



A COMPREHENSIVE ANALYSIS OF THE CHEMICAL PROFILE OF VOLATILE COMPOUNDS IN NARCISSUS TAZETTA L. SUBSP. TAZETTA THROUGH HIERARCHICAL CLUSTERING METHODS

NARCISSUS TAZETTA L. SUBSP. TAZETTA UÇUCU BİLEŞİKLERİNİN KİMYASAL PROFİLİNİN HİYERARŞİK KÜMELEME YÖNTEMLERİYLE KAPSAMLI ANALİZİ

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ABSTRACT

Objective: This study aimed to analyze the chemical profile of volatile and non-volatile components of *Narcissus tazetta* subsp. *tazetta* (daffodil) is grown in Türkiye. The chemical profiles of fresh and dried flowers were analyzed using Headspace Solid-Phase Microextraction (HS-SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) methods. The study also aimed to investigate the effects of the drying process on the chemical profile and contribute to the plant's pharmacological, commercial, and ecological potential.

Material and Method: Fresh and dried flower samples collected from the Muğla region were analyzed using the HS-SPME method. Fresh samples were immediately analyzed to prevent the loss of volatile compounds, while dried samples were air-dried at room temperature until they reached a constant weight and stored at 4°C. The mass spectra of volatile compounds were evaluated using GC/MS in conjunction with the Başer Library and other reference databases. Hierarchical Cluster Analysis (HCA) was applied for statistical analysis and clustering.

Result and Discussion: The main volatile compounds identified in fresh flowers were (E)-β-ocimene (62.8%), 1,8-cineole (12.9%), and linalool (7.6%). In dried flowers, (E)-β-ocimene (43.1%), (Z)-3-hexenal (18.7%), and (Z)-3-hexenyl acetate (9.8%) were prominent. Significant changes were observed in volatile compounds after drying, with monoterpene hydrocarbons decreasing and new aldehyde and ester compounds emerging. The influence of regional and environmental factors on the chemical profile was emphasized. The Muğla samples showed differences compared to samples from İzmir, Siirt, and Şırnak. HCA successfully grouped the compounds based on structural and functional similarities, statistically highlighting the chemical differences between fresh and dried flowers.

Keywords: Chemical profile, hierarchical cluster analysis, *Narcissus tazetta*, volatile compounds

ÖZ

Amaç: Bu çalışmada, Türkiye'de yetişen *Narcissus tazetta* subsp. *tazetta* (nergis) bitkisinin uçucu ve uçucu olmayan bileşenlerinin kimyasal profili incelenmiştir. Taze ve kurutulmuş çiçeklerin kimyasal profilleri, Tepe Boşluklu- Katı Faz Mikroekstraksiyon (TB-KFME) ve Gaz Kromatografisi-Kütle Spektrometresi (GK-KS) yöntemleriyle analiz edilmiştir. Çalışma, kurutma işleminin kimyasal

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Submitted / Gönderilme : 19.10.2024

Accepted / Kabul : 03.01.2025

Published / Yayınlanma : 19.05.2025

profil üzerindeki etkilerini araştırmayı ve bitkinin farmakolojik, ticari ve ekolojik potansiyeline katkı sağlamayı amaçlamaktadır.

Gereç ve Yöntem: Muğla bölgesinden toplanan taze ve kurutulmuş çiçek örnekleri, HS-SPME yöntemiyle analiz edilmiştir. Taze örnekler uçucu bileşik kaybını önlemek için hemen analiz edilirken, kurutulmuş örnekler gölgede, oda sıcaklığında sabit ağırlığa gelene kadar kurutulmuş ve 4°C'de saklanmıştır. Uçucu bileşiklerin kütle spektrumu, GK-KS ile Başer Library ve diğer referans kütüphaneler kullanılarak değerlendirilmiştir. Verilerin istatistiksel analizi ve kümeleneşmesi için Hiyerarşik Kümeleme Analizi (HCA) uygulanmıştır.

Sonuç ve Tartışma: Taze çiçeklerde ana uçucu bileşikler arasında (E)- β -osimen (%62.8), 1,8-sineol (%12.9) ve linalol (%7.6) bulunmuştur. Kurutulmuş çiçeklerde, (E)- β -osimen (%43.1), (Z)-3-hekzenal (%18.7) ve (Z)-3-hekzenil asetat (%9.8) ön plana çıkmıştır. Kurutma işlemiyle uçucu bileşiklerde önemli değişimler gözlemlenmiş, özellikle monoterpen hidrokarbonlar azalırken, yeni aldehit ve ester bileşikler ortaya çıkmıştır. Bölgesel ve çevresel faktörlerin kimyasal profil üzerindeki etkisi vurgulanmıştır. Muğla örnekleri, İzmir, Siirt ve Şırnak'taki örneklerle karşılaştırıldığında farklılık göstermiştir. HCA, bileşiklerin yapısal ve işlevsel benzerliklerine göre gruplandırılmasını sağlayarak, taze ve kurutulmuş çiçekler arasındaki kimyasal farklılıkları istatistiksel olarak ortaya koymuştur.

Anahtar Kelimeler: Hiyerarşik kümeleme analizi, kimyasal profil, *Narcissus tazetta*, uçucu bileşikler

INTRODUCTION

Narcissus tazetta L., commonly known as daffodil, belongs to the Amaryllidaceae family and is widely distributed across various regions, including Türkiye. It is represented in Türkiye by two subspecies, *N. tazetta* L. subsp. *tazetta* and *N. tazetta* L. subsp. *aureus* (Jord. & Fourr.) Baker [1]. This species holds great ethnobotanical significance due to its historical applications in traditional medicine, ornamental use, and the unique aroma of its flowers, which is valued in perfumery and aromatherapy [2,3].

N. tazetta has been extensively used in traditional medicine for its antispasmodic, emetic, analgesic, and antitumor properties [2]. In East Asian countries such as China and Japan, the bulbs and flowers of *N. tazetta* are traditionally applied to treat inflammation, wounds, and skin disorders [4]. In Türkiye, while its medicinal applications are less documented, the plant plays a cultural role as an ornamental species, and its aromatic extracts are used in folk remedies and fragrance formulations [5].

The therapeutic value of *N. tazetta* primarily stems from its rich alkaloid content, a characteristic feature of the Amaryllidaceae family. Among its key alkaloids, galanthamine is widely recognized for its acetylcholinesterase inhibitory activity and is clinically utilized in Alzheimer's disease and other cognitive disorders [3]. Lycorine exhibits significant antiviral, anti-inflammatory, and antitumor properties, with prominence for its ability to inhibit cancer cell proliferation [6]. Similarly, narciclasine demonstrates potent anti-inflammatory and immunomodulatory effects while being actively studied for its antitumor potential [4]. Although less studied, another notable alkaloid, tazettine shows emerging evidence for its neuroprotective and cytotoxic activities [3]. Together with phenolic and flavonoid compounds, these alkaloids form the chemical foundation underlying the pharmacological properties of *N. tazetta*.

The volatile profile of *N. tazetta* is dominated by monoterpenes such as (E)- β -ocimene, linalool, and 1,8-cineole, contributing to its distinct aroma and bioactivity. These monoterpenes are known for their significant roles in plant defense mechanisms, particularly in deterring herbivores and attracting pollinators [3]. Non-volatile constituents include alkaloids, phenolic acids, and flavonoids, enhancing the plant's antioxidant capacity and therapeutic potential. For example, phenolic compounds and flavonoids contribute to the plant's antioxidant activity and exhibit anti-inflammatory and neuroprotective properties [5]. These findings emphasize the dual importance of volatile and non-volatile components in defining the pharmacological and ecological value of *N. tazetta*.

N. tazetta has notable commercial value as an ornamental plant and a source of essential oils. Its aromatic compounds are widely utilized in perfumery, while its alkaloid content positions it as a potential raw material for pharmaceutical applications [6]. Moreover, recent advancements in analytical

methods have paved the way for a deeper understanding of its chemical diversity, opening new opportunities for its utilization in various industries.

This study aims to comprehensively analyse of the volatile and non-volatile compounds of *N. tazetta* subsp. *tazetta*, grown in Türkiye, uses advanced analytical techniques such as Headspace Solid-Phase Microextraction (HS-SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). By examining fresh and dried flowers, this study seeks to understand the impact of drying on the plant's chemical profile, contributing to its broader pharmacological, commercial, and ecological context.

MATERIAL AND METHOD

General Experimental Procedures

N. tazetta subsp. *tazetta* fresh flowers were collected from the Datça (Muğla) region of Türkiye during their natural flowering season (O.Tugay 17.983 & D.Ulukuş; KNYA Herbarium no: 29.972). Fresh flowers were analyzed immediately to preserve their volatile profiles. At the same time, dried samples were prepared by air-drying at room temperature in a shaded environment until a constant weight was reached. The samples were stored in airtight containers at 4°C prior to analysis to prevent loss or degradation of volatile compounds.

Analysis of Volatile Compounds Using HS-SPME

The fresh and dried flowers of the plant were directly analyzed using HS-SPME. A manual SPME device (Supelco, Taufkirchen, Germany) equipped with a fibre coated with a 65 µm-thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB) was employed to extract the volatiles. For the analysis, 100 mg of fresh plant material was placed in a 10 ml vial, sealed with parafilm to allow saturation of the headspace with volatiles. The fibre was then inserted through the parafilm and exposed to the material's headspace for 15 min at room temperature [7].

Determination of Volatile Components Mass Spectra Using GC/MS

Gas Chromatography-Mass Spectrometry (GC/MS) was used to determine the mass spectra of volatile oils. The study utilized an Agilent 5975 GC/MSD system with an HP-Innowax polar column (60 m × 0.25 mm i.d., 0.25 µm film thickness) and helium as the carrier gas (flow rate: 0.8 ml/min). The injection port temperature was set to 250 °C. Analyses were performed using 70 eV electron energy within a mass range of 35-450 m/z.

A total temperature program of 80 min was applied: starting at 60°C for 10 min, increasing at a rate of 4°C/min to 220°C, holding at 220°C for 10 minutes, and then increasing at a rate of 1°C/min to 240°C. Data evaluation was conducted using the "Başer Library of Essential Oil Components" as well as Wiley and MassFinder 4 Library Search Software [8]. The results are presented in Table 1.

Application of Hierarchical Cluster Analysis (HCA)

Minitab 19 (State College, PA, USA) was used for statistical analysis. The number of clusters was determined by using a cutoff point (Euclidean distance) that enables the formation of consistent clusters and by examining the rescaled distances in the dendrogram. Hierarchical Cluster Analysis (HCA) was employed to assess the similarity between the chemical compositions of volatile compounds [9].

RESULT AND DISCUSSION

Volatile Components in *Narcissus tazetta* Flowers

In this study, the volatile compound profile of both fresh and dried flowers of *N. tazetta* subsp. *tazetta* was comprehensively analyzed. Using the Solid-Phase Microextraction (SPME) method, significant differences were observed in the chemical profiles of the flowers before and after the drying process. The aroma profile of fresh flowers was notably altered post-drying, with some volatile compounds decreasing in concentration or disappearing entirely. Monoterpene hydrocarbons and oxygenated monoterpenes were identified as the most dominant compound groups in both fresh and

dried samples, highlighting the natural aromatic richness of the plant, particularly in its monoterpenes and their oxygenated derivatives. Additionally, the findings indicated that these compounds, which were abundant in fresh flowers, decreased or nearly vanished after the drying process. These results provide crucial insights into the effects of drying on the volatile compound profile of the plant.

These analyses revealed that the major volatile compounds identified in the fresh flowers were (*Z*)- β -ocimene (62.8%), 1,8-cineole (12.9%), and linalool (7.6%), while in the dried flowers, (*Z*)- β -ocimene (43.1%), (*Z*)-3-hexenal (18.7%), and (*Z*)-3-hexenyl acetate (9.8%) were found (Table 2).

The analysis further revealed that (*E*)- β -ocimene, the most abundant compound in fresh flowers (62.8%), was followed by a group of oxygenated monoterpenes. The high (*E*)- β -ocimene levels and other monoterpenes in fresh samples suggest a distinctive and intense aromatic structure. In dried flowers, however, the concentration of these compounds notably declined while new compounds categorized as “other” emerged. The proportion of monoterpene hydrocarbons decreased to 43.1% in dried samples, and oxygenated monoterpenes were reduced to a low 0.3%. These changes indicate that the drying process reshapes the volatile profile of the plant, leading to a marked transformation compared with the fresh flower profile. The substantial reduction of oxygenated monoterpenes specifically underscores the chemical transformations occurring throughout the drying process.

Table 1. The volatile components of *N. tazetta* subsp. *tazetta*

RRI ^[a]	Compound	Fresh flowers (%) ^[b]	Dried flowers (%) ^[b]
1032	α -Pinene	1.1	-
1093	Hexanal	-	3.6
1136	Isoamyl acetate	4.0	-
1174	Myrcene	1.8	-
1213	1,8-Cineole	12.9	-
1225	(<i>Z</i>)-3-Hexenal	-	18.7
1246	(<i>Z</i>)- β -Ocimene	1.0	-
1266	(<i>E</i>)- β -Ocimene	62.8	43.1
1327	(<i>Z</i>)-3-Hexenyl acetate	0.6	9.8
1340	(<i>E</i>)-2-Hexenyl acetate	-	1.3
1360	1-Hexanol	-	0.3
1382	<i>cis</i> -Alloocimene	0.8	-
1391	(<i>Z</i>)-3-Hexen-1-ol	-	4.0
1409	<i>trans</i> -Alloocimene	0.2	-
1412	(<i>E</i>)-2-Hexen-1-ol	-	0.9
1496	2-Ethyl hexanol	-	0.1
1553	Linalool	7.6	0.3
1706	α -Terpineol	0.6	-
1747	Benzyl acetate	3.9	1.6
1838	2- Phenylethyl acetate	0.9	0.5
1896	Benzyl alcohol	-	0.9
1935	Phenyl ethyl alcohol	-	0.4
Monoterpene hydrocarbons		67.7	43.1
Oxygenated monoterpenes		21.1	0.3
Sesquiterpene hydrocarbons		-	-
Oxygenated sesquiterpenes		-	-
Fatty acid		-	-
Others		9.4	42.1
Total		98.2	85.5

^[a]: Relative retention indices calculated against *n*-alkanes; ^[b]: calculated from FID data

The major volatile compounds identified in this study, particularly (*E*)- β -ocimene, 1,8-cineole, and linalool, align with findings from previous research on *N. tazetta*. Studies conducted by Chen et al. (2013) and Melliou et al. (2007) have similarly reported (*E*)- β -ocimene as the predominant compound,

with concentrations exceeding 60% [4,10]. The presence of linalool, observed in our fresh flower samples at 7.6%, is consistent with the findings of Seleem and Salem (2020), who reported similar concentrations using ethyl ether extraction [6]. These alignments validate the robustness of the HS-SPME method employed in our study and confirm its suitability for analyzing the volatile compounds of *N. tazetta* species.

Table 2. Studies on the volatile compounds of *Narcissus tazetta*

HCA no	Plant name	Country	Plant parts	Method	Major compounds	Ref.
1	<i>N. tazetta</i> subsp. <i>tazetta</i>	Muğla/ Türkiye	Fresh flowers	HS-SPME	(<i>E</i>)- β -Ocimene (62.8%), 1,8-cineole (12.9%), linalool (7.6%)	Our study
2	<i>N. tazetta</i> subsp. <i>tazetta</i>	Muğla/ Türkiye	Dried flowers	HS-SPME	(<i>E</i>)- β -Ocimene (43.1%), (<i>Z</i>)-3-hexenal (18.7%), (<i>Z</i>)-3-hexenyl acetate (9.8%)	Our study
3	<i>N. tazetta</i>	İzmir/ Türkiye	Flowers	HS-SPME	Benzyl acetate (30.4%), β -cimene (10.9%), 3-hexenyl acetate (8.5%)	[5]
4	<i>N. tazetta</i>	Siirt/ Türkiye	Flowers	HS-SPME	3-Hexenyl acetate (37.5%), benzyl acetate (30.1%), β -cimene (10.3%)	[5]
5	<i>N. tazetta</i>	Şirnak/ Türkiye	Flowers	HS-SPME	Benzyl acetate (34.1%), β -cimene (33.6%), 3-hexenyl acetate (12.9%)	[5]
6	<i>N. tazetta</i>	Egypt	Flowers	Ethyl ether extract	α -Pinene (15.38%), α -terpinene (15.27%), ethyl cinnamate (13.68%), and linalool (11.60%)	[6]
7	<i>N. tazetta</i>	Egypt	Flowers	Ethyl ether extract	Linalool (19.34%), methyl cinnamate (11.91%), ethyl cinnamate (10.61%), and limonene (8.31%)	[6]
8	<i>N. tazetta</i>	Egypt	Flowers	Ethyl ether extract	α -Pinene (22.24%), ethyl cinnamate (15.89%), α -terpineol (14.86%), and linalool (13.42%)	[6]
9	<i>N. tazetta</i> subsp. <i>tazetta</i>	Greece	Flowers	Water distillation	(<i>E</i>)- β -Ocimene (61.12%), 3-phenylpropyl acetate (6.4%), and benzyl acetate (6.04%)	[10]
10	<i>N. tazetta</i> var. <i>chinensis</i>	China	Single and double flowers	HS-SPME	(<i>E</i>)- β -Ocimene (62.73%, 66.06%) and benzyl acetate (11.65%, 25.02%), respectively	[4]

However, distinct differences were observed in the composition of dried flowers, where (*Z*)-3-hexenal (18.7%) and (*Z*)-3-hexenyl acetate (9.8%) were prominent. This shift in chemical profile is rarely addressed in existing literature, as most studies have predominantly focused on fresh flowers. The present work fills this gap, demonstrating the impact of drying on volatile compound transformation, likely influenced by enzymatic and oxidative processes.

Additionally, the total volatile content of fresh flowers (98.2%) and dried flowers (85.5%) reported in this study is notably higher compared to previous works [5]. This finding emphasizes our methodology's analytical precision while suggesting that regional and environmental factors unique to the Muğla region of Türkiye may contribute to these variations.

Another unique aspect of this study is the regional diversity of volatile profiles among *N. tazetta* samples from Türkiye. The identification of (*E*)- β -ocimene as the dominant compound aligns with findings from studies conducted in Egypt, Greece, and China, suggesting that this compound is a key chemotaxonomic marker for the species. However, the variations in secondary compounds such as benzyl acetate and 3-hexenyl acetate indicate potential influences of regional climatic and soil conditions, which merit further ecological investigation.

Furthermore, the comparison of results from the Muğla region with those from İzmir, Siirt, and Şırnak, as reported by Zarifikhosroshahi et al. (2021), highlights a significant variation in compound distribution [5]. These regional differences underscore the role of local environmental factors and cultivation conditions, which may profoundly affect volatile compounds' biosynthesis.

A significant strength of this study lies in applying of hierarchical clustering analysis (HCA), which enabled the classification of compounds into distinct groups based on their structural and functional similarities. This analytical approach, rarely implemented in previous studies on *N. tazetta*, enhances our understanding of the relationships between compounds and provides a more comprehensive overview of the volatile profile (Figure 1).

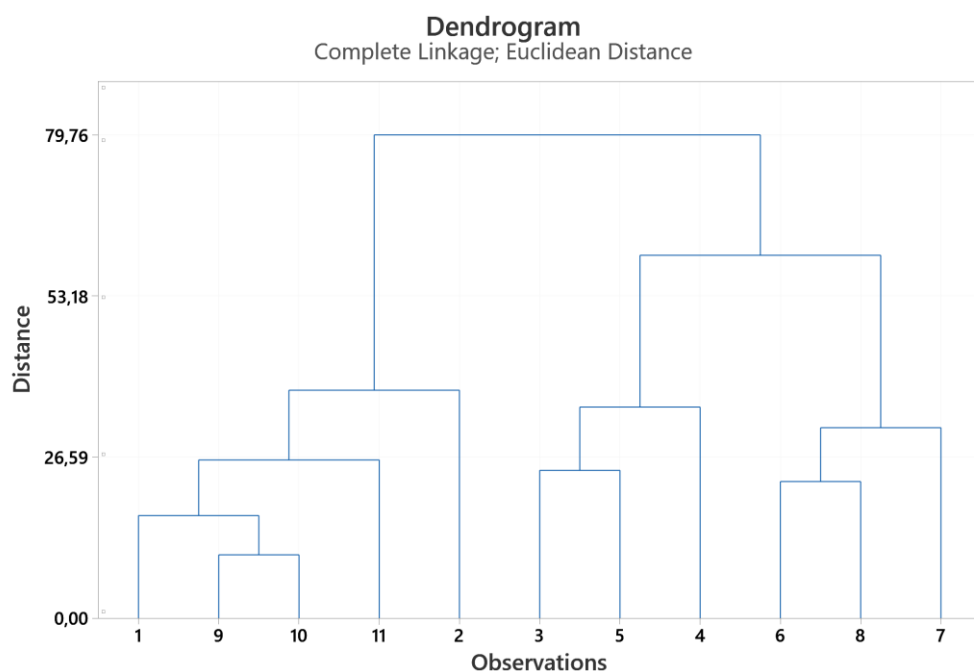


Figure 1. Hierarchical clustering dendrogram showing the similarity relationships among volatile compounds of *N. tazetta* subsp. *tazetta* fresh and dried flowers. The analysis highlights the clustering of compounds based on structural and functional similarities, with notable distinctions between fresh and dried flower profiles

The volatile compound profiles of *N. tazetta* subsp. *tazetta* fresh and dried flowers were analyzed using hierarchical clustering analysis (HCA). The results highlighted notable differences and similarities in the chemical composition of the samples. Compounds in clusters 9 and 10 exhibited the highest similarity (86.88%), indicating a close chemical relationship between these groups. In contrast, no statistical similarity was observed among clusters in the range of 1 to 3, showcasing clear compositional divergence. Furthermore, cluster 1 was found to be most like cluster 9 (78.79%), while cluster 2 demonstrated a lower similarity with cluster 1 (37.61%).

The chemical profiles revealed significant differences between fresh and dried flowers. Fresh flowers were dominated by (*E*)- β -ocimene (62.8%) and 1,8-cineole (12.9%), while dried flowers contained (*Z*)-3-hexenal (18.7%) and (*Z*)-3-hexenyl acetate (9.8%) as major components. The total volatile content was higher in fresh flowers (98.2%) than in dried flowers (85.5%), reflecting the impact of drying processes. These results suggest that enzymatic activity and oxidative processes during drying lead to substantial chemical transformations in the volatile profile.

Regional comparisons demonstrated significant variations in chemical profiles across different locations in Türkiye. The samples from the Muğla region showed a distinct composition when compared to previous studies conducted in İzmir, Siirt, and Şırnak. On an international scale, the dominance of (*E*)- β -ocimene aligns with findings from Greece, China, and Egypt, confirming its role as a

chemotaxonomic marker for *N. tazetta*. However, variations in secondary compounds, such as benzyl acetate and 3-hexenyl acetate, underscore the influence of environmental factors, including soil composition and climatic conditions in Muğla.

Drying significantly influenced the chemical profiles, with the emergence of aldehydes and esters such as (Z)-3-hexenal and (Z)-3-hexenyl acetate, which were absent in fresh flowers. These findings highlight the chemical transformations caused by drying, a process that has been underexplored in previous research. The study demonstrates how drying alters the biosynthesis and stability of specific volatile compounds, offering new insights into their potential applications.

A key strength of this study is the application of HCA. This advanced method allowed for the classification of compounds based on structural and functional similarities, providing a deeper understanding of their relationships. Compared to traditional approaches, HCA offered a statistically robust and visually effective framework for comparing the chemical profiles of fresh and dried flowers, thus enhancing the interpretative power of the findings.

(E)- β -Ocimene, as one of the main volatile compounds identified in *Narcissus tazetta* subsp. *tazetta*, plays a significant role in both ecological and pharmacological contexts. In the literature, this compound has been reported to exhibit diverse biological activities. Its antimicrobial properties are particularly noteworthy, with demonstrated efficacy against various bacterial and fungal pathogens, suggesting its potential as a natural antimicrobial agent. Furthermore, (E)- β -ocimene possesses antioxidant capacity, as it effectively neutralizes free radicals, thereby offering protection against oxidative stress-related conditions. Additionally, its anti-inflammatory effects are associated with the inhibition of pro-inflammatory cytokine production.

Moreover, (E)- β -ocimene has shown cytotoxic activity in several studies, with evidence of its effectiveness against specific cancer cell lines. These properties highlight the pharmacological relevance of this compound, reinforcing its potential applications in therapeutic and industrial contexts.

The findings of this study underscore the dual importance of (E)- β -ocimene, not only as a major contributor to the volatile profile of *N. tazetta* but also as a bioactive compound with significant pharmacological implications. Future studies focusing on the biological activities of this compound could provide deeper insights into its therapeutic potential and further expand the utility of *N. tazetta* in pharmacology and industry [11-14].

In conclusion, the findings of this study provide a robust framework for understanding the chemical diversity of *N. tazetta* and its potential applications. The high concentrations of (E)- β -ocimene and linalool suggest possible uses in perfumery and aromatherapy, while the identification of unique compounds in dried flowers could open new avenues for exploring their biological activities.

Future studies could focus on investigating the ecological and environmental factors that influence the volatile compound profiles in different regions. Understanding these variations could provide insights into the role of local conditions in shaping the chemical composition of *Narcissus tazetta*. Additionally, exploring the enzymatic pathways involved in the transformation of compounds during the drying process could shed light on the biochemical mechanisms driving these changes. Furthermore, conducting bioactivity assays to evaluate the pharmacological potential of the identified compounds would help uncover their therapeutic applications and pave the way for their utilization in pharmaceutical and industrial contexts.

In conclusion, this study not only confirms the chemical richness of *N. tazetta* subsp. *tazetta* but also introduces novel findings related to dried flowers and regional variations. By integrating advanced analytical methods such as HCA, it sets a benchmark for future research on the chemical ecology of this and related species.

AUTHOR CONTRIBUTIONS

Concept: D.K., B.D.; Design: D.K., B.D.; Control: B.D.; Sources: D.K., B.D, O.T., D.U.; Materials: O.T., D.U.; Data Collection and/or Processing: D.K, B.D.; Analysis and/or Interpretation: D.K. B.D.; Literature Review: D.K; Manuscript Writing: D.K., O.T., D.U., B.D.; Critical Review: D.K., O.T., D.U., B.D.; Other: D.K., O.T., D.U., B.D.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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