

# Characterization of chemical profiles of essential oils and volatiles of *Pulicaria gnaphalodes* (Vent.) Boiss. using classical hydrodistillation, classical steam distillation, microwave-assisted hydrodistillation, solvent free microwave extraction, headspace, headspace-solidphase microextraction combined with gas chromatographic-mass spectrometric determination

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## Abstract

In this study, the essential oils and volatiles from the flowers, leaves, and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. were characterized for the first time using microwave-assisted hydrodistillation (MAHD), solvent-free microwave extraction (SFME), headspace-assisted (HS) extraction, and solid-phase microextraction (SPME). The results were also compared with those obtained from traditional separation techniques, including classical hydrodistillation (CHD) and classical steam distillation (CSD). While some common compounds were identified across all profiles, notable differences emerged due to the varying extraction methods employed. In terms of chemical categories, oxygenated monoterpenes were found to be the most prevalent group of natural compounds contributing to the overall chemical profiles. Moreover, 1,8-cineole was identified as the major constituent component of all the characterized profiles.

## Keywords:

Classical hydrodistillation (CHD)

Classical steam distillation (CSD)

Headspace (HS)

Headspace-solid phase microextraction (HS-SPME)

Microwave-assisted hydrodistillation (MAHD)

*Pulicaria gnaphalodes* (Vent.) Boiss.

Solvent free microwave extraction (SFME)

## 1. Introduction

Asteraceae, also known as the Aster, Daisy, or Compositae family, is one of the largest families of flowering plants. This family comprises over 32,000 species across more than 1,900 genera, making it one of the most extensive plant families globally (Xu et al., 2017). These plants are found in various habitats, from subpolar to tropical regions, and thrive in diverse environments including deserts and temperate areas.

*Pulicaria gnaphalodes* (Vent.) Boiss. (Asteraceae) is a perennial herb belonging to the Asteraceae family, commonly found in regions ranging from Iraq to Central Asia, including the Western Himalayas and the Arabian Peninsula (Kasote et al., 2024). This plant is characterized by its densely branched stems and a distinctive covering of long, white, lanate tomentum, particularly at the base (Harati et al., 2023). This species typically thrives in temperate climates and is often found in various ecological niches across its native range. Its adaptability to different environments contributes to its widespread presence. It has a wide range of traditional medicinal uses, although specific applications can vary by region (Pourhossein Alamdary et al., 2023; Kasote et al., 2024). However, further research into its phytochemical properties could provide insights into its potential benefits.

The main objective of the current report is to provide a detailed information regarding the quantitative and qualitative analysis and characterization of the chemical makeup of essential oils and volatile compounds

obtained from the flowers, leaves, and stems of *P. gnaphalodes* (Fig. 1), a wild and herbal plant found in Sahraye Jalali, Shahrood, Semnan Province, Iran, utilizing both traditional and modern separation methods.

## 2. Experimental

### 2.1. Chemicals and supplies

The normal alkane mixtures were obtained from Fluka (Buchs, Switzerland). High-purity helium and nitrogen served as carrier gases for the gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) equipment.

### 2.2. Plant material and botanical identification

The plant samples (Fig. 1) were gathered in April 2024 during the flowering period, while wearing polystyrene gloves, in Sahraye Jalali, Shahrood, Semnan Province, Iran, located at 36°24'48"N 54°58'41"E. The plant was first identified by a local botanist and a voucher specimen has been submitted to the Herbarium of the Research Institute of Forests and Rangelands in Tehran, Iran, for further verification (HRIFRT56q).



**Fig. 1.** The photograph from the aerial parts of *Pulicaria gnaphalodes* (Vent.) Boiss. growing wild in the sampling area (Sahraye Jalali, Shahrood, Semnan Province, Iran (36°24'48"N 54°58'41"E).

### 2.3. Classical hydrodistillation (CHD)

Classical hydrodistillation is a traditional method used for extracting essential oils from a large number of plant materials. This technique has been widely employed in both laboratory and industrial settings due to its effectiveness in producing high-quality oils (Perović et al., 2024). This approach which is usually conducted using a Clevenger glassware set up consists of three sequential steps, namely heating, condensation and separation. Despite its simplicity and effectiveness, CHD is a time-consuming process and may cause thermal degradation in many cases (Freitas et al., 2023). In addition, a significant amount of water is needed for the process, which may not be environmentally sustainable in some contexts.

To extract the essential oils from various parts of *P. gnaphalodes*, 150-g portions of air-dried flowers, leaves, and stems of this herbal species were individually subjected to hydrodistillation using a Clevenger-type glass apparatus for three hours. The resulting essential oils were pale yellow in color, then dried with anhydrous sodium sulfate and stored at 4 °C, away from light, until analysis.

#### 2.4. Classical steam distillation (CSD)

Classical steam distillation operates on the principle of using steam to vaporize volatile compounds. The steam is introduced into a simple system containing the plant material, causing the essential oils to evaporate. The vapor then travels to a condenser, where it is cooled and condensed back into liquid form. The same three steps involved in CHD are also observed in CSD technique (Shrivastava, 2023). CSD can be utilized for the separation of both essential oils and hydrosols. The practical limitations of are the same as CHD, as well.

In one part of this study, the essential oils extracted from the aforementioned organs, *i.e.*, flowers, leaves, and stems of *P. gnaphalodes* were isolated using a setup that included a glass column connected at its lower and upper sections to a water flask and a condenser, respectively. In this straightforward system, the plant sample was subjected to a continuous, gentle flow of water vapor, which facilitated the release of the essential oils. The resulting essential oils were subsequently separated through decantation.

#### 2.5. Operational characteristics hydrodistillation (MAHD)

Microwave-assisted hydrodistillation (MAHD) is an innovative extraction technique that combines microwave energy with traditional hydrodistillation method to enhance the extraction of essential oils from plant materials (Elnour et al., 2024). This method offers several advantages over conventional extraction techniques such as more flexibility, friendly-used nature, increased efficiency, more cost-, time- and energy-effective identity, and higher yields of the essential oils (Kirkinci et al., 2024). A comprehensive overview of the application of the MAHD technique has been provided in our previous papers (Mohammadhosseini, 2017; Hashemi-Moghaddam et al., 2018). Briefly, a microwave oven (Samsung, South Korea) operating at a frequency of 2450 MHz, with sufficient internal capacity to house the glass Clevenger setup, was utilized. This oven was connected to a condenser located outside the system. It was determined that 50-g portions of the dried parts of *P. gnaphalodes* were sufficient to complete the extraction process.

#### 2.6. Operational characteristics of the solvent-free microwave extraction (SFME)

Solvent-free microwave extraction (SFME) is an advanced extraction technique that utilizes microwave energy to extract essential oils from plant materials without the use of solvents. This method is gaining popularity due to its efficiency, environmental benefits, and ability to produce high-quality extracts. In this approach, the plant material is placed in a microwave reactor where it is exposed to microwave radiation. This causes rapid heating and vaporization of the volatile compounds, which can then be collected in a condenser. SFME is an efficient technique which can be utilized for the extraction of both essential oil and bioactive compounds (Yingngam, 2023). Similar to MAHD, SFME is a green and time-saving approach and can results in obtaining essential oils with satisfied yield and quality.

The fundamental principles of the solvent-free microwave extraction (SFME) method have been thoroughly discussed in our previous reports, which closely resemble those of the MAHD method (Mohammadhosseini et al., 2015; Mohammadhosseini, 2021). The primary distinction between these two approaches lies in the soaking 50-g portions of the dried and ground plant material (*P. gnaphalodes*) in the SFME method, followed by the immediate drainage of excess water. This process primarily serves to hydrate the outer layers of the plant material, thereby conditioning them for more effective extraction of essential oils from the corresponding secretory glands.

## 2.7. Operational details of HS-GC-MS

Headspace analysis combined with gas chromatography-mass spectrometry (GC-MS) is an effective method for analyzing volatile organic compounds (VOCs) in various samples (Kwon et al., 2023; Wijeweera Patabandige et al., 2024). This technique is particularly useful for extracting and identifying compounds from complex matrices, *e.g.*, food (Siderhurst et al., 2024), pharmaceuticals (You et al., 2023), as well as environmental samples and detecting pollutants in soil and water samples (Skok and Bazel, 2023).

In headspace sampling, a sealed vial containing the sample is heated, allowing volatile compounds to partition into the gas phase above the sample. This gas layer, or "headspace," is then analyzed by GC-MS. This method is advantageous for its simplicity and efficiency, especially when dealing with solid or viscous samples that may be difficult to be extracted directly (Koonani and Ghiasvand, 2023; Karimi and Gross, 2024).

This approach has found a number of uses in a variety of disciplines, *e.g.*, food and beverage industries, pharmaceuticals, environmental monitoring and detecting pollutants in soil and water samples. The main superiorities of this technique include:

- i) Reducing the need for extensive sample preparation.
- ii) Minimizing contamination risks associated with direct sampling methods.
- iii) High potentiality for the rapid analysis of multiple samples, making it suitable for high-throughput settings (Skok and Bazel, 2023).

To maintain the headspace, a commercially available Combi PAL system, equipped with a headspace autosampler, heater, and agitator, was utilized. Accordingly, the vials were positioned in the headspace tray. To complete the extraction of volatiles using the HSA-GC-MS method, 20 mL vials were heated to an optimized temperature of 95 °C and allowed to stand for 30 minutes under mild agitation. In this analysis, the temperatures of both the sampling needle and transmission line were adjusted at 100 °C.

## 2.8. Operational details of headspace solid-phase microextraction (HS-SPME)

Headspace-solid phase microextraction (SPME) is a solvent-free extraction method that utilizes a coated fiber to extract volatile and semi-volatile compounds from various matrices, including gases, liquids, and solids. The analytes are adsorbed onto the fiber coating, which is then desorbed for analysis using some analytical methods like gas chromatography (GC) or liquid chromatography (LC) (Agatonovic-Kustrin et al., 2023).

The SPME device consists of a fused-silica fiber coated with a stationary phase suitable for the target analytes. After a predetermined extraction time, the fiber is removed and inserted into the GC or LC system for thermal or solvent desorption, respectively. SPME has been widely applied in a broad range of fields involving food analysis (Aspromonte et al., 2024), biological samples (Feng et al., 2024), forensic science (Bhatnagar et al., 2023), among others.

In this study, HS-SPME sampling technique was conducted using a manual holder and a 1-cm fused-silica fiber tip coated with a combination of polydimethylsiloxane and carboxen (PDMS-CAR) (Mohammadosseini, 2015). The SPME fiber was conditioned according to the manufacturer's instructions. For sampling, one gram of the powdered plant material was placed in a 20-mL sample vial, which was sealed with septum-type caps and heated to 70 °C for fifteen minutes. Subsequently, the PDMS-CAR fiber was extended through the needle to the outer position by piercing the septum with the SPME needle, exposing the fiber coating to the headspace above the sample. After an additional 15 minutes of sampling, the fiber was retracted into the needle and transferred to the GC-MS injection port, utilizing the splitless mode at a temperature of 250 °C for five minutes.

## 2.9. GC and GC-MS analyses

Gas chromatographic analyses were conducted using a Shimadzu 15A gas chromatograph equipped with a split/splitless injector (ratio 1:30) and a flame ionization detector, both operating at 250 °C. In the separation process, high-purity nitrogen was employed as the carrier gas, with a flow rate of 1 mL/min through a DB-5 capillary column measuring 50 m × 0.2 mm and featuring a film thickness of 0.32 µm. According to our temperature programming, the column temperature was initially maintained at 60 °C for 3 minutes, then increased to 220 °C at a rate of 5 °C/min, and finally held constant at 220 °C for an additional 5 minutes. Relative percentage amounts were calculated from the peak areas using a CR5 Shimadzu CR pack, without the application of correction factors.

GC/MS analysis was conducted using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The effluent from the GC column was introduced directly into the mass spectrometer source. The column temperature programming was consistent with the GC analysis. The flow rate of the helium carrier gas was set at 1 mL/min. Additionally, the final temperature of the column was precisely 230 °C, while the detector temperature was maintained at 250 °C. All mass spectra were acquired at 70 eV (E1) over the range of 30-350 amu, with an electron multiplier voltage of 1800 eV and scan times of 2 scans per second.

### 3. Results and Discussion

Essential oils are complex mixtures of secondary metabolites produced by plants, primarily consisting of terpenes and their derivatives (Karpuz Ağören and Akkol, 2024; Siddiqui et al., 2024). These compounds serve various ecological roles, including protection against pests, attraction of pollinators (Raguso, 2020), and seed dispersal (Mugao et al., 2020). Recent studies have highlighted the diverse chemical compositions (Caputo et al., 2020) and biological activities (Caputo et al., 2020; Candela et al., 2021; Cunha et al., 2022) of essential oils derived from different plant species. It is important to note that essential oils and the volatile fractions of plant materials are both derived from plants, yet they differ significantly in their composition, extraction methods, and characteristics. The term “volatile fractions” refers to all volatile components present in plant materials which can be obtained through various methods, e.g., solvent extraction, cold pressing as well as HS and HS-SPME techniques. Furthermore, volatile fractions may contain a wider range of compounds, including non-aromatic volatiles that do not contribute to the plant's characteristic scent.

#### 3. Prevalence of various natural compounds constituting groups in the essential oil and volatile profiles of *Pulicaria gnaphalodes* (Vent.) Boiss.

The chemical profiles of the various parts of *P. gnaphalodes* were characterized by monoterpene hydrocarbons (MH), oxygenated monoterpene (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpene (OS) and non-terpene hydrocarbons (NH) when using the CHD, CSD, MAHD, SFME, HS and HS-SPME approaches.

Using the CHD method:

i) Flowers: Twelve monoterpene hydrocarbons (13.9%), fourteen oxygenated monoterpenes (60.5%), four sesquiterpene hydrocarbons (15.2%), three oxygenated sesquiterpenes (8.5%) and one non-terpene hydrocarbon (0.6%) were identified (see Table 1 and typical Fig. 2).

ii) Leaves: Ten monoterpene hydrocarbons (23.1%), thirteen oxygenated monoterpenes (64.1%), two sesquiterpene hydrocarbons (8.3%), three oxygenated sesquiterpenes (3.3%) and one non-terpene hydrocarbon (0.1%) were the main components (Table 1).

iii) Stems: Ten monoterpene hydrocarbons (18.7%), fourteen oxygenated monoterpenes (51.5%), three sesquiterpene hydrocarbons (24.9%), three oxygenated sesquiterpenes (3.6%) and one non-terpene hydrocarbon (0.9%) constituted the stem oil (Table 1).

Using the SD method (see Table 2) in the:

i) Flowers: Nine monoterpene hydrocarbons (22.5%), fourteen oxygenated monoterpenes (51.6%), four sesquiterpene hydrocarbons (24.7%) and three oxygenated sesquiterpenes (1.0%) were identified (Table 2).

ii) Leaves: Eleven monoterpene hydrocarbons (15.8%), thirteen oxygenated monoterpenes (61.9%), two sesquiterpene hydrocarbons (20.4%), three oxygenated sesquiterpenes (1.6%) and one non-terpene hydrocarbon (0.2%) were among the main recognized components (Table 2).

iii) Stems: Eleven monoterpene hydrocarbons (14.4%), fourteen oxygenated monoterpenes (68.1%), four sesquiterpene hydrocarbons (10.7%), three oxygenated sesquiterpenes (4.9%) and one non-terpene hydrocarbon (1.2%) contributed as the constituting groups of the stem oil (Table 2).

In the MAHD oil profiles (see Table 3) of:

i) Flowers: Thirteen monoterpene hydrocarbons (8.8%), fifteen oxygenated monoterpenes (66.1%), five sesquiterpene hydrocarbons (18.5%), four oxygenated sesquiterpenes (5.6%) and one non-terpene hydrocarbon (0.1%) were identified (Table 3).

ii) Leaves: Ten monoterpene hydrocarbons (8.4%), fifteen oxygenated monoterpenes (62.4%), five sesquiterpene hydrocarbons (20.4%) and four oxygenated sesquiterpenes (7.9%) were characterized (Table 3).

iii) Stems: Nine monoterpene hydrocarbons (6.2%), fifteen oxygenated monoterpenes (56.8%), five sesquiterpene hydrocarbons (27.4%), three oxygenated sesquiterpenes (9.0%) and one non-terpene hydrocarbon (0.2%) contributed to the respective profile (Table 3).

In the SFME oil profiles (see Table 4) of:

i) Flowers: Twelve monoterpene hydrocarbons (30.6%), nine oxygenated monoterpenes (60.3%), five sesquiterpene hydrocarbons (4.0%), four oxygenated sesquiterpenes (4.5%) and one non-terpene hydrocarbon (0.2%) were recognized (Table 4).

ii) Leaves: Twelve monoterpene hydrocarbons (9.1%), twelve oxygenated monoterpenes (77.0%), five sesquiterpene hydrocarbons (7.9%), four oxygenated sesquiterpenes (4.8%) and one non-terpene hydrocarbon (0.1%) were the most abundant components (Table 4).

iii) Stems: Twelve monoterpene hydrocarbons (8.1%), ten oxygenated monoterpenes (62.3%), five sesquiterpene hydrocarbons (13.6%), four oxygenated sesquiterpenes (14.2%) and one non-terpene hydrocarbon (0.8%) were characterized (Table 4).

By using the HS approach (see Table 5), in the profiles of:

i) Flowers: Eleven monoterpene hydrocarbons (18.8%), thirteen oxygenated monoterpenes (63.4%), four sesquiterpene hydrocarbons (6.6%) and four oxygenated sesquiterpenes (10.4%) were the prevailing components (Table 5).

ii) Leaves: Eleven monoterpene hydrocarbons (18.7%), thirteen oxygenated monoterpenes (66.1%), four sesquiterpene hydrocarbons (8.9%), four oxygenated sesquiterpenes (5.4%) and one non-terpene hydrocarbon (0.4%) were involved (Table 5).

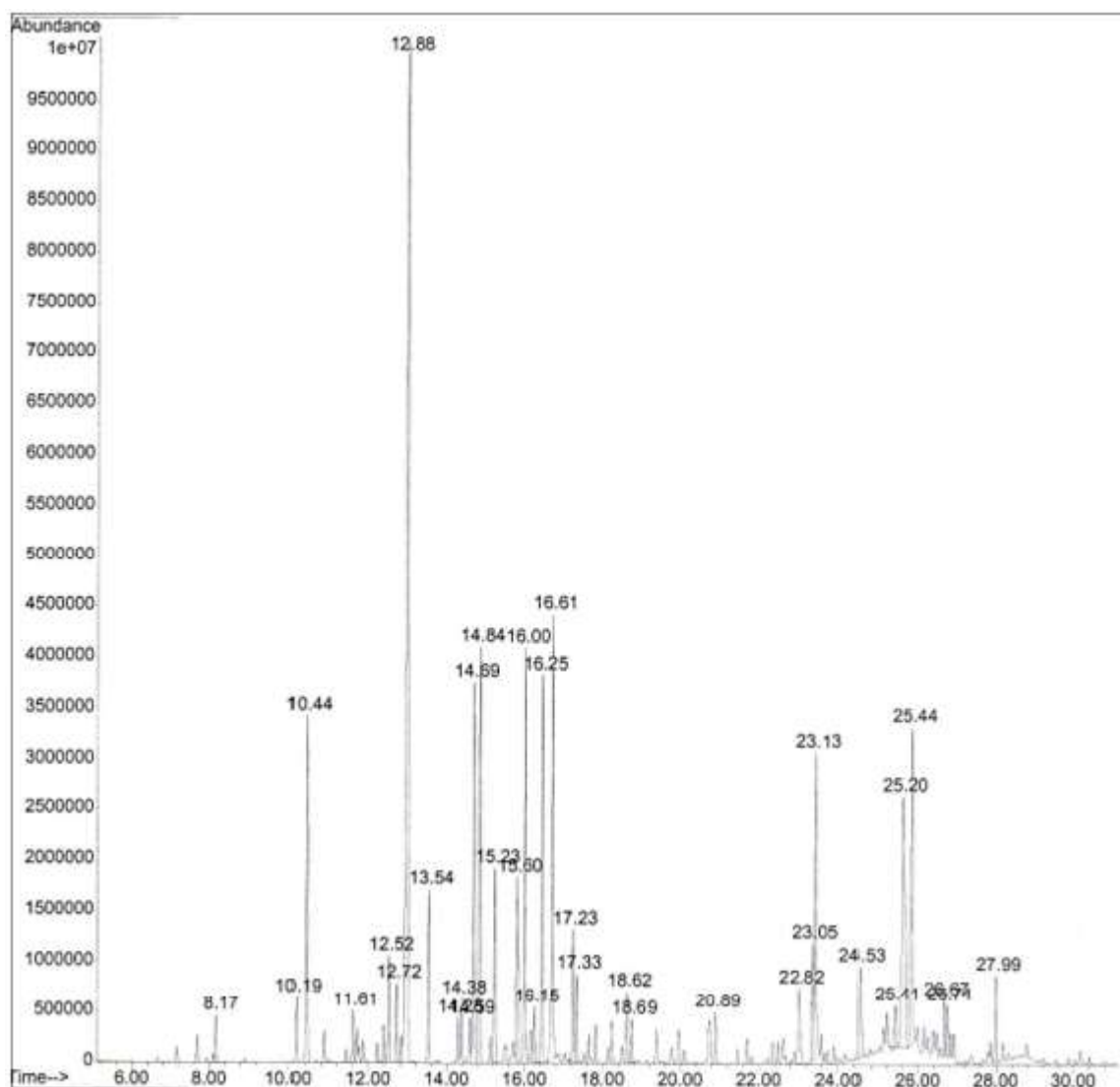
iii) Stems: Eleven monoterpene hydrocarbons (23.0%), thirteen oxygenated monoterpenes (56.9%), four sesquiterpene hydrocarbons (12.5%), four oxygenated sesquiterpenes (6.6%) and one non-terpene hydrocarbon (0.5%) were the major constituents (Table 5).

In the SPME profiles (see Table 6) of:

i) Flowers: Nine monoterpene hydrocarbons (23.4%), twelve oxygenated monoterpenes (60.4%), four sesquiterpene hydrocarbons (8.8%), and four oxygenated sesquiterpenes (6.0%) were identified (Table 6).

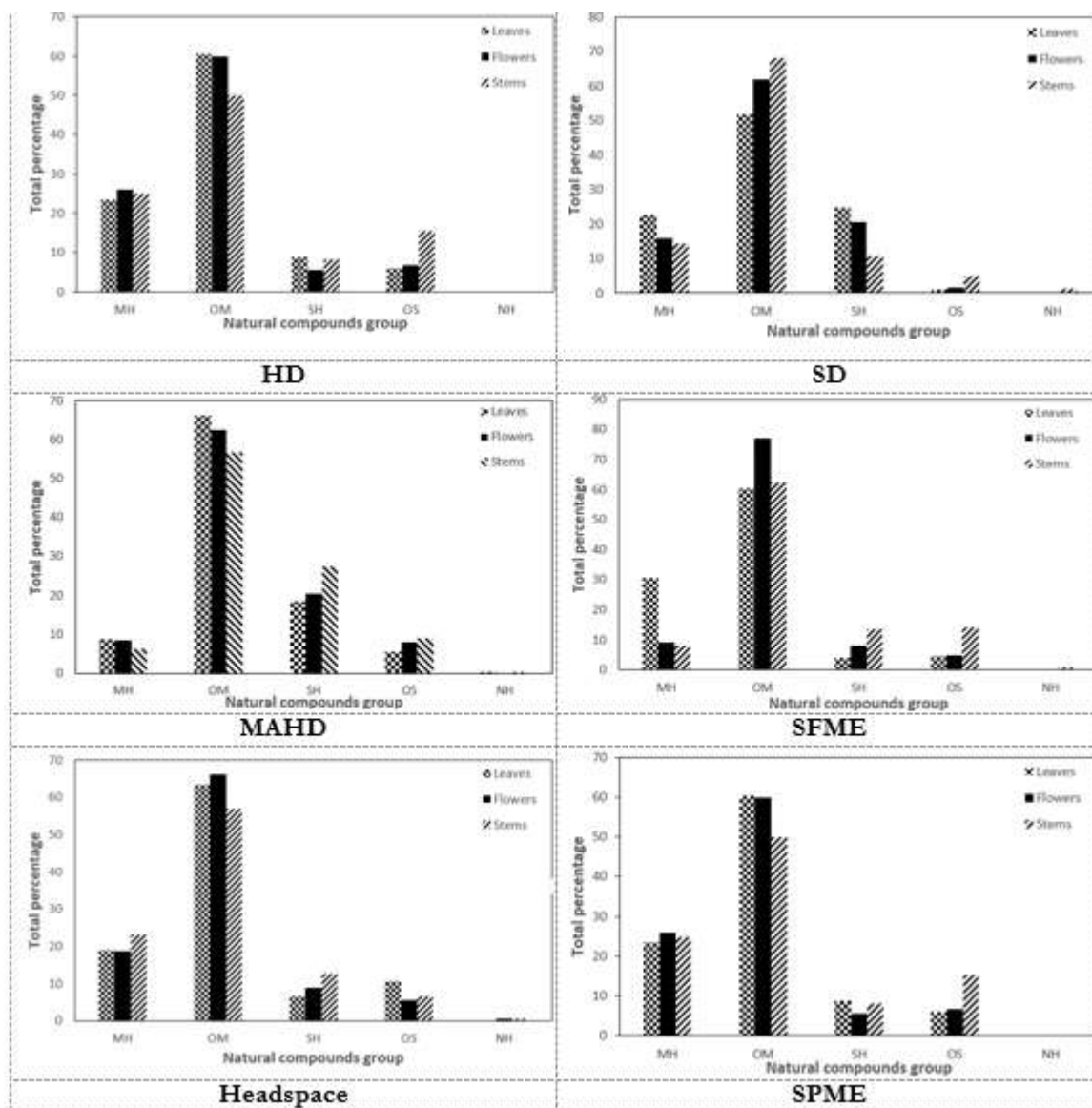
ii) Leaves: Nine monoterpene hydrocarbons (26.0%), twelve oxygenated monoterpenes (59.8%), four sesquiterpene hydrocarbons (5.5%) and four oxygenated sesquiterpenes (6.8%) were distinguished (Table 6).

iii) Stems: Ten monoterpene hydrocarbons (24.9%), eleven oxygenated monoterpenes (49.9%), four sesquiterpene hydrocarbons (8.1%) and four oxygenated sesquiterpenes (15.4%) were present (Table 6).



**Fig. 2.** The typical chromatogram of the essential oils from the flowers of *Pulicaria gnaphalodes* (Vent.) Boiss. growing wild in Sahraye Jalali, Shahrood, Semnan Province, Iran (36°24'48"N 54°58'41"E).

In Fig. 3, a comparison is presented regarding the relative percentages of the natural compound groups that constitute the chemical profiles of the essential oils and volatile fractions of *P. gnaphalodes* organs, utilizing the methods described.



**Fig. 3.** A simple graphical comparison of the groups of natural compounds (MHs, OMs, SHs, OSs, NHs) that contribute to the chemical profiles of essential oils and volatiles from the flowers, leaves, and stems of *P. gnaphalodes*, utilizing various classical and fairly advanced methods.

### 3.2. Chemical profiles of *P. gnaphalodes* Ven. in similar reports

To the best of our knowledge, some sporadic reports could be found in the literature dealing with the qualitative and quantitative analysis of the essential oils from foliage of *P. gnaphalodes*. In this sense, Khani and Asghari (2012) have characterized the constituent components of *P. gnaphalodes* and reported chrysanthenyl acetate (22.4%), 2L-4L-dihydroxy eicosane (18.5%), verbenol (16.6%), dehydroaromadendrene (12.5%),  $\beta$ -pinene (6.4%), and 1,8-cineole (5.6%) as the most abundant components. In a related study, the essential oils extracted from the aerial parts of *P. gnaphalodes*, which grow wild in Iran, were analyzed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). This analysis identified thirty-three compounds that accounted for approximately 91.6% of the total oil. Among these, oxygenated sesquiterpenes (47.5%) and sesquiterpene hydrocarbons (26.0%) were found to be the predominant fractions. In contrast, the total content of monoterpene hydrocarbons was significantly lower, at 12.7%. Notably, the major constituents identified in a similar attempt included calamenene-10-one (12.2%), longifolol (6.0%), curcumen-15-al-ar (5.6%), 14-hydroxy- $\delta$  cadinene (5.5%), calamene 10-ol-*trans* (5.0%), and curcumenol (4.9%) (Salarbashi et al., 2013).



In another report, Shariatifarn et al. (2014) have studied the essential oils from the aerial parts of *P. gnaphalodes* in soybean oil. In this report, fifty-eight compounds were identified altogether representing 90.7% of the essential oil composition among which the main ingredients were found to be  $\alpha$ -pinene (30.2%), 1,8-cineole (12.1%),  $\beta$ -citronellol (9.6%), mertenol (6.6%),  $\alpha$ -terpineol (6.1%), 4-terpineol (5.9%) and chrysanthenone (2.9%). In the study of Gandomi et al. (2015), the chemical composition of *P. gnaphalodes* essential oil was evaluated by gas chromatography-mass spectrometric analysis. Accordingly, among the 34 components,  $\alpha$ -pinene (32.2%) and 1,8-cineole (10.9%) were characterized as the major constituent components.

Hassanabadi et al. (2022) have recently analyzed the essential oil profiles of some *P. gnaphaloides* populations. According to the finding of this report, the main constituents of the characterized chemical profiles were found to be: 1,8-cineole (11.8-24.0%), terpinen-4-ol (4.8-20.3%),  $\alpha$ -terpineol (7.9-11.5%), geraniol (1.6-7.5%), and cedr-8(15)-en-9- $\alpha$ -ol (2.5-11.0%).

### 3.3. Chemical profiles of other *Pulicaria* species worldwide

In literature, a number of reports could be found dealing with the analysis of a variety of *Pulicaria* species. In this context, a simple perusal of the literature resembles that all the available data concerning the essential oil composition of various species of *Pulicaria* as an herbal genus are from Middle East countries like Saudi Arabia, United Arab Emirates, Iran, etc. as well as African countries like Algeria, Sudan, Tunisia, Morocco, etc. However, only few reports are available for this genus species in European or American countries. Anyway, as seen in Table 7, a wide range of natural compounds have been characterized as the major constituents of the reported profiles of diverse *Pulicaria* species. As expected, the majority of the identified profiles were dominated by high frequency of oxygenated monoterpenes, e.g., *tau*-cadinol (Yusufoglu et al., 2021),  $\alpha$ -cadinol (Djermene et al., 2023), chrysanthenone (Chaib et al., 2017), carvotanacetone (Cristofari et al., 2011; Awadh Ali et al., 2012b; Gherib et al., 2016; Shahat et al., 2017; Issa et al., 2020), thymol (Hanbali et al., 2005; Sharifi-Rad et al., 2014), camphor (Assaeed et al., 2020; Mohamed et al., 2020), 4-terpineole (Ravandeh et al., 2011), 1,8-cineole (Seidi Damyeh and Niakousari, 2017), carvacrol (Mustafa et al., 2020). On the other hand, some of the chemical profiles of some of the other species of *Pulicaria* are composed of high quantities of sesquiterpene hydrocarbons (Al Yousuf et al., 2001; Awadh Ali et al., 2012a; Fadel et al., 2020) and oxygenated sesquiterpenes (Al Yousuf et al., 2001; Awadh Ali et al., 2012a; Yusufoglu et al., 2021). However, in minor cases, monoterpene hydrocarbons like  $\alpha$ -pinene (Nematollahi et al., 2006) and  $\alpha$ -ocimene (Lougraimzi et al., 2022) have been reported as the most abundant constituents.

**Table 1**

Chemical composition of the essential oils from the flowers, leaves and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. using classical hydrodistillation (CHD) combined with gas chromatography-mass spectrometry

<sup>a</sup>.

Num.	Compound	RI (Lit.) <sup>b</sup>	NCCG <sup>1</sup>	Percentage		
				Flowers	Leaves	Stems
1	$\alpha$ -Thujene	924	MH	0.9	0.1	1.3
2	$\alpha$ -Pinene	932	MH	4.9	18.1	10.4
3	$\alpha$ -Fenchene	945	MH	0.5	0.5	0.8
4	$\beta$ -Pinene	974	MH	0.7	2.1	0.1
5	$\alpha$ -Phellandrene	1002	MH	1.0	-	3.2
6	$\alpha$ -Terpinene	1014	MH	1.3	0.1	0.4
7	<i>p</i> -Cymene	1020	MH	1.1	0.3	-
8	$\beta$ -Phellandrene	1025	MH	0.4	0.1	0.8
9	1,8-Cineole	1026	OM	20.2	7.9	25.1
10	( <i>Z</i> )- $\beta$ -Ocimene	1032	MH	0.1	-	-
11	( <i>E</i> )- $\beta$ -Ocimene	1044	MH	0.2	0.2	0.5
12	$\gamma$ -Terpinene	1054	MH	2.2	1.4	0.3
13	$\alpha$ -Terpinolene	1086	MH	0.6	0.2	0.9
14	Linalool	1095	OM	2.1	4.1	3.7
15	dehydro-Sabina ketone	1117	OM	0.7	0.3	0.1
16	<i>exo</i> -Fenchol	1118	OM	5.3	-	0.1
17	Chrysanthenone	1124	OM	5.9	1.1	1.3
18	Camphor	1141	OM	2.6	0.2	0.4
19	<i>cis</i> -Chrysanthenol	1160	OM	6.1	0.7	0.5
20	Borneol	1165	OM	0.8	0.2	0.1
21	Terpinen-4-ol	1174	OM	5.5	24.4	9.5
22	$\alpha$ -Terpineol	1186	OM	6.8	0.3	0.1
23	Citronellol	1223	OM	1.8	0.5	0.4
24	Nerol	1227	OM	1.2	0.1	0.4
25	Bornyl acetate	1254	OM	0.6	24.2	2.7
26	Thymol	1289	OM	0.9	0.1	7.1
27	Methyl eugenol	1403	NH	0.6	0.1	0.9
28	Germacrene D	1484	SH	1.4	4.2	4.0
29	$\alpha$ -Muurolene	1500	SH	1.4	-	-
30	$\gamma$ -Cadinene	1513	SH	1.6	-	11.2
31	$\delta$ -Cadinene	1522	SH	10.8	4.1	9.7
32	Germacrene D-4-ol	1574	OS	1.6	0.6	1.1
33	<i>epi</i> - $\alpha$ -Cadinol	1638	OS	0.2	0.6	0.9
34	$\alpha$ -Cadinol	1652	OS	6.7	2.1	1.6
Total				98.7	98.9	99.6

<sup>a</sup> The compounds have been sorted according to their elution order on an HP-5MS column.

<sup>b</sup> Retention indices in the literature

<sup>c</sup> NCCG: Natural compound constituting group

**Table 2**

Chemical composition of the essential oils from the flowers, leaves and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. using classical steam distillation (CSD) combined with gas chromatography-mass spectrometry<sup>a</sup>.

Num.	Compound	RI (Lit.) <sup>b</sup>	NCCG <sup>c</sup>	Percentage		
				Flowers	Leaves	Stems
1	$\alpha$ -Thujene	924	MH	4.1	1.2	3.1
2	$\alpha$ -Pinene	932	MH	8.2	6.1	3.3
3	$\alpha$ -Fenchene	945	MH	1.0	0.2	-
4	$\beta$ -Pinene	974	MH	6.5	5.1	5.4
5	$\alpha$ -Phellandrene	1002	MH	0.3	0.5	0.1
6	$\alpha$ -Terpinene	1014	MH	0.7	1.1	1.0
7	<i>p</i> -Cymene	1020	MH	-	0.6	0.4
8	$\beta$ -Phellandrene	1025	MH	1.1	0.2	0.1
9	1,8-Cineole	1026	OM	30.1	19.5	31.2
10	( <i>Z</i> )- $\beta$ -Ocimene	1032	MH	-	0.1	0.1
11	( <i>E</i> )- $\beta$ -Ocimene	1044	MH	0.4	0.3	0.2
12	$\gamma$ -Terpinene	1054	MH	0.2	0.4	0.5
13	$\alpha$ -Terpinolene	1086	MH	-	-	0.2
14	Linalool	1095	OM	1.4	0.7	1.1
15	dehydro-Sabina ketone	1117	OM	1.2	2.4	4.0
16	<i>exo</i> -Fenchol	1118	OM	0.4	0.2	0.5
17	Chrysanthenone	1124	OM	0.1	0.1	0.7
18	Camphor	1141	OM	1.1	0.5	0.5
19	<i>cis</i> -Chrysanthenol	1160	OM	5.0	2.1	1.5
20	Borneol	1165	OM	2.7	1.4	0.7
21	Terpinen-4-ol	1174	OM	1.1	3.1	1.8
22	$\alpha$ -Terpineol	1186	OM	2.2	5.5	0.8
23	Citronellol	1223	OM	1.7	1.0	1.1
24	Nerol	1227	OM	0.1	-	0.1
25	Bornyl acetate	1254	OM	3.3	24.9	16.0
26	Thymol	1289	OM	1.2	0.5	8.1
27	Methyl eugenol	1403	NH	-	0.2	1.2
28	Germacrene D	1484	SH	2.4	6.5	5.1
29	$\alpha$ -Muurolene	1500	SH	0.1	-	0.1
30	$\gamma$ -Cadinene	1513	SH	5.4	-	0.4
31	$\delta$ -Cadinene	1522	SH	16.8	13.9	5.1
32	Germacrene D-4-ol	1574	OS	0.4	0.1	1.5
33	<i>epi</i> - $\alpha$ -Cadinol	1638	OS	0.1	0.4	1.9
34	$\alpha$ -Cadinol	1652	OS	0.5	1.1	1.5
Total				99.8	99.9	99.3

<sup>a</sup> The compounds have been sorted according to their elution order on an HP-5MS column.

<sup>b</sup> Retention indices in the literature

<sup>c</sup> NCCG: Natural compound constituting group

**Table 3**

Chemical composition of the essential oils from the flowers, leaves and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. using microwave assisted hydrodistillation (MAHD) combined with gas chromatography-mass spectrometry<sup>a</sup>.

Num.	Compound	RI (Lit.) <sup>b</sup>	NCCG1	Percentage		
				Flowers	Leaves	Stems
1	$\alpha$ -Thujene	924	MH	0.1	1.0	0.9
2	$\alpha$ -Pinene	932	MH	2	5.1	4
3	$\alpha$ -Fenchene	945	MH	0.1	0.1	0.1
4	$\beta$ -Pinene	974	MH	0.1	0.1	0.1
5	Myrcene	988	MH	3.7	-	-
6	$\alpha$ -Phellandrene	1002	MH	0.1	0.1	0.2
7	$\alpha$ -Terpinene	1014	MH	0.4	-	-
8	p-Cymene	1020	MH	0.5	0.4	-
9	$\beta$ -Phellandrene	1025	MH	0.2	0.2	0.1
10	1,8-Cineole	1026	OM	44.1	39.4	33.0
11	(Z)- $\beta$ -Ocimene	1032	MH	0.2	0.1	0.1
12	(E)- $\beta$ -Ocimene	1044	MH	0.1	0.1	0.3
13	$\gamma$ -Terpinene	1054	MH	1.2	1.2	0.4
14	$\alpha$ -Terpinolene	1086	MH	0.1	-	-
15	Linalool	1095	OM	0.2	1.2	2.0
16	endo-Fenchol	1114	OM	0.4	0.1	0.2
17	dehydro-Sabina ketone	1117	OM	0.5	0.5	1.1
18	exo-Fenchol	1118	OM	0.1	0.4	0.2
19	Chrysanthenone	1124	OM	0.5	0.1	0.1
20	Camphor	1141	OM	4.1	6	6.3
21	cis-Chrysanthenol	1160	OM	0.1	0.2	0.2
22	Borneol	1165	OM	1	1.2	1
23	Terpinen-4-ol	1174	OM	0.8	0.2	2.5
24	$\alpha$ -Terpineol	1186	OM	9.5	6.1	3.2
25	Citronellol	1223	OM	0.8	0.2	0.2
26	Nerol	1227	OM	0.1	0.7	0.3
27	Bornyl acetate	1254	OM	0.8	0.7	0.3
28	Thymol	1289	OM	3.1	5.4	6.2
29	Methyl eugenol	1403	NH	0.1	-	0.2
30	Germacrene D	1484	SH	8.4	7	11.2
31	$\alpha$ -Muurolene	1500	SH	2.4	0.9	1
32	$\gamma$ -Cadinene	1513	SH	0.6	9.2	4.1
33	$\delta$ -Cadinene	1522	SH	1.3	2.4	7.7
34	$\alpha$ -Cadinene	1537	SH	5.8	0.9	3.4
35	Germacrene D-4-ol	1574	OS	0.6	2.6	3.1
36	epi- $\alpha$ -Cadinol	1638	OS	0.7	1.8	2.8
37	$\alpha$ -Cadinol	1652	OS	3.6	2.9	3.1
38	(2E,6E)-Farnesol	1742	OS	0.7	0.6	-
Total				99.1	99.1	99.6

<sup>a</sup> The compounds have been sorted according to their elution order on an HP-5MS column.

<sup>b</sup> Retention indices in the literature

<sup>c</sup> NCCG: Natural compound constituting group

**Table 4**

Chemical composition of the essential oils from the flowers, leaves and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. using solvent free microwave extraction (SFME) combined with gas chromatography-mass spectrometry<sup>a</sup>.

Num.	Compound	RI (Lit.) <sup>b</sup>	NCCG <sup>1</sup>	Percentage		
				Flowers	Leaves	Stems
1	$\alpha$ -Thujene	924	MH	3.4	2.0	1.3
2	$\alpha$ -Pinene	932	MH	9.1	3.2	1.8
3	$\alpha$ -Fenchene	945	MH	-	0.2	0.8
4	$\beta$ -Pinene	974	MH	6.5	1.2	1.5
5	Myrcene	988	MH	0.2	0.1	0.1
6	$\alpha$ -Phellandrene	1002	MH	0.4	0.2	0.1
7	$\alpha$ -Terpinene	1014	MH	0.4	0.2	0.2
8	<i>p</i> -Cymene	1020	MH	0.2	0.1	0.5
9	$\beta$ -Phellandrene	1025	MH	1.0	1.1	1.2
10	1,8-Cineole	1026	OM	28.5	31.4	27.9
11	( <i>Z</i> )- $\beta$ -Ocimene	1032	MH	4.5	-	0.2
12	( <i>E</i> )- $\beta$ -Ocimene	1044	MH	4.2	0.3	0.1
13	$\gamma$ -Terpinene	1054	MH	0.2	0.4	-
14	$\alpha$ -Terpinolene	1086	MH	0.5	0.1	0.3
15	Linalool	1095	OM	6.0	3.1	3.5
16	<i>exo</i> -Fenchol	1118	OM	-	0.7	-
17	Camphor	1141	OM	10.0	12.1	5.4
18	<i>cis</i> -Chrysanthenol	1160	OM	0.2	0.6	0.4
19	Borneol	1165	OM	2.1	2.4	2.3
20	Terpinen-4-ol	1174	OM	2.8	1.2	4.9
21	$\alpha$ -Terpineol	1186	OM	4.0	17.3	4.2
22	Citronellol	1223	OM	-	0.1	0.4
23	Nerol	1227	OM	-	0.5	-
24	Bornyl acetate	1254	OM	1.7	1.1	1.2
25	Thymol	1289	OM	5.0	6.5	12.1
26	Methyl eugenol	1403	NH	0.2	0.1	0.8
27	Germacrene D	1484	SH	2.1	2.0	6.3
28	$\alpha$ -Muurolene	1500	SH	0.4	0.2	0.3
29	$\gamma$ -Cadinene	1513	SH	0.4	5.2	0.9
30	$\delta$ -Cadinene	1522	SH	1.0	0.4	0.5
31	$\alpha$ -Cadinene	1537	SH	0.1	0.1	5.6
32	Germacrene D-4-ol	1574	OS	0.2	0.1	3.7
33	<i>epi</i> - $\alpha$ -Cadinol	1638	OS	0.2	2.4	3.5
34	$\alpha$ -Cadinol	1652	OS	4.0	2.1	6.3
35	(2 <i>E</i> ,6 <i>E</i> )-Farnesol	1742	OS	0.1	0.2	0.7
Total				99.6	98.9	99

<sup>a</sup> The compounds have been sorted according to their elution order on an HP-5MS column.

<sup>b</sup> Retention indices in the literature

<sup>c</sup> NCCG: Natural compound constituting group

**Table 5**

Chemical composition of the volatile fractions from the flowers, leaves and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. using headspace (HS) combined with gas chromatography-mass spectrometry (HS-GC-MS)<sup>a</sup>.

Num.	Compound	RI (Lit.) <sup>b</sup>	NCCG <sup>c</sup>	Percentage		
				Flowers	Leaves	Stems
1	$\alpha$ -Thujene	924	MH	1.3	1.9	3.1
2	$\alpha$ -Pinene	932	MH	4	6.3	5.1
3	$\alpha$ -Fenchene	945	MH	0.8	0.3	0.4
4	$\beta$ -Pinene	974	MH	3.9	1.1	2
5	Myrcene	988	MH	0.5	0.9	1
6	$\alpha$ -Phellandrene	1002	MH	0.4	0.2	0.1
7	$\alpha$ -Terpinene	1014	MH	1.2	2.2	0.7
8	<i>p</i> -Cymene	1020	MH	0.1	0.2	0.1
9	$\beta$ -Phellandrene	1025	MH	0.4	0.1	0.4
10	1,8-Cineole	1026	OM	17.4	12.1	14.7
11	$\gamma$ -Terpinene	1054	MH	5.1	3.1	8
12	$\alpha$ -Terpinolene	1086	MH	1.1	2.4	2.1
13	Linalool	1095	OM	4.1	6.2	2.5
14	<i>endo</i> -Fenchol	1114	OM	2	0.8	2.1
15	dehydro-Sabina ketone	1117	OM	0.4	3.9	4.1
16	<i>exo</i> -Fenchol	1118	OM	0.5	0.6	0.4
17	Chrysanthenone	1124	OM	0.3	0.7	0.7
18	Camphor	1141	OM	11.1	12.7	7.7
19	<i>cis</i> -Chrysanthenol	1160	OM	0.9	1.1	0.8
20	Borneol	1165	OM	3	2.8	3
21	Terpinen-4-ol	1174	OM	2.3	5.5	6.1
22	$\alpha$ -Terpineol	1186	OM	9.1	8.4	4.1
23	Bornyl acetate	1254	OM	8.3	8.0	3.5
24	Thymol	1289	OM	4	3.3	7.2
25	Methyl eugenol	1403	NH	-	0.4	0.5
26	Germacrene D	1484	SH	2.5	3.1	6.6
27	$\gamma$ -Cadinene	1513	SH	1.8	2.1	0.3
28	$\delta$ -Cadinene	1522	SH	1.1	3.3	5
29	$\alpha$ -Cadinene	1537	SH	1.2	0.4	0.6
30	Germacrene D-4-ol	1574	OS	2.1	2.0	2.5
31	<i>epi</i> - $\alpha$ -Cadinol	1638	OS	3.9	0.6	0.7
32	$\alpha$ -Cadinol	1652	OS	2.9	1.6	1
33	(2E,6E)-Farnesol	1742	OS	1.5	1.2	2.4
Total				99.2	99.5	99.5

<sup>a</sup> The compounds have been sorted according to their elution order on an HP-5MS column.

<sup>b</sup> Retention indices in the literature

<sup>c</sup> NCCG: Natural compound constituting group

**Table 6**

Chemical composition of the volatile fractions from the flowers, leaves and stems of *Pulicaria gnaphalodes* (Vent.) Boiss. using solid phase microextraction (SPME) combined with gas chromatography-mass spectrometry (SPME -GC-MS)<sup>a</sup>.

Num.	Compound	RI (Lit.) <sup>b</sup>	NCCG <sup>1</sup>	Percentage		
				Flowers	Leaves	Stems
1	$\alpha$ -Thujene	924	MH	0.1	0.6	0.8
2	$\alpha$ -Pinene	932	MH	7.4	6.5	3.3
4	$\beta$ -Pinene	974	MH	9.7	3.4	3.6
5	Myrcene	988	MH	1.9	6	2.4
6	$\alpha$ -Phellandrene	1002	MH	3.3	7.1	3.3
8	<i>p</i> -Cymene	1020	MH	0.5	0.5	0.7
9	$\beta$ -Phellandrene	1025	MH	0.2	0.4	0.3
10	1,8-Cineole	1026	OM	17.1	15.4	16.6
11	( <i>Z</i> )- $\beta$ -Ocimene	1032	MH	0.1	0.2	0.3
12	( <i>E</i> )- $\beta$ -Ocimene	1044	MH	0.2	1.3	1.8
15	Linalool	1095	OM	8.5	6.1	8.4
16	<i>exo</i> -Fenchol	1118	OM	1.1	1.3	0.9
17	Camphor	1141	OM	10.2	7.1	5
18	<i>cis</i> -Chrysanthenol	1160	OM	0.8	0.5	0.9
19	Borneol	1165	OM	1.8	3.6	5.7
20	Terpinen-4-ol	1174	OM	6.1	5.7	1.4
21	$\alpha$ -Terpineol	1186	OM	2.6	5.1	4.3
22	Citronellol	1223	OM	0.7	2.8	2.6
23	Nerol	1227	OM	1.7	1.4	3.3
24	Bornyl acetate	1254	OM	2.4	2.3	3.4
25	Thymol	1289	OM	7.4	8.5	5.8
27	Germacrene D	1484	SH	3.3	0.8	2.6
29	$\gamma$ -Cadinene	1513	SH	0.9	2.1	4.4
30	$\delta$ -Cadinene	1522	SH	3.4	0.7	0.3
31	$\alpha$ -Cadinene	1537	SH	1.2	1.9	0.8
32	Germacrene D-4-ol	1574	OS	0.3	0.9	4.6
33	<i>epi</i> - $\alpha$ -Cadinol	1638	OS	1.8	2.7	1.9
34	$\alpha$ -Cadinol	1652	OS	2.4	2.3	7.6
35	(2 <i>E</i> ,6 <i>E</i> )-Farnesol	1742	OS	1.5	0.9	1.3
Total				98.6	98.1	98.3

<sup>a</sup> The compounds have been sorted according to their elution order on an HP-5MS column.

<sup>b</sup> Retention indices in the literature

<sup>c</sup> NCCG: Natural compound constituting group

**Table 7**Major constituents of essential oils of diverse *Pulicaria* species in different parts of the world.

Ser. Num.	<i>Pulicaria</i> species	Organ(s)	Main constituent(s)	Sampling area	Reference
1	<i>P. arabica</i>	Aerial parts	<i>tau</i> -Cadinol (38.6%)	Saudi Arabia	(Yusufoglu et al., 2021)
		Aerial parts	$\alpha$ -Cadinol (35.0%), $\delta$ -cadinene (22.5%), $\tau$ -muurolol (12.6%), and $\tau$ -cadinol (11.7%)	Algeria	(Djermane et al., 2023)
		NA <sup>a</sup>	Carvotanacetone (37.0%), (-)-carvomenthone (27.2%) and 2-(1,1-dimethylethyl)-1,4-dimethoxy-benzene (6.9%)	NA	(Nasr et al., 2023)
2	<i>P. crispa</i> (Forsk.)	NA	1,4-Ditert butylbenzene (22.8%), caryophyllene (13.2%), carvone (11.8%), and neryl(s)-2-methylbutanoate (10.3%)	Sudan	(Mohamed et al., 2020)
		Aerial parts	<i>tau</i> -Cadinol (53.5%)	Saudi Arabia	(Yusufoglu et al., 2021)
		Aerial parts	$\beta$ -Caryophyllene oxide (34.0%), modephene (23.3%), geranyl isovalerate (6.7%), geranyl propionate (6.3%), 4-cadinadiene (5.0%), humulene (4.0%), and $\beta$ -caryophyllene (2.7%)	Saudi Arabia	(AIMotwaa and Al-Otaibi, 2022)
3	<i>P. dysenterica</i> (L.) Bernh f	Aerial parts	<i>ar</i> -Curcumene (28.3%), <i>epi</i> - $\alpha$ -cadinol (16.4%) and ( <i>E</i> )-coniferyl alcohol (11.0%)	Iran	(Mumivand et al., 2010)
4	<i>P. glutinosa</i> Jaub.		$\beta$ -Elemene (11.8-16.4%), $\tau$ -cadinol (12.4-14.2%) and $\alpha$ -cadinol (8.4-10.5%)	United Arab Emirates	(Al Yousuf et al., 2001)
5	<i>P. inuloides</i>	Aerial parts	2-Methyl-5-(1-methyl)-2-cyclohexen-1-one (55.1%) and methyl-benzene (20.6%)	NA	(Al-Hajj et al., 2014)
		NA	$\delta$ -Cadinene (24.0%), $\alpha$ - <i>epi</i> -cadinol (15.0%), $\alpha$ -cadinol (12.7%), $\alpha$ -muurolene (7.8%) and $\gamma$ -cadinene (6.6%)	Algeria (Southwest)	(Fadel et al., 2020)
6	<i>P. incisa</i> (Lam.) DC.	Aerial parts	Chrysanthenone (45.3%) and 2,6-dimethylphenol (12.6%)	Hoggar	(Chaib et al., 2017)
		Leaves	Carvotanacetone (66.0%) and chrysanthenone (13.3%)	Egypt	(Shahat et al., 2017)
		Flowers	Carvotanacetone (50.9%) and chrysanthenone (24.3%)		
		Aerial parts	$\beta$ -Gurjunene (13.1%), $\beta$ -bourbonene (11.5%), camphor (8.8%), $\gamma$ -muurolene (7.9%), 1,8-cineole (eucalyptol) (6.1%)	Morocco	(Lougraimzi et al., 2020)
		Aerial parts	$\alpha$ -Ocimene (15.2%), $\tau$ -cadinol (6.8%), $\alpha$ -cadinol (4.5%), alloaromadendrene (4.4%) $\delta$ -cadinene (4.1%)	Morocco	(Lougraimzi et al., 2022)
7	<i>P. mauritanica</i>	Aerial parts	Carvotanacetone (87.3%)	NA	(Cristofari et al., 2011)
		Aerial parts	Carvotanacetone (89.2-96.1%)	Algeria	(Gherib et al., 2016)
8	<i>P. odora</i> L	Roots	Thymol (47.8%) and isobutyrate (30.0%)	NA	(Hanbali et al., 2005)
		Aerial parts	NA	Italy	(Sgadari et al., 2023)
9	<i>P. somalensis</i>	Above-ground parts	Juniper camphor (24.7%), $\alpha$ -sinensal (7.7%), 6- <i>epi</i> -shyobunol (6.6%), $\alpha$ -zingiberene (5.8%), $\alpha$ -bisabolol (5.3%), and T-muurolol	Saudi Arabia	(Assaeed et al., 2020)



			(4.7%)		
		Aerial parts	$\alpha$ -Cadinol (9.7%), $\gamma$ -eudesmol (9.1%), $\alpha$ -eudesmol (5.2%) and $\beta$ -eudesmol (5.2%)	Saudi Arabia	(Yusufoglu et al., 2021)
10	<i>P. stephanocarpa</i>	Leaves	( <i>E</i> )-Caryophyllene (13.4%), ( <i>E</i> )-nerolidol (8.5%), caryophyllene oxide (8.5%), $\alpha$ -cadinol (8.2%), spathulenol (6.8%) and $\tau$ -cadinol (4.7%)	Soqotra	(Awadh Ali et al., 2012a)
11	<i>P. undulata</i> (L.) C.A. Mey.	NA	$\alpha$ -Pinene (45.7%) and 1,8-cineole (27.1%)	Iran	(Nematollahi et al., 2006)
		Aerial parts	4-Terpineole (20.1%), 1 <i>S</i> - <i>cis</i> -calamenene (13.4%), junipene (8.7%), <i>cis</i> -sabinene hydrate (8.3%), $\gamma$ -terpinene (7.0%), linalool (5.6%) and $\alpha$ -terpinene (4.0%)	Iran	(Ravandeh et al., 2011)
		Leaves	Carvotanacetone (91.4%) and 2,5-dimethoxy- <i>p</i> -cymene (2.6%)	Yemen	(Awadh Ali et al., 2012b)
		Aerial parts	Carvotanacetone (14.8%), $\delta$ -cadinene (8.2%), $\alpha$ -cadinol (4.7%) and thujanol (4.7%)	Algeria	(Boumaraf et al., 2016)
		NA	1,8-Cineole (14.7%) and chrysanthenone (10.2%)	NA	(Seidi Damyeh and Niakousari, 2017)
		NA	Carvotanacetone (80.1%)	NA	(Issa et al., 2020)
		NA	Camphor (44.5%), and thymyl acetate (10.3%)	Sudan	(Mohamed et al., 2020)
		NA	Carvacrol (46.5%), xanthoxylin (18.1%) and carvotanacetone (8.7%)	Egypt	(Mustafa et al., 2020)
12	<i>P. vulgaris</i>	Aerial parts	Thymol (50.2%), <i>p</i> -menth-6-en-2-one (carvotanacetone, 20.2%), thymol isobutyrate (16.9%), menthan-2-one (4.3%), 1-methyl-1,2-propanedione (4.1%), 2,5-dimethoxy- <i>p</i> -cymene (4.0%), myrcene (1.9%), myrtenol (1.2%), and linalool (1.1%)	Iran	(Sharifi-Rad et al., 2014)
		Aerial parts	Hexadecanoic acid (21.7%), $\beta$ -caryophyllene (14.3%) and geranyl propionate (8.2%)	Italy	(Casiglia et al., 2016)
		Aerial parts	$\gamma$ -Irone (39.2%), 7- <i>epi</i> -silphiperfol-5-ene (19.3%), 2,5-dimethoxy- <i>p</i> -cymene (8.5%) and $\gamma$ -himachalene (8.0%)	Tunisia	(Zardi-Bergaoui et al., 2020)

NA: Not available.

#### 4. Concluding remarks

For a long time, there has been a growing interest in the potential applications of extracts from aromatic plants and their isolated secondary metabolites across various scientific fields, including medicine, pharmaceuticals, pharmacognosy, and phytochemistry. Our research primarily aimed to analyze the essential oils and volatile fractions found in the flowers, leaves, and stems of *P. gnaphalodes*, a herbal remedy commonly used in the traditional medicine. For this investigation, we employed six different extraction methods: classical techniques (hydrodistillation: CHD and steam distillation: CSD) and advanced methods (microwave-assisted hydrodistillation: MAHD, solvent-free microwave extraction: SFME, headspace solid-phase microextraction: HS-SPME, and headspace: HS), along with gas chromatography-mass spectrometry for analysis. Characterization and screening the chemical profiles of the essential oils and volatiles from the plant material organ (*P. gnaphalodes*), e.g., flowers, leaves and stems, revealed that all the chemical profiles consisted mainly of oxygenated monoterpenes with 1,8-cineole as the most prevalent compound. An overview of the obtained data in this study also points out that in some chemical profiles, e.g., in the HD, SFME, HS and HS-SPME profiles, the second rank is due to MHs from frequency point of view, while in one profile (MAHD), SHs were the second abundant group of natural compounds. However, in another profile (SD), the total amount of MHs and SHs were close together and comparable.

#### Abbreviations

**CHD**: Classical Hydrodistillation; **CSD**: Classical Steam Distillation; **GC**: Gas Chromatography; **GC-MS**: Gas Chromatography-Mass Spectrometry; **HS**: Headspace; **HS-SPME**: Headspace-Solid Phase Microextraction; **LC**: Liquid Chromatography; **MAHD**: Microwave-Assisted Hydrodistillation; **MH**: Monoterpene Hydrocarbons; **NH**: Non-Terpene Hydrocarbons; **OM**: Oxygenated Monoterpenes; **OS**: Oxygenated Sesquiterpene; ***P. gnaphalodes***: *Pulicaria gnaphalodes*; **PDMS-CAR**: Polydimethylsiloxane and Carboxen; **SFME**: Solvent Free Microwave Extraction; **SH**: Sesquiterpene Hydrocarbons.

#### 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.

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#### Conflict of interest

The author declares that there is no conflict of interest.

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