The first report on the screening of the GC/MS profiles of the hydrodistilled essential oils and volatile components from the aerial parts of *Verbascum sinuatum* L.

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Abstract
This study elucidates the chemical composition of the essential oil and volatile fractions from the aerial parts of *V. sinuatum* L. using classical hydrodistillation (CHD) and headspace-solid phase microextraction (HS-SPME) techniques, identifying 23 compounds via GC-MS analysis. In the CHD essential oil, non-terpene hydrocarbons (NH) predominated at 64.8%, followed by oxygenated diterpenes (OD) at 14.2%, sesquiterpene hydrocarbons (SH) at 8.0%, and oxygenated sesquiterpenes (OS) at 7.8%. The principal constituents were hexadecanoic acid (28.9%), phytol (14.2%), and linoleic acid (7.3%), accounting for 94.8% of the total. Conversely, HS-SPME profiles emphasized NH (70.1%) and OS (19.1%), with hexadecanoic acid (49.3%), (*E*)-phytol acetate (8.1%), *n*-heneicosane (4.1%), and spathulenol (4.0%) comprising 92.5% overall. These findings reveal method-dependent variations in terpenoid and fatty acid content, highlighting CHD's efficacy in extracting higher quantities of oxygenated diterpenes and HS-SPME's sensitivity to volatiles, offering insights into phytochemical diversity for potential pharmacological applications.

Keywords:
Classical hydrodistillation (CHD)
Essential oils
GC/MS
Headspace-solid phase microextraction (HS-SPME)
Scrophulariaceae
Verbascum sinuatum L.

1. Introduction

Medicinal and herbal plants have been inextricably linked to human health and survival for millennia, serving as the foundation of traditional medicine systems across the globe and providing a primary source of healthcare for a vast majority of the world's population (Fabricant and Farnsworth, 2001; Singh, 2023). In the contemporary era, their significance has been further magnified, as they represent an invaluable reservoir for the discovery of novel pharmaceutical lead compounds (A.M. Abdel-Rahman et al., 2022; Pardeshi et al., 2022); a prime example is aspirin, derived from *Salix alba* (Alamgir, 2017), and the anti-malarial artemisinin from *Artemisia annua* (Al-Khayri et al., 2022). Beyond their therapeutic applications, these plants are deeply embedded in cultural practices and contribute substantially to local economies, underscoring their multifaceted role in human life (Sharif and Jabeen, 2024).

The Scrophulariaceae, as traditionally circumscribed, represents a complex and historically contentious family within the Lamiales order, showcasing a remarkable evolutionary narrative of adaptation and taxonomic revision

(Xu et al., 2019). Morphologically, it was characterized by typically zygomorphic, often bilabiate flowers with a superior, bicarpellate ovary that matures into a capsule, and foliage that is usually opposite or whorled (Bejenaru et al., 2024). This classic assemblage once included over 200 genera, such as the emblematic *Antirrhinum* (snapdragons), *Digitalis* (foxgloves, source of the cardiac glycoside digoxin), *Verbascum* (mulleins), and *Scrophularia* (figworts) (Bejenaru et al., 2024). However, molecular phylogenetic studies over the past three decades have fundamentally reorganized our understanding, revealing the family, in its broad sense, to be polyphyletic (Tank et al., 2006). The Scrophulariaceae family, to which the genus *Verbascum* belongs, is particularly renowned for its rich diversity of species with documented ethnobotanical uses (Blanco-Salas et al., 2021). Within this family, the genus *Verbascum*, commonly known as mullein, comprises more than 400 species worldwide. These species are celebrated for their medicinal properties, such as *V. thapsus*, which is widely used for its demulcent and emollient effects on the respiratory tract (Tatli and Akdemir, 2004; Riaz et al., 2013.; Blanco-Salas et al., 2021; Jan et al., 2022). In the Iranian flora, forty-five species of *Verbascum*, along with several hybrids, are found, of which twenty are endemic (Rechinger, 1981; Sotoodeh et al., 2017).

Verbascum sinuatum L., commonly known as the wavy-leaved mullein, is a species that has attracted attention for its traditional uses and phytochemical potential. It is a biennial or perennial herb native to the Mediterranean region and parts of Western Asia, characterized by its sinuate-pinnatifid leaves and yellow flowers (Tatli and Akdemir, 2004). The plant commonly known as Gul-e Mahoor or Khargoushak is referred to by various names in different regions of Iran, including 'Alaf-e Khargoush' (hare's herb), 'Khargoushak', 'Gul-e Mahoor', and 'Alaf-e Mahoor'. In traditional texts, it is recorded under the names 'Gholunos', 'Bousir', and 'Azan al-Dab' (bear's ears). Since ancient times, this plant has been used for the treatment of respiratory ailments (Mirhaidar, 2005). In traditional Persian folk medicine, physicians used this plant to treat coughs, bronchitis, and fever. European immigrants brought it with them to America. Since antiquity, it has been used to treat coughs, the common cold, pharyngeal and throat inflammation, tonsillitis, diarrhea, hemorrhoids, and urinary tract infections (Mirhaidar, 2005).

Ethnobotanical records suggest its use in various folk medicines for treating ailments such as intestinal bleeding, inflammation, migraine headaches, abdominal and bowel diseases, respiratory conditions, as well as snakebites and wounds (Shinwari and Gilani, 2003; Ghorbani, 2005; Turker and Gurel, 2005; Sher, 2011; Georgiev et al., 2012; Riaz et al., 2013.; Alipieva et al., 2014; Mihailović et al., 2016). Throughout its native range in the Mediterranean basin and Western Asia, *V. sinuatum* L. has secured a notable position in the traditional and folk medicine of various cultures, where it has been empirically employed for generations to treat a diverse array of ailments. In Turkish folk medicine, the plant is highly valued for its emollient and wound-healing properties, frequently applied topically as a poultice or ointment prepared from its leaves to soothe burns, haemorrhoids, and various skin inflammations (Tatli and Akdemir, 2004). Furthermore, its flowers and leaves are commonly brewed into a herbal tea consumed to alleviate respiratory conditions such as bronchitis, asthma, and persistent coughs, leveraging its expected expectorant and demulcent actions. Similarly, in regions of Greece and other parts of the Eastern Mediterranean, infusions of the aerial parts of *V. sinuatum* have been traditionally used for their attributed anti-inflammatory and analgesic effects, often taken internally to ease abdominal pains or used as a gargle for sore throats (Tetik et al., 2013). Its use also extends to gastrointestinal complaints, including diarrhoea, indicating a broader application for its potential antimicrobial and astringent properties. These ethnobotanical records, passed down through oral traditions, not only underscore the historical importance of *V. sinuatum* as a versatile therapeutic agent but also provide a critical ethnopharmacological foundation that justifies the scientific investigation of its phytochemical composition and biological activities to validate its traditional uses.

According to Wikipedia (https://en.wikipedia.org/wiki/*Verbascum_sinuatum*), this valuable species is distributed across various regions worldwide, including the Middle East, Europe, Africa, and America (see Table 1). However, a critical review of the existing literature reveals a significant gap concerning a comprehensive analysis of its volatile chemical constituents. While the essential oils of other *Verbascum* species, like *V. thapsus* and *V. olympicum*, have been studied to some extent, the data on the essential oil composition and volatile profile of *V. sinuatum* remain notably scarce and fragmented. Some preliminary studies have reported the presence of various compounds, but a detailed and systematic quantification is lacking. Therefore, the present research is undertaken to provide a comprehensive quantification and characterization of the essential oils and volatile fractions obtained from *V. sinuatum*. This work aims to elucidate its complete volatile phytochemical profile, which is essential for understanding its full medicinal potential, chemotaxonomic significance, and possible applications in pharmacology and aromatherapy.

2. Experimental

2.1. Chemicals and supplies

In this study, a certified reference standard mixture comprising a homologous series of straight-chain *n*-alkanes from nonane (C_9) to pentacosane (C_{35}), each at an identical concentration of 40 mg/L in hexane, was procured from Fluka (Buchs, Switzerland). High-purity helium and nitrogen were used as carrier gases for gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analyses. For analyte preconcentration and introduction, a headspace solid phase microextraction (HS-SPME) setup was employed, utilizing a manual SPME holder fitted with a 1-cm fused-silica fiber. The stationary phase coating the fiber was a bifunctional composite of polydimethylsiloxane and carboxen (PDMS/CAR, 75 µm film thickness; obtained from Supelco, Bellefonte, PA, USA), selected for its efficacy in adsorbing analytes across a broad volatility range. All extractions were performed in dedicated 10-milliliter clear glass vials (22 mm × 46 mm) sealed with crimp-top closures, supplied by MicroLiter Analytical Supplies Inc. (Suwanee, GA, USA). These vials were sealed using aluminum caps fitted with 20-mm septa composed of a polytetrafluoroethylene (PTFE) and silicone laminate, sourced from Supelco. This septum material was specifically chosen to minimize sorptive losses and prevent contamination during the high-temperature incubation phase of the HS-SPME procedure.

2.2. Plant material

The aerial parts of the plant (*V. sinuatum* L.) (Fig. 1) were collected during the flowering stage in June 2024 from the mountainous regions of the Abr Forest, located 45 kilometers north of Shahrood in Semnan Province. The specimens were taxonomically identified by Dr. Gholami (taxonomist) and vouchered at the Herbarium of the Agricultural Research Center of Semnan Province (herbarium number: VS2024.k). The collected plant samples were cleaned, shade-dried, and subsequently powdered using a mechanical grinder.

2.3. Classical hydrodistillation (CHD)

Hydrodistillation represents a classic and longstanding method that is extensively employed for extracting essential oils from a wide variety of plant tissues. This technique has found widespread application in both laboratory-scale and industrial-scale operations owing to its reliability in yielding high-quality oils (Périno et al., 2019). Typically performed with a Clevenger-type apparatus, the process involves three main stages: heating, vapor condensation, and phase separation. Although conventional and/or classical hydrodistillation (CHD) is valued for its straightforwardness and efficacy, it is often lengthy and can lead to thermal decomposition of sensitive compounds in many instances (Roldán-Gutiérrez et al., 2008). Furthermore, the method requires substantial volumes of water, raising concerns about environmental sustainability in certain applications.

To isolate the essential oils from *V. sinuatum* L., 150 g samples of air-dried aerial parts of this medicinal plant were separately subjected to hydrodistillation in a Clevenger-type apparatus for 2.5 hours. The obtained pale

yellowish oils were subsequently dried over anhydrous sodium sulfate and stored at 4 °C in the dark until subsequent analyses.

2.4. Description of headspace-solid phase microextraction (HS-SPME) technique performance: Sequential steps and details

The standardized procedure for performing HS-SPME comprises five critical stages:

i) Sample preparation: The sample—whether solid, liquid, or viscous—is precisely weighed or measured into a sealed vial, often with the addition of salts or pH modifiers to enhance volatility or matrix effects.

ii) Equilibration/Conditioning: The sealed vial is heated in a controlled-temperature environment, such as a heating block or autosampler agitator. This step drives volatile compounds from the sample matrix into the headspace, establishing a thermodynamically stable equilibrium between the two phases.

iii) Fiber selection: A critical optimization step involves selecting a fiber with a coating material whose polarity and thickness are compatible with the target analytes' chemical properties (*e.g.*, molecular weight, polarity and volatility). Common coatings include polydimethylsiloxane (PDMS) for non-polar compounds, polyacrylate (PA) for polar compounds, and mixed phases like PDMS/divinylbenzene (DVB) or Carboxen™/PDMS for a broader analyte range.

iv) Headspace extraction: The selected fiber, housed within a protective needle, is pierced through the vial's septum and manually or automatically exposed to the headspace. Analytes adsorb/absorb onto the fiber's stationary phase coating. Extraction time and temperature are controlled to optimize uptake, which can be equilibrium-based or time-weighted for quantitative analysis.

v) Thermal desorption and analysis: Following extraction, the fiber is immediately retracted into the needle, withdrawn from the vial, and inserted into the heated injection port of a gas chromatograph (GC) or gas chromatograph-mass spectrometer (GC-MS). Rapid heating of the fiber (typically 250-300 °C) desorbs the concentrated analytes directly onto the analytical column in a narrow band, ensuring high-resolution separation and detection.

In the present study, HS-SPME sampling was executed with a manual SPME holder assembly. A 1-cm fused silica fiber coated with a biphasic sorbent of polydimethylsiloxane and Carboxen™ (PDMS/CAR) was employed to target a wide spectrum of volatile compounds. Prior to use, the fiber underwent thermal conditioning in a GC injection port according to the manufacturer's protocol to remove any contaminant residues.

For the analytical procedure, a representative 1.0 g aliquot of finely powdered plant material from distinct organs of *V. sinuatum* L. was transferred into a 20-mL headspace vial. The vial was immediately sealed with a polytetrafluoroethylene (PTFE)/silicone septum cap to ensure an airtight environment. The sealed vial was then placed on a heating block and incubated at 70 °C for a 20-minute period to allow for full thermal equilibration and headspace saturation.

Subsequently, the needle of the manual SPME holder, containing the pre-conditioned PDMS/CAR fiber retracted within it, was used to pierce the vial's septum. The fiber was then extended from the needle to expose the coated segment directly to the headspace above the heated plant material. The extraction proceeded for a duration of 20 minutes, after which the fiber was retracted back into the protective needle sheath. The needle was swiftly removed from the sample vial and inserted into the GC-MS injection port.

Thermal desorption of the extracted volatiles from the fiber coating was performed in splitless injection mode to ensure maximum transfer efficiency to the capillary column. The injector temperature was maintained at 250 °C for a period of 5 minutes to guarantee complete desorption of analytes, including those with higher molecular weights, before initiating the GC-MS temperature program for chromatographic separation and mass spectrometric identification.

2.5. Apparatus

The chemical composition of the volatile fractions isolated from the aerial parts of *V. sinuatum* L.. was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Gas chromatographic (GC) analysis was performed on a Shimadzu 15A gas chromatograph, configured with a split/splitless injector and a flame ionization detector (FID) maintained at 250 °C. Separations were achieved using a DB-5 fused silica capillary column, with ultrapure nitrogen as the carrier gas. The oven temperature program was initiated at 60 °C (held for 3 min), then increased to 220 °C at a rate of 5 °C/min, with a final hold time of 5 min. Complementary GC/MS analysis was conducted on a Hewlett-Packard 5973 system fitted with an HP-5MS capillary column, employing an identical temperature program. The mass spectrometer operated with an ion source temperature of 250 °C. Mass spectra were acquired in electron ionization (EI) mode at 70 eV, with an electron multiplier voltage of 1800 V, scanning a mass range of *m/z* 30-350.

3. Results and discussion

3.1. Phytochemistry of *Verbascum* species

V. sinuatum L. produces a diverse array of bioactive compounds, predominantly flavonoids, phenolic acids, phenylethanoid glycosides, and iridoid glycosides from its aerial parts, especially the flowers and leaves. These compounds have been isolated using techniques such as maceration and heat-assisted extraction, demonstrating significant therapeutic potential in antioxidant and antimicrobial applications. Over more than three decades of phytochemical research, these constituents have been shown to support the traditional uses of *V. sinuatum* in Mediterranean folk medicine (Gondal et al., 2020; Selseleh et al., 2020; Amini et al., 2022; Garcia-Oliveira et al., 2022; Donn et al., 2023). Table 2 presents and compares various classes of natural compounds characterized in different species of the genus *Verbascum*. As illustrated in this table, numerous flavonoids, phenolic compounds, phenylethanoid glycosides, and iridoid glycosides have been isolated and identified across many species within this valuable genus.

3.2. Characterization of the essential oil and volatile fraction from the aerial parts of *V. sinuatum* L.

The therapeutic potential of medicinal plants is fundamentally attributed to their complex and diverse phytochemical constituents. These secondary metabolites, including flavonoids, phenylethanoid glycosides, iridoids, and saponins, are responsible for a wide spectrum of biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Georgiev et al., 2011). Among these, the volatile components, particularly essential oils, constitute a crucial fraction due to their significant pharmacological properties and their role in plant defense and communication. The chemical profile of these volatile fractions is highly variable, influenced by genetic, environmental, and ontogenetic factors, making the chemical characterization of each species a subject of considerable research interest. While the non-volatile secondary metabolites of *V. sinuatum*, such as its characteristic phenylethanoid glycosides (*e.g.*, verbascoside), iridoids, and flavonoids, have been the subject of several isolation and identification studies confirming their significant contribution to the plant's antioxidant and anti-inflammatory activities, the existing literature data concerning a comprehensive qualitative and particularly quantitative analysis of its essential oils and volatile fractions remain notably scarce and fragmented (Georgiev et al., 2011). To the best of our knowledge, this paper is the first to conduct both qualitative and quantitative analyses of the essential oil and volatiles from the aerial parts of the studied plant species (*V. sinuatum* L.).

Using an HP-5MS capillary column, GC-MS analysis allowed the identification of 23 compounds through comparison of their mass spectra, retention indices, and co-injection with authentic standards. The constituents of the essential oil, isolated from the aerial parts of *V. sinuatum* L. via classical hydrodistillation (CHD), are detailed in Table 3.

The average yield of essential oil, determined from three replicate hydrodistillations and expressed as the weight of oil per gram of dried plant material, was 0.12% (*w/w*). In the hydrodistilled oil, the 23 identified

compounds accounted for 94.8% of the total composition. The predominant components were hexadecanoic acid (28.9%), phytol (14.2%), linoleic acid (7.3%), *n*-pentacosane (6.8%), *n*-heneicosane (5.9%), germacrene D (5.4%), *n*-tricosane (5.2%) and (*E*)-phytol acetate (5.0%).

In contrast, the volatile fraction obtained from the same aerial parts using HS-SPME was dominated by hexadecanoic acid (49.3%), (*E*)-phytol acetate (8.1%), *n*-heneicosane (4.1%) and spathulenol (4.0%) as the principal constituents.

3.3. Frequencies of terpenoids and non-terpenoids in the essential oil and volatile profiles of *V. sinuatum* L.

The chemical profiles of the different parts of *V. sinuatum* L were characterized by sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OS), oxygenated diterpenes (OD), and non-terpene hydrocarbons (NH) when using the HD and HS-SPME methods.

In terms of general categories, the essential oil obtained by CHD from the aerial parts of *V. sinuatum* L. contained seven sesquiterpene hydrocarbons (8.0%), five oxygenated monoterpenes (7.8%), one oxygenated diterpene (14.2%) and ten non-terpene hydrocarbons (64.8%) (Fig. 2). The abundance of these compounds followed this order: NH > OD > SH > OS.

In contrast, the volatile profile of the same aerial parts obtained by HS-SPME was characterized by three sesquiterpene hydrocarbons (1.1%), five oxygenated sesquiterpenes (19.1%), one oxygenated diterpene (2.2%), and nine non-terpene hydrocarbons (70.1%) (Fig. 2). Together, these components accounted for 92.5% of the total detected volatiles, with an abundance ranking of NH > OS > OD > SH.

3.4. Chemical composition of the essential oils of the other *Verbascum* species

Essential oils from *Verbascum* species in the Scrophulariaceae family display remarkable chemical diversity in their major constituents (see Table 4), primarily comprising alcohols, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aldehydes, esters and aliphatic hydrocarbons, with compositions influenced by species-specific biosynthesis (Yu et al., 2025), plant organ differentiation, geographical origin, and environmental conditions (Dodoš et al., 2019; Németh-Zámbori, 2020). For instance, 1-octen-3-ol emerges as a dominant alcohol in flowers of *V. undulatum* (up to 22.5%) (Melliou et al., 2007) and *V. creticum* (up to 23.9%) (Vaglica et al., 2025), imparting mushroom-like aromas linked to fungal interactions and contributing to potent antimicrobial and antifungal activities (Lee et al., 2024), while *V. pinetorum* features high 1,8-cineole (16.9%) and α-selinene (16.4%) (Boğa et al., 2016b), monoterpene and sesquiterpene profiles associated with expectorant, anti-inflammatory, and analgesic properties traditional in phytotherapy for respiratory disorders (Juergens et al., 2020; Yan et al., 2025). In contrast, *V. wiedemannianum* showcases organ-specific profiles—pentadecane (58.2%) (Iskender et al., 2009) in flowers indicating hydrocarbon dominance possibly for pollinator attraction (Bohman et al., 2020), (2*E*)-hexanal (33.2%) in leaves (Iskender et al., 2009) suggesting green leaf volatile pathways for herbivore defense (Scala et al., 2013), and hexadecanoic acid (24.6%) in stems pointing to fatty acid derivatives—underscoring intra-plant variability that challenges standardized extraction (Pansini et al., 2023). The widespread *V. thapsus* (common mullein) differs notably with 6,10,14-trimethyl-2-pentadecanone (14.3%) and (*E*)-phytol (9.3%) (Morteza-Semnani et al., 2012), diterpenoid-like compounds supporting its ethnomedicinal use in cough syrups due to mucolytic and soothing effects on mucous membranes (Murgia et al., 2021). These chemotaxonomic patterns reveal evolutionary adaptations within the genus, where Mediterranean endemics favor oxygenated terpenoids for stress tolerance, unlike temperate species leaning toward aliphatic chains; such insights validate Scrophulariaceae's harmacological potential but necessitate expanded GC-MS/MS investigations across genotypes, seasons, and stressors to fully elucidate structure-activity relationships, optimize yields via hydrodistillation or supercritical extraction, and develop species-authentic markers for quality control in herbal markets.

Taking into account the findings of the recent report by Farid et al. (2020) on the GC-MS and LC-ESI-MS analysis of biologically active fractions from *V. letourneuxii*, the major compounds identified in the diethyl ether fraction are 1,11-bis(methoxycarbonylethenyl)-10,2-dihydroxycycloeicosane (19.4%), *n*-propyl 9,12-octadecadienoate (16.0%), (*E*)-9-octadecenoic acid ethyl ester (15.2%), hexadecanoic acid ethyl ester (9.9%), and phytol (7.2%). In contrast, the primary constituents characterized in the chloroform fraction include 16-octadecenoic acid methyl ester (14.7%), hexadecanoic acid methyl ester (12.5%), 9,12-octadecadienoic acid methyl ester (9.7%), (*E*)-9-octadecenoic acid ethyl ester (9.0%), phytol (8.1%), 9,12-octadecadienoic acid ethyl ester (5.2%), diisooctyl phthalate (5.0%), and hexadecanoic acid ethyl ester (4.2%). Furthermore, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester, 16-octadecenoic acid methyl ester, and (*E*)-9-octadecenoic acid ethyl ester have been reported as anti-inflammatory and cancer-preventive agents (Singh et al., 2008). The cytotoxic activity of the chloroform and diethyl ether fractions may be attributed to compounds detected by GC analysis, such as phytol, which has demonstrated cytotoxic activity against various cell lines (Sanseera et al., 2012). These compounds and their methyl esters likely contribute to the pronounced synergistic effects observed in both the chloroform and diethyl ether fractions. Additionally, these fractions exhibited moderate antioxidant activity and the highest cytotoxic effects, which could be attributed to their high content of saturated fatty acids (Farid et al., 2020).

3.5. Comparison of the chemical profiles of this report with the previously works in the literature

The volatile profile of *V. sinuatum* L. aerial parts from Table 3, dominated by non-terpene hydrocarbons (NH, 64.8% CHD/70.1% HS-SPME) like hexadecanoic acid (28.9%/49.3%) and alkanes (*e.g.*, *n*-pentacosane 6.8%/3.7%), oxygenated diterpenes (OD, 14.2%/2.2%) such as phytol (14.2%/2.2%), sesquiterpene hydrocarbons (SH, 8.0%/1.1%) including germacrene D (5.4%/0.5%), and oxygenated sesquiterpenes (OS, 7.8%/19.1%) like (*E*)-phytol acetate (5.0%/8.1%), aligns with lipid-rich chemotypes in Table 4, such as *V. wiedemannianum* (high alkanes like pentacosane: 5.0–6.2%, palmitic acid: 7.1–24.6%) (Iskender et al., 2009) and *V. letourneuxii* (fatty acid esters: 9.9–16.0% for diethyl ether fraction and 4.2-14.7% for chloroform fraction, phytol: 7.2–8.1%) (Farid et al., 2020), while sharing sesquiterpenoids with *V. pinetorum* (α-selinene: 16.4%) (Boğa et al., 2016b) and *V. flavidum* (α-selinene: 8.9%) (Boğa et al., 2016a); however, it contrasts with monoterpene/alcohol-dominant species like *V. creticum* (1-octen-3-ol: 23.9%, *cis*-3-hexen-1-ol: 9.4%) (Vaglica et al., 2025) and *V. undulatum* (1-octen-3-ol 22.5%) (Melliou et al., 2007), ketone-rich *V. thapsus* (6,10,14-trimethyl-2-pentadecanone 14.3%, (*E*)-phytol 9.3%), (Morteza-Semnani et al., 2012) and highlighting method-dependent (CHD *vs.* HS-SPME) and species-specific variations in terpenoid/fatty acid pathways, with *V. sinuatum* as a hybrid chemotype for potential chemotaxonomic and pharmacological insights.

4. Concluding remarks

In summary, this investigation demonstrates that the essential oil and volatiles extracted from the aerial parts of *V. sinuatum* L. using CHD and HS-SPME techniques are rich in non-terpene hydrocarbons and oxygenated compounds. Hexadecanoic acid emerged as the predominant constituent in both methods, albeit at higher levels in HS-SPME (49.3% *vs.* 28.9%). CHD favored the extraction of oxygenated diterpenes such as phytol (14.2%), whereas HS-SPME enhanced the presence of sesquiterpenoids, including (*E*)-phytol acetate (8.1%) and spathulenol (4.0%), highlighting the influence of extraction techniques on yield and chemical profile. These findings confirm the chemotypic uniqueness of this plant within the *Verbascum* genus, which may be associated with bioactivities such as antimicrobial and anti-inflammatory effects. Future research should investigate seasonal variations, employ bioassay-guided fractionation for therapeutic validation, and conduct comparative metabolomic studies across different habitats to elucidate ecological adaptations and broaden ethnopharmacological applications.

Author contribution

Conceptualization and literature search were performed by MM. The first draft of the manuscript was prepared by MM. MM also critically analyzed and gave suggestions to finalize the manuscript. The author read and approved the final manuscript.

Acknowledgements

Financial and technical support from the Office for Research Affairs of the Islamic Azad University, Shahrood Branch are gratefully acknowledged.

Conflict of interest

The author declares that there is no conflict of interest.

Figure 1. The photograph of the aerial parts of *Verbascum sinuatum* L. (Sampling area: Abr Forest, located 45 kilometers north of Shahrood, Iran).

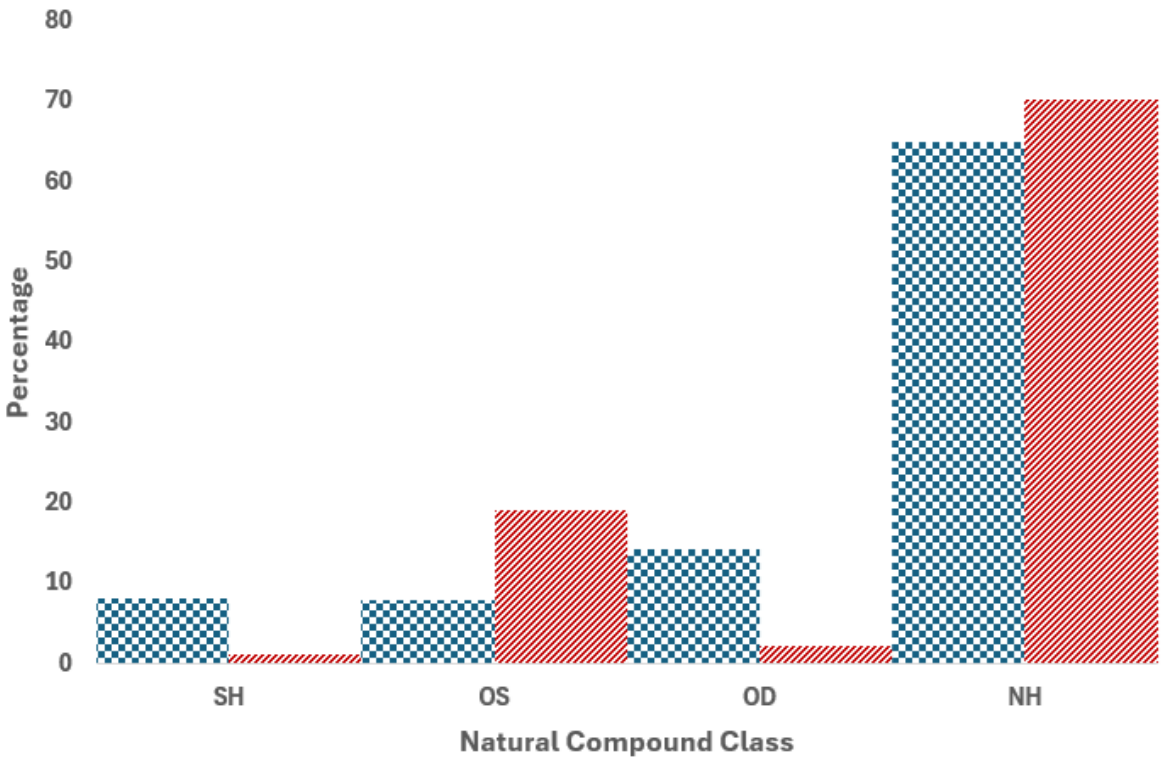

Figure 2. Comparison of the natural compounds groups contributing to the chemical profiles of the essential oils and volatile profiles from the aerial parts of *V. sinuatum* L. using classical hydrodistillation (CHD) and Headspace-solid phase microextraction (HS-SPME).

Table 1. Distribution of *Verbascum sinuatum* L. in various parts of the world.

Region	**Key Countries/Examples**	**Habitat**
Mediterranean Europe	Spain, Portugal, France, Italy (incl. Sicily, Sardinia), Greece (incl. Crete), Croatia, Albania and Malta	Coastal areas, waysides and chaparrals
Eastern Mediterranean & Middle East	Turkey, Syria, Lebanon, Palestine, Jordan, Cyprus, Iraq and Iran	Open shrublands, waste ground, Jordan Valley
North Africa	Egypt (Sinai), Libya, Tunisia, Algeria and Morocco	Arid steppes and disturbed sites
Central Asia (Irano-Turanian)	Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan, Armenia and Georgia	Heavy soils in steppe regions worldplants

Table 2. Representation of the isolated compounds, the relevant natural compounds constituting groups of diverse *Verbascum* species and the literature.

***Verbascum* species**	**Class**	**Isolated compounds**	**Plant part/Source**	**Reference**
V. cheirantifolium, *V. densiflorum*, *V. erianthum*, *V. macrocarpum*, *V. punalense*, *V. sinuatum*, *V. songaricum*, *V. speciosum*, *V. stachidiforme*, and *V. thapsus*	Flavonoids	Naringenin, plantagonine, luteolin, rhamnetin, myricetin, hesperetin, cynaroside, apigenin, apigetrin, hyperoside and chrysin	Air-dried aerial flowering parts	(Selseleh et al., 2020; Donn et al., 2023)
V. cheirantifolium, *V. densiflorum*, *V. erianthum*, *V. haussknechtianum*, *V. macrocarpum*, *V. punalense*, *V. saccatum* *V. sinuatum*, *V. songaricum*, *V. speciosum*, *V. stachidiforme*, *V. szovitsianum*, and *V. thapsus*	Phenolic compounds	Harpagoside, protocatechuic acid, gentisic acid, *p*-coumaric acid, ferulic acid, salicylic acid, rosmarinica cid, rutin and quercetin, quinic acid, ursolic acid, cinnamic acid, gallic acid, chlorogenic acid, caffeic acid	Flowers (various extracts); Air-dried aerial parts	(Selseleh et al., 2020; Amini et al., 2022)
V. sinaiticum, V. sinuatum	Phenylethanoid glycosides	Verbascoside, isoverbascoside, *p*-coumaroyl-6-*O*-rhamnosyl aucubin (isomers I/II), forsythoside	Air-dried aerial parts (hydroethanolic extracts/infusion)	(Gondal et al., 2020; Garcia-Oliveira et al.,

				2022)
V. sinuatum	Iridoid glycosides	Aucuboside, pulverulentoside, harpagide, and harpagoside, 6-*O*-β-d-xylopyranosyl aucubin, sinuatol (6-*O*-α-l-rhamnopyranosylaucubin), sinuatoside (6-*O*-(3-*O*-β-d-xylopyranosyl)α-d-galactopyranosyl aucubin), catalpol	Air-dried aerial parts (methanolic extracts)	(Bianco et al., 1981)

Table 3

Chemical composition of essential oils and volatile fractions from the aerial parts of *Verbascum sinuatum* L. obtained by classical hydrodistillation (CHD) and headspace solid phase microextraction (HS-SPME) using gas chromatography–mass spectrometry (GC-MS)[a].

Num.	Compound	RI (Cal.)[b]	RI (Lit.)[c]	NCCG[d]	Percentage	
					CHD	HS-SPME
1	Isopentyl isovalerate	1100.2	1102	NH	0.2	-
2	*cis*-Muurola-4,5-diene	1446.3	1448	SH	0.5	-
3	*trans*-Muurola-4,5-diene	1449	1451	SH	0.9	-
4	*allo*-Aromadendrene	1457.1	1458	SH	0.1	0.5
5	Germacrene D	1486.2	1484	SH	**5.4**	0.5
6	β-Selinene	1490.1	1489	SH	0.7	0.1
7	δ-Selinene	1492.3	1492	SH	0.2	-
8	α-Muurolene	1500	1500	SH	0.2	-
9	Spathulenol	1576	1577	OS	1.2	**4.0**
10	γ-Eudesmol	1629.2	1630	OS	0.8	0.9
11	β-Eudesmol	1648.5	1649	OS	0.2	3.2
12	α-Bisabolol	1713.3	1714	OS	0.6	2.9
13	*n*-Hexadecanol	1875	1874	NH	0.1	2.1
14	Hexadecanoic acid	1960.2	1959	NH	**28.9**	**49.3**
15	Phytol	1940.9	1942	OD	**14.2**	2.2
16	*n*-Heneicosane	2102	2100	NH	**5.9**	**4.1**
17	Linoleic acid	2134.4	2132	NH	**7.3**	1.4
18	Oleic acid	2143.1	2141	NH	2.6	1.3
19	(*E*)-Phytol acetate	2219.5	2218	OS	**5.0**	**8.1**
20	*n*-Tricosane	2300.3	2300	NH	**5.2**	3.1
21	*n*-Tetracosane	2404.2	2400	NH	3.9	2.9
22	*n*-Pentacosane	2503.5	2500	NH	**6.8**	3.7
23	Heptacosane	2703.1	2700	NH	3.9	2.2
Total					**94.8**	**92.5**

[a] The compounds have been sorted according to their elution order on an HP-5MS column.

[b] Calculated Retention indices

[c] Retention indices in the literature

[d] NCCG: Natural compound constituting group

Table 4. The major constituents of the essential oils of the other *Verbascum* species in the literature.

Species	Major constituents (% of total oil)	Reference
V. pinetorum	1,8-Cineole (16.9%), α-selinene (16.4%), 2,5-di-tert octyl-*p*-benzoquinone (7.8%), dihydro carvyl acetate (3.5%), τ-muurolene (3.4%), 1,3-di-tert butyl benzene (3.2%), heneicosane (3.1%) and heptacosane (3.1%)	(Boğa et al., 2016b)
V. undulatum	1-Octen-3-ol (22.5%) and α-bisabolol (10.6%)	(Melliou et al., 2007)
V. wiedemannianum	**Flower:** Pentadecane (58.2%), tetradecane (9.0%), tricosane (6.4%), pentacosane (6.0%) and benzene acetaldehyde (3.9) **Leaf:** (2*E*)-Hexenal (33.2%), palmitic acid (7.1%), (2*E*,4*Z*)-heptadienal (6.6%), pentacosane (6.2%) and *allo*-ocimene (5.8%) **Stem:** Palmitic acid (24.6%), tetracosane (18.3), pentacosane (5.9%), pentadecenal (5.9%) and (2*E*)-hexenal (5.2%)	(Iskender et al., 2009)
V. creticum	1-Octen-3-ol (23.9%), *cis*-3-hexen-1-ol (9.4%), phenylethanal (4.6%) and 2-methyl-benzofuran (4.6%)	(Vaglica et al., 2025)
V. thapsus	6,10,14-Trimethyl-2-pentadecanone (14.3%) and (*E*)-phytol (9.3%)	(Morteza-Semnani et al., 2012)
*V. flavidum (*Boiss.) Freyn & Bornm	Arachidic acid (16.4%) and α-selinene (8.9%)	(Boğa et al., 2016a)
V. letourneuxii	**Diethyl ether fraction:** 1,11-Bis(methoxycarbonylethenyl)-10,2-dihydroxy-cycloeicosane (19.4%), *n*-propyl 9,12-octadecadienoate (16.0%), (*E*)-9-octadecenoic acid ethyl ester (15.2%), hexadecanoic acid ethyl ester (9.9%) and phytol (7.2%)	(Farid et al., 2020)
	Chloroform fraction: 16-octadecenoic acid methyl ester (14.7%), hexadecanoic acid methyl ester (12.5%), 9,12-octadecadienoic acid methyl ester (9.7%), (*E*)-9-octadecenoic acid ethyl ester (9.0%), phytol (8.1%), 9,12-octadecadienoic acid ethyl ester (5.2%), diisooctyl phthalate (5.0%) and hexadecanoic acid ethyl ester (4.2%)	

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