied in splitless mode at 240 °C. A 1 µL solution of essential oil in hexane (GC grade) was injected, initially held at 60 °C for 2 min, followed by an increase in temperature at 3 °C/min until 240 °C was reached. After the separation of volatile compounds from the gas chromatography column, the individual mass spectra for each compound were obtained. Compound identification was performed by comparing the mass spectra of each component with reference compounds in the Wiley and NIST libraries, and the retention times of the identified compounds were compared with literature data (Adams 2007).

3. Result and Discussion

The yield of essential oil, calculated as a percentage, was 0.69% (w/w) based on the hydrodistillation process conducted with 25 g of dried *Calendula arvensis* L. (Field Marigold) flowers.

The volatile components exhibited high matching rates (at least 80%) and their chemical structures were identified by comparing their retention times. GC-MS/FID analysis of the essential oil revealed the structures of 74 natural compounds, while the structures of the two compounds remained undetermined. The total volatile compounds in the essential oil were identified at a rate of 99.15%. Table 1 presents the names, classes, retention times, Kovats indices, and literature Kovats indices of the compounds found in Field Marigold flowers, as determined by GC-MS/FID analysis.

Table 1.
GC-MS/FID Analysis Results of Essential oil Obtained from *Calendula arvensis* L. (Field Marigold) Flowers

No	Retention Time (RT)	% Area	Compound Name	Compound Class	Kovats Index	Literature Kovats Index
1	7.24	0.17	Hexanal	Aldehyde	798	798
2	8.42	0.01	Furfural	Aldehyde	830	830
3	9.16	0.02	(E)-2-Hexenal	Aldehyde	849	849
4	10.65	0.02	2-Nonanone	Ketone	889	889
5	11.11	0.03	Heptanal	Aldehyde	901	901
6	12.30	8.79	α-Thujene	Monoterpene	927	927
7	12.60	6.04	α-Pinene	Monoterpene	933	933
8	13.25	0.05	Camphene	Monoterpene	947	947
9	13.81	0.03	Benzaldehyde	Aldehyde	959	959
10	14.42	0.52	Sabinene	Monoterpene	972	972
11	14.56	0.20	β-Pinene	Monoterpene	975	975
12	15.06	0.16	6-Methyl-5-hepten-2-one	Ketone	986	986
13	15.56	0.04	(E,E)-2,4-Heptadienal	Aldehyde	997	997
14	15.89	0.20	α-Fellandren	Monoterpene	1004	1004
15	16.17	0.08	3-Carene	Monoterpene	1010	1010
16	16.49	0.16	α-Terpinene	Monoterpene	1016	1016

Table 1 continue

17	16.88	0.08	p-Cymene	Monoterpene	1024	1024
18	17.07	0.12	Limonene	Monoterpene	1028	1028
19	17.22	0.06	Eucalyptol	Monoterpene	1031	1031
20	17.83	0.09	Benzyl Acetaldehyde	Aldehyde	1043	1043
21	18.57	0.30	γ-Terpinene	Monoterpene	1058	1058
22	20.02	0.05	α-Terpinolen	Monoterpene	1088	1088
23	24.41	0.48	Terpinen-4-ol	Monoterpeneoid	1179	1179
24	26.46	0.06	β-Cyclocitral	Monoterpeneoid	1222	1222
25	29.77	0.11	Thymol	Monoterpeneoid	1294	1294
26	30.81	0.08	(E,E)-2,4-Decadienal	Aldehyde	1317	1317
27	32.36	0.08	α-Cubebene	Sesquiterpene	1352	1352
28	32.66	0.05	Eugenol	Terpenoid	1359	1359
29	33.35	0.14	Ylangene	Sesquiterpene	1375	1375
30	33.54	0.47	α-Copaene	Sesquiterpene	1379	1379
31	33.95	0.14	β-Bourbonene	Sesquiterpene	1388	1388
32	34.15	0.12	β-Cubebene	Sesquiterpene	1393	1393
33	35.02	0.16	α-Gurjunene	Sesquiterpene	1414	1414
34	35.47	1.40	Caryophyllene	Sesquiterpene	1424	1424
35	35.72	0.13	α-Ionone	Monoterpeneoid	1430	1430
36	36.73	0.34	(E)-Geranyl Acetone	Monoterpeneoid	1454	1455
37	36.90	0.98	Humulene	Sesquiterpene	1459	1459
38	37.19	0.11	γ-Muurolene	Sesquiterpene	1466	1466
39	37.28	0.20	epi-Bisiklosesquiphellan-drene	Sesquiterpene	1468	1469
40	37.84	0.67	α-Amorphene	Sesquiterpene	1481	1481
41	37.94	1.75	(Z)-(-)-2,4a,5,6,9a-Hexahy-dro-3,5,5,9-tetrame-thyl(1H)benzocycloheptene	Sesquiterpene	1484	1484
42	38.06	1.72	Germacrene D	Sesquiterpene	1487	1487
43	38.17	0.53	trans-β-Ionone	Monoterpeneoid	1489	1489
44	38.27	0.33	β-Selinene	Sesquiterpene	1492	1492
45	38.80	2.13	α-Muurolene	Sesquiterpene	1505	1505
46	39.02	0.17	α-Farnesene	Sesquiterpene	1510	1510
47	39.41	4.17	γ-Cadinene	Sesquiterpene	1520	1520
48	39.83	16.28	δ-Cadinene	Sesquiterpene	1531	1531
49	40.11	0.56	Cadine-1,4-diene	Sesquiterpene	1538	1539
50	40.31	0.60	α-Cadinene	Sesquiterpene	1543	1544
51	40.74	0.41	Germacrene B	Sesquiterpene	1554	1555
52	41.20	0.25	Ledol	Sesquiterpenoid	1565	1565
53	41.56	0.14	(Z)-3-Hexenyl benzoate	Ester	1574	1575
54	41.83	0.34	Not Found		1582	-
55	42.17	0.32	Kusimone	Sesquiterpenoid	1590	1592
56	42.37	0.51	Not Found		1595	-
57	42.60	2.79	Hexadecane	Hydrocarbon	1601	1600
58	43.15	0.63	β-Oplophenone	Sesquiterpenoid	1616	1611
59	43.35	0.42	Tetradecanal	Aldehyde	1621	1621
60	43.54	0.21	γ-Eudesmol	Sesquiterpenoid	1626	1626
61	43.86	1.22	β-Eudesmol	Sesquiterpenoid	1635	1635
62	44.49	10.99	δ-Cadinol	Sesquiterpenoid	1652	1652

Table 1 continue

63	44.80	1.26	γ-Cadinol	Sesquiterpenoid	1660	1658
64	45.10	26.34	τ-Cadinol	Sesquiterpenoid	1668	1665
65	46.08	0.46	Heptadecane	Hydrocarbon	1699	1700
66	46.69	0.26	Pentadecanal	Aldehyde	1711	1711
67	47.25	0.21	Farnesol	Sesquiterpenoid	1726	1725
68	47.59	0.49	Valerenol	Sesquiterpenoid	1736	1736
69	48.52	0.08	Benzil benzoat	Ester	1762	1762
70	49.29	0.26	2(1H)Naftalenon, 3,5,6,7,8,8a-hegzahidro-4,8a-dimetil-6-(1-metilethenil)-	Sesquiterpenoid	1784	1790
71	50.10	0.13	Etilen glikol difenil eter	Ether	1807	1810
72	50.94	0.39	Hexadecanal	Aldehyde	1832	1830
73	51.40	0.32	Hexahydrofarnesyl acetone	Sesquiterpenoid	1845	1845
74	53.19	0.17	Nonadecane	Hydrocarbon	1898	1900
75	59.54	0.16	Heneicosane	Hydrocarbon	2098	2100
76	65.47	0.08	Tricosane	Hydrocarbon	2297	2300
	100.00					

The compounds with the highest percentage in the essential oil were τ-cadinol (26.34%), δ-cadinene (16.28%), eucalyptol (10.99%), α-thujene (8.79%), and α-pinene (6.04%). The volatile compound found in the highest amount in the essential oil was τ-cadinol. The chemical classification of the compounds found in the Field Marigold flower essential oil is provided in Table 2.

Table 2.
Chemical Classification of Compounds Found in Essential oil Obtained from *Calendula arvensis* L. (Field Marigold) Flowers

Compound Class	Number of Compounds	Percentage (%)	Main Component
Aldehydes	11	1.54	Tetradecanal
Ethers	1	0.13	Ethylene glycol diphenyl ether
Esters	2	0.22	(Z)-3-Hexenyl benzoate
Hydrocarbons	5	3.66	Hexadecane
Ketones	2	0.18	6-Methyl-5-hepten-2-one
Monoterpenes	13	16.65	α-Thujene
Monoterpenoids	6	1.65	(E)-β-Ionone
Sesquiterpenes	21	32.59	δ-Cadinene
Sesquiterpenoids	12	42.48	τ-Cadinol
Terpene (as)	1	0.05	Eugenol
Unknown	2	0.85	
Total	76	100	

According to the GC-MS/FID results of the essential oil, the 76 compounds identified in the essential oil of Field Marigold flowers were categorized into 11 groups according to their chemical classifications. Upon evaluating the chemical compound classes, the chemical structure class identified as sesquiterpenoids were determined as the highest quantity within the chemical composition of the essential oil, with 42.18 %. The other compound classes were sesquiterpenes (32.59 %) and monoterpenes (16.65 %). Upon closer examination of Table 2, it is evident that the compound classes with the highest number of compounds were sesquiterpenes with 21 compounds, monoterpenes with 13 compounds, sesquiterpenoids with 12 compounds, and aldehydes with 11 compounds. In total, terpenes, terpenoids, and similar compounds accounted for 93.42% of the composition and were represented by 53 compounds in the flower samples (Table 2). In a study on *C. arvensis* collected from Istanbul, the essential oil obtained from the above-ground parts, with a yield of 0.38% (v/w), was analyzed by GC-MS. The study reported that the oil consisted of 36 compounds, and the major compounds were δ-cadinene (14.8%), epicubebol (10.7%), α-cadinol (8.5%), cubenol (7.7%), and cubebol (7.2%) (Servi et al., 2020). Tosun et al. (2012) investigated the effect of essential oil isolation from *C. arvensis* L. (fresh plant material) collected from Akçaabat, Trabzon, using hydrodistillation (HD) and microwave distillation (MD). According to the study results, they identified a total of 45 and 44 compounds in *C. arvensis* oil, constituting 88.3% and 84.8% of the composition, respectively. The major terpene components in *C. arvensis* oils have been reported to be α-selinene (HD, 16.0%; MD, 0.0%), α-pinene (HD: 11.9%, MD: 12.3%), (Z)-α-santalol (HD: 8.2%, MD: 7.4%), δ-amorphene (HD: 0.0%, MD: 8.0%), and (Z)-sesquilavandulol (HD, 4.8%; MD, 0.0%). The main groups of volatile compounds were sesquiterpenes (HD, 30.5%; MD, 23.4%) and monoterpenes (HD, 26.3%; MD, 24.3%). In a different study, the essential oils of *C. officinalis* flowers were analyzed by GC-MS, revealing the major components to be α-cadinol (20.6%), trans-β-ocimene (19.6%), carvenone (17.9%), carvacrol (16.8%), cadinene (10.1%), 1,8 cineole (7.65%), limonene (4.2%), and α-pinene (2.11%) (Sahingil, 2019). Another study by Okoh et al. (2008) reported the essential oil of fresh *C. officinalis* flowers and identified the major essential oil components as α-thujene (26.9%), *T*-muurolol (24.9%), and δ-cadinene (13.1%). It has also been mentioned that sesquiterpenoids dominate the chemical classification of compounds in fresh flowers, accounting for 26%. Comparing the results of this study with the literature, it can be observed that the percentage of essential oil, the number of identified compounds, the dominant volatile compounds (cadinol, α-thujene, δ-cadinene, and α-pinene), and the chemical compound classifications (sesquiterpenoids, sesquiterpenes, and monoterpenes) were similar. Variations in the results can be attributed to factors such as geographical location, climate, natural variations, collection time, storage conditions, and analytical parameters (Karataş et al., 2022). Differences in the composition of volatiles can vary depending on the environmental conditions, cultivation, and preparation methods (El-Hawary et al., 2018).

4. Conclusion

This study aimed to investigate the chemical composition of the essential oil obtained from the dried flowers of *Calendula arvensis* L., known as Field Marigold. The study revealed that yield of the essential oil was determined to be 0.69% through hydrodistillation. The structures of 74 compounds were identified through GC-MS/FID analysis, although two compounds could not be specifically identified. The major components in the flower essential oil were τ-cadinol, δ-cadinene, eucalyptol, α-thujene, and α-pinene. In terms of the chemical classification of volatile compounds, sesquiterpenoids, sesquiterpenes, and monoterpenes were the most abundant compounds. The results from this study are believed to be beneficial for various industries such as the food, cosmetic, perfumery, and pharmaceutical sectors. In addition, they may contribute to future research on this plant.

Further research should be conducted to explore the potential applications of the identified compounds in these industries and investigate their specific properties and benefits. Further studies could focus on optimizing the extraction and isolation techniques for these compounds, potentially increasing the yield and purity of the essential oil. Further studies could evaluate the impact of different growing conditions, harvest times, and storage methods on the chemical composition of Field Marigold essential oil. These endeavors will provide valuable insights into the practical applications of this natural resource.

Acknowledgements

This publication was presented as an oral presentation at the 5th International Symposium on Non-Wood Forest Products.

Author Contribution

The planning, designing, gathering of examples, conducting analyses, and writing of the article were carried out by Onur Tolga OKAN.

Conflict of Interest

The author have no conflicts of interest to declare that are relevant to the content of this article.

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