Egypt. J. Chem. **Vol. 66**, No.SI 13 pp. 2201 - 2209 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Phytochemical Screening of Two Medicinal Plants: *Calendula officinalis* L. and *Ammi visnaga* L., Collected from the Meknes Region, Morocco

Asmae Benabderrahmane [a,b] *, Majid Atmani [a], Wijdane Rhioui [a,b], Abdellatif Boutagayout [b], Saâdia Belmalha [b].

[a] *Laboratory of Functional Ecology and Environmental Engineering, Faculty of Sciences and Techniques, Sidi Mohamed Ben Abdellah University, Fez, Morocco*

[b] *Department of Plant Protection and Environment, National School of Agriculture, Meknes, Morocco*

Abstract

Calendula officinalis L. (*Asteraceae*) and *Ammi visnaga* L. (*Apiaceae*) are two herbaceous annual medicinal plants that are specific to the Mediterranean region and are part of Morocco's diverse flora. These two plants have numerous benefits. The objective of this study is to investigate the presence of various biological compounds in the aqueous and methanolic extracts of these plants using phytochemical tests. Plant samples were collected from the Meknes region, then dried and crushed. Two types of extracts were prepared: aqueous and organic (methanolic). The aqueous extracts were subjected to standard assays to identify specific chemical compounds, while both types of extracts were assessed for total phenols and total flavonoids. Additionally, gas chromatography/mass spectrometry (GC/MS) analysis was performed for the methanolic extract. The phytochemical screening revealed the diversity of terpenoids, alkaloids, flavonoids, and coumarins in both plants. A comparison of the extracts revealed higher concentrations of phenols and flavonoids in the infused extracts compared to the decocted and methanolic extracts. The GC/MS analysis revealed a wide array of characteristic molecules for each plant. The abundance of these active compounds contributes to the remarkable properties of these plants, which may explain their various therapeutic applications and utilization in traditional medicine.

Keywords: *Ammi visnaga* L.; *Calendula officinalis* L.; GC/MS; Methanolic extract.

1. Introduction

Morocco is characterized by its climatic and soil diversity due to its geographical position and orographical context. This ecological diversity is evident in its wide variety of flora. The Moroccan flora contains many plant species that have been utilized for aromatic and medicinal purposes for a long time. Today, these plants are used in traditional pharmacopeia, the food and pharmaceutical industries, as well as the perfumery and cosmetics industries [1].

Calendula officinalis L. (CO), a member of the *Asteraceae* family, and *Ammi visnaga* L. (AV), a member of the *Apiaceae* family, both belong to the diverse flora of Morocco. These two annual herbaceous medicinal plants are prevalent in the Mediterranean region and have been extensively studied for their beneficial properties [1, 2].

C. officinalis derives from the Latin *calends*, meaning the first day of each month, referring to its long blooming season [3]. Its yellow-to-orange flowers follow the rays of the sun, which has led to

*Corresponding author e-mail: asmae.benabderrahmane@usmba.ac.ma.; (Asmae Benabderrahmane).
Received date 2023-04-24; revised date 2023-05-25; accepted date 2023-06-08
DOI: 10.21608/EJCHEM.2023.207170.7900

its association with the astrological sign of summer, Leo. Additionally, it has been linked to the treatment of heart and health conditions caused by heat [4]. This plant species contains a variety of phytochemical compounds, including carbohydrates, phenolics, lipids, steroids, terpenoids, quinones, and carotenoids, which have many benefits for human health [3].

A. visnaga, also known as Khella Baldi or toothpick weed, was renowned in ancient times as Pharaoh's bread. It was used to treat kidney diseases and as an antispasmodic and vasodilator. Therefore, extracts of this plant's seeds are used in medicine for the treatment of coronary heart disease and bronchial asthma [5]. The literature indicates that *A. visnaga* contains a variety of chemical constituents, such as γ -pyrones, coumarins, flavonoids, and essential oils. The quality and quantity of these secondary metabolites depend on the part of the plant that is analyzed, as well as the culture conditions [6]. Khellin, visnagin, and visnadin are the active ingredients of *A. visnaga* fruits, which are used in the pharmaceutical industry [1].

In traditional Moroccan medicine, aqueous extracts are predominantly used, either through decoction, infusion, or direct application of fresh or dried plant material. Therefore, within the framework of the valorization of the medicinal and aromatic plants of Morocco, the objective of this study is to demonstrate the richness in biological compounds of the aqueous and methanolic extracts of *A. visnaga* and *C. officinalis*. In addition, gas chromatography/mass spectrometry (GC/MS) analysis was conducted on the methanolic extracts of both plants.

2. Materials and methods

2.1. Plant Material

In absolute terms, nothing is more effective in phytotherapy than a freshly harvested plant. However, a perfectly dehydrated herb that has been properly preserved can be as effective as a fresh plant if all conditions are met.

The two plants were harvested manually using a secateur at two locations in the Meknes region, with the GPS coordinates 33.999373, -5.556719, and 33.973559, -5.576539. The harvesting of *C. officinalis* occurred in late March and early April at temperatures averaging around 20 °C, while *A. visnaga* was harvested in June at temperatures averaging around 25 °C over the course of several days. Only the floral parts of the plants were collected.

The aerial parts, including flowers and leaves, must not be washed, as this prolongs the drying process. The material was quickly transported to the drying room and laid flat on a clean white cloth on the floor in a single layer, ensuring adequate air circulation and heat distribution. Overlapping of plant material should be avoided to prevent the development of mold and mildew due to poor ventilation. The drying took place in the laboratory of the Department of Plant Protection and Environment at the National School of Agriculture in Meknes, in a well-ventilated place with moderate luminosity and free of direct sunlight [7]. The weight of the plant material was monitored daily using an electric scale to determine the optimal drying endpoint and create a drying curve. The evaluation was done manually, aiming to achieve a dry but non-brittle texture, typically targeting a maximum humidity level of 10% [8]. The grinding was performed using an electric grinder with a stainless steel tank to obtain a homogeneous powder. The conservation was carried out in a glass jar with a hermetic lid and placed in a dry place protected from light and humidity. The taxonomic identification was conducted using the complete reference guide *Flore pratique du Maroc* [9] . The two specimens were systematically cataloged and stored in the herbarium managed by the Department of Plant Protection and Environment at the National School of Agriculture in Meknes.

2.2. *Extract preparation*

2.2.1. Aqueous extract

An infusion was prepared by pouring 80 ml of boiling distilled water over 2 g of the plant powder and leaving it to infuse for 12 h at room temperature [10]. The decoction was obtained by boiling a mixture of 2 g of plant powder and 80 ml of distilled water for 15 minutes [11]. After cooling, both extracts were filtered through Whatman No. 1 filter paper and stored at 4 °C until further use.

2.2.2 Organic extract

Soxhlet extracts were produced from an equal amount of plant material for both plants. A total of 10 g of each powder obtained was mixed with 100 ml of organic solvent (methanol) heated at 70 °C. The extraction was done until the color of the plant powder disappeared [11, 12]. The extracts were all

gathered in small bottles, dried, and filtered using Rotavapor [13].

2.3. Phytochemical screening

The phytochemical screening of the aqueous extracts was carried out on the basis of standard tests using reagents that yield characteristic color changes and precipitation reactions to determine the presence of chemical compounds. Salkowski's test was used to detect terpenoids [14], the Dragendorff reagent for alkaloids [15], an alkaline reagent test for flavonoids [11], and the Keller-Kilian test for cardiac steroidal glycosides [16]. The $FeCl_3$ test was used to reveal the presence of catechic and gallic tannins [16]. For the condensed tannins, hydrochloric acid was used [17]. The coumarins were determined using NaOH and observed under UV light [17], and the saponosides were identified using the foam test [14]. Organic acids were detected by adding a few drops of bromothymol blue to a decoction [17]. The Liebermann-Burchard test was performed to detect steroids [15]. The unsaturated sterol search involved adding sulfuric acid [17].

2.4. Total phenolic content

The total phenolic content in the plant's aqueous and organic extracts was determined following a modified version of the method described by Kim *et al.* (2003) and Waterhouse (2002) [18, 19]. In this method, 2 ml of Folin-Denis reagent (diluted 10-fold in distilled water) was added to 400 µl of each extract with a concentration of 1 mg/ml. After 4 minutes, 1600 µl of a solution of sodium carbonate (75 mg/ml in distilled water) was added to the mixture. After 2 h of incubation in the dark and at room temperature, the absorbance was measured at 765 nm using a UV-visible spectrophotometer. The same procedure was repeated for the gallic acid standard solution, and the calibration line was constructed (ranging from 0 to 300 µg/ml in methanol).

2.1. Total flavonoid content

The total flavonoid content was assessed following the method of Chang *et al.* (2002) with some modifications [20]. To 2 ml of each extract (at a concentration of 1 mg/ml), 2 ml of an $AlCl_3$ solution (2% in methanol) was added. This process was replicated for the aqueous and methanolic extracts of both plants. After 10 minutes of incubation in the dark and at room temperature, the absorbance was measured at room temperature. The absorbance was measured at 430 nm against the blank using a UV-visible spectrophotometer. The calibration was performed using quercetin (ranging from 0 to 40 µg/ml in methanol).

2.6. *GC/MS analysis*

The GC/MS analysis of each sample was carried out using a Shimadzu (TQ8040) series GC/MS system (Tokyo, Japan) equipped with an AOC-20i Plus auto-injector. The analysis was performed on a capillary column Rxi-5MS (30 m × 0.25 mm i.d., 0.25 µm). The oven temperature was initially programmed at 50 °C for 5 min, increased to 290 °C at a rate of 10 °C/min, and held for 10 min. The injector and detector temperatures were set at 200 °C. The ionization energy was 70 eV, with a mass range of 40–650 atomic mass units (AMUs). The management of the GC/MS system, parameter settings for GC and MS, and data receipt and processing were performed using Shimadzu GC/MS solution ver. 4 software (Tokyo, Japan). The compounds were identified based on a comparison of their mass spectra with data in the *Wiley Registry 11th Edition/NIST 2017 Mass Spectral Library* (Wiley, National Institute of Standards and Technology).

3. Results and discussions

3.1 *Drying process*

The drying of the calendula took 10 days from harvest to weight stabilization, resulting in a 79% reduction in the initial quantity. Each 100 g of fresh *C. officinalis* yielded 21 g of the dried plant. The drying of *A. visnaga* took 12 days, resulting in a reduction of 70%. For every 100 g, 30 g of the plant remained (Figure 1).

The drying method in the shade has proven to be beneficial, as it minimizes the deterioration of the chemical compounds of a plant, such as oxidation, and ensures the maintenance of volatile contents, especially for heat-sensitive chemicals. However, the drawback of this method is the excessively long drying period, which can be solved with other time- and compound-preserving processes, such as convection, freezing, or microwave methods [21]. Our drying time is similar to those of Okoh *et al.* (2008) and Zrira *et al.* (2008), who mentioned a

duration of 7 days at room temperature for the same type of plant samples but did not mention whether weight stabilization occurred [22, 23].

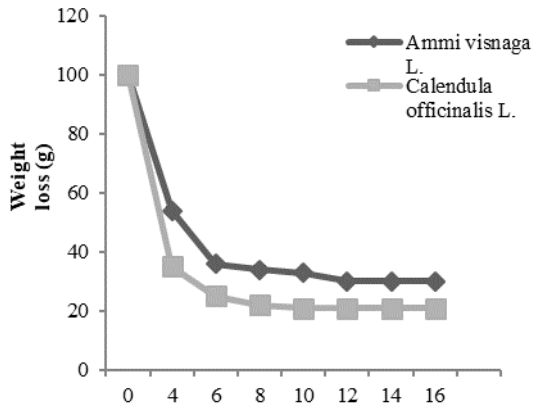

Fig 1. Drying kinematics of *Calendula officinalis* L. and *Ammi visnaga* L.

3.2. *Phytochemical screening*

The decoction was used to detect the presence of organic acids, while the infusion was utilized to highlight other chemical groups. The phytochemical screening revealed the presence of terpenoids, alkaloids, flavonoids, cardiac glycosides, unsaturated sterols, and coumarins in both plants. *C. officinalis* exhibited the presence of catechic tannins, while *A. visnaga* contained gallic tannins. *A. visnaga* displayed a high level of organic acids and steroids, while *C. officinalis* had no organic acids and only traces of steroids. There was a total absence of saponosides and condensed tannins (also named proanthocyanidins or catechin tannins) in *A. visnaga*, while some traces were observed in *C. officinalis* (Table 1).

The results of *A. visnaga* presented in Table 1 showcase the richness of this plant in secondary metabolites, which aligns with the findings of Zaher *et al.* (2019) and El Karkouri *et al*. (2020), who reported the presence of various phytochemical compounds such as polyphenols, flavonoids (flavanols and catechols), tannins (simple tannins), and coumarins [24, 25]. Regarding *C. officinalis,* the results of this work correspond to those of Ghaima *et al.* (2013), who mentioned the presence of various compounds such as alkaloids, phenols, and saponins [26]. The same confirmation was obtained from the review conducted by Verma *et al.* (2018) [27].

This research additionally highlights the presence of organic acids and gallic tannins in *A. visnaga* and catechic tannins in *C. officinalis*, which were not revealed in the aforementioned studies. These differences could potentially be attributed to variations in climate, soil type, or the methods employed for drying and preservation.

Table 1. Preliminary phytochemical screening tests of *Ammi visnaga* L. and *Calendula officinalis* L. aqueous extract.

Chemical compounds	**Result**	***Ammi visnaga* L.**	***Calendula officinalis* L.**
Terpenoid	Brown red color	+++	+++
Alkaloid	orange or red precipitate	+++	+++
Flavonoid	dark yellow color	+++	+++
Glycosides	Red color	+++	+++
Catechic tannins	Green color	-	+++
Gallic tannins	Blue black color	+++	-
Condensed tannins (proanthocyanidin)	persistent red color	-	+
Organic acids	canary yellow color	+++	-
Steroids	Red color	+++	+
Unsaturated sterols	Gradual red color	+++	+++
Coumarins	yellow fluorescence	+++	+++
Saponosides	persistent foam	-	+

3.3. *Total phenols and total flavonoids*

The absorbance values of the different extract solutions, together with the equivalent standard solution as described previously, allowed for the determination of total phenols and total flavonoids, as presented in Table 2. A comparison of the two types of aqueous extracts indicates that the infused sample contained a higher concentration of phenols and flavonoids than the decocted sample, with values for both plants being very similar. Notably, the *C. officinalis* infusion exhibited a measurement of 361.20 µg EAG/mg E of phenols and 85.34 µg QE/mg E of flavonoids. In the methanolic extract, *A. visnaga* displayed the highest level of phenols with a value of 156.89 µg EAG/mg and flavonoids with 10.1160 µg QE/mg E. The comparative analysis of the aqueous and organic extracts demonstrates higher phenol and flavonoid content in the aqueous extract of the infusion type for both plants.

Indeed, the studied extracts of both plants displayed interesting values of phenols and flavonoids. Aourabi *et al.* (2018) and Belkacem *et al.* (2016) also reported significant values of phenols and

flavonoids in *A. visnaga* [28, 29]. However, other studies that analyzed the composition of these secondary metabolites in essential oil samples of this species reported lower values. This difference could be partly explained by variations in phytochemical compositions between extracts and essential oils [30]. Likewise, similar amounts of flavonoids and phenols were detected in *C. officinalis* in the study conducted by Rigane *et al.* (2013) [31]. Efstratiou *et al.* (2012) found that methanolic extracts were superior to ethanolic extracts for phenol extraction [32]. Aqueous extracts, specifically those obtained through infusion, exhibited the highest amounts of phenols and total flavonoids, followed by methanolic extracts. This can be attributed to the influence of extraction conditions such as temperature and exposure time, as reported in the study by Antony *et al.* (2022) [33]. They suggested that phenolics are typically thermolabile compounds, and the stability of flavonoids depends on the amount of substituent in the flavonoid molecule [34]. This demonstrates the importance of selecting the appropriate extraction method.

Table 2. Total polyphenols and total flavonoids contents on aqueous and methanolic extracts of *Ammi visnaga* L. (AV) and *Calendula officinalis* L. (CO).

	Aqueous extract				Methanolic extract	
	AV deco	AV inf	CO deco	C inf	AV	CO
Total polyphenol µgEAG/mg E	11,65	337,68	32,00	361,20	168,32	159,89
Total flavonoïd µgQE/mg E	11,5728	81,6200	11,8444	85,3400	10,1160	9,4000

*AV: Ammi visnaga L. CO: Calendula officinalis L., deco: decoction, inf: infusion

3.4. *GC/MS analysis*

The GC/MS analysis of the methanolic extract of *C. officinalis* revealed the presence of seventeen chemical compounds. Table 3 and Figure 2 provide details such as the compound name, chemical formula, molecular weight, height, peak area, and retention time. Some of the noteworthy components include 2,4-di-tert-butylphenol, tetracosane, methyl hydroxylinolenate, eicosane, humulane-1,6-dien-3-ol, and stigmasterol.

Al-Musawi and Al-Hussaini (2019) detected 37 compounds in an aqueous-methanolic extract, and its largest peak areas were attributed to hydroxyacetic acid hydrazide, 3-ethoxypropanenitrile, and 2-methyladenosine [35]. Hasan and Alnaqqash (2020) also identified other components using a different GC/MS protocol [36]. The various results obtained highlight the complexity and diversity of this plant's phytochemicals, emphasizing the importance of using various separation and extraction methods to identify as many of these compounds as possible.

For *A. visnaga*, the GC/MS analysis identified eight chemical compounds listed in Table 4 and Figure 3. The most prominent compound detected was cembrene, followed by edulisin III, khellin, suberosin, and visnagin.

Zaher *et al.* (2021) identified a total of 46 compounds, including edulisin III, binapacryl, khellin, and visnagin, using a different protocol [37]. The number of GC/MS analyses conducted on the methanolic extracts of *A. visnaga* appears to be limited. Conversely, several studies have focused on the essential oil of this plant, such as the research conducted by Khadhri *et al.* and Khalfallah *et al.* in 2011, which identified compounds such as linalool, isoamyl 2-methyl butyrate, isopentyl isovalerate, and 2,2-dimethylbutanoic acid [38, 39]. Alaatabi *et al.* (2020) reported that the main compounds, including khellin, visnagin, and edulisin III, were identified in the methanolic extract of *A. visnaga* [40].

Table 3. Chemical compounds of *Calendula officinalis* L. (CO) methanolic extract identified by GCMS.

	CO compounds	Retention time (min)	Area	Peak area %	Height %	Chemical formula	Molecular weight
1	Gamma.-Curcumene	27.132	87949	2.01	2.67	$C_{15}H_{24}$	204.35
2	Zingiberene	27.654	154886	3.54	4.77	$C_{15}H_{24}$	204.35
3	Sesquiphellandrene	28.535	95065	2.17	3.09	$C_{15}H_{24}$	204.35
4	2,4-di-tert-butylphenol	30.310	858091	19.60	25.17	$C_{14}H_{22}O$	206,32
5	Trans-Sesquisabinene hydrate	31.672	96009	2.19	2.80	$C_{15}H_{26}O$	222.37
6	Methyl Palmitate	40.517	129479	2.96	3.54	$C_{17}H_{34}O_2$	270.5
7	Hexadecanoic acid	41.576	114483	2.62	2.88	$C_{16}H_{32}O_2$	256.42

8	Methyl hydroxylinolenate	45.169	332572	7.60	8.16	$C_{19}H_{32}O_3$	308.5
9	Linolenic acid methyl ester	45.861	168322	3.85	3.61	$C_{19}H_{32}O_2$	292.5
10	Eicosane	46.058	277353	6.34	6.59	$C_{20}H_{42}$	282.5
11	Dotriacontane	51.882	189719	4.33	4.86	$C_{32}H_{66}$	450.9
12	Hexatriacontane	57.256	164121	3.75	4.00	$C_{36}H_{74}$	507.0
13	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	57.348	163934	3.75	4.32	$C_{30}H_{52}O_2$	444.7
14	Humulane-1,6-dien-3-ol	58.517	312026	7.13	5.44	$C_{15}H_{26}O$	222.37
15	4-Protoadamantanone	59.795	64186	1.47	1.68	$C_{10}H_{14}O$	150.22
16	Tetracosane	62.226	485443	11.09	10.77	$C_{24}H_{50}$	338.7
17	Stigmasterol	63.354	683441	15.61	5.66	$C_{29}H_{48}O$	412.7

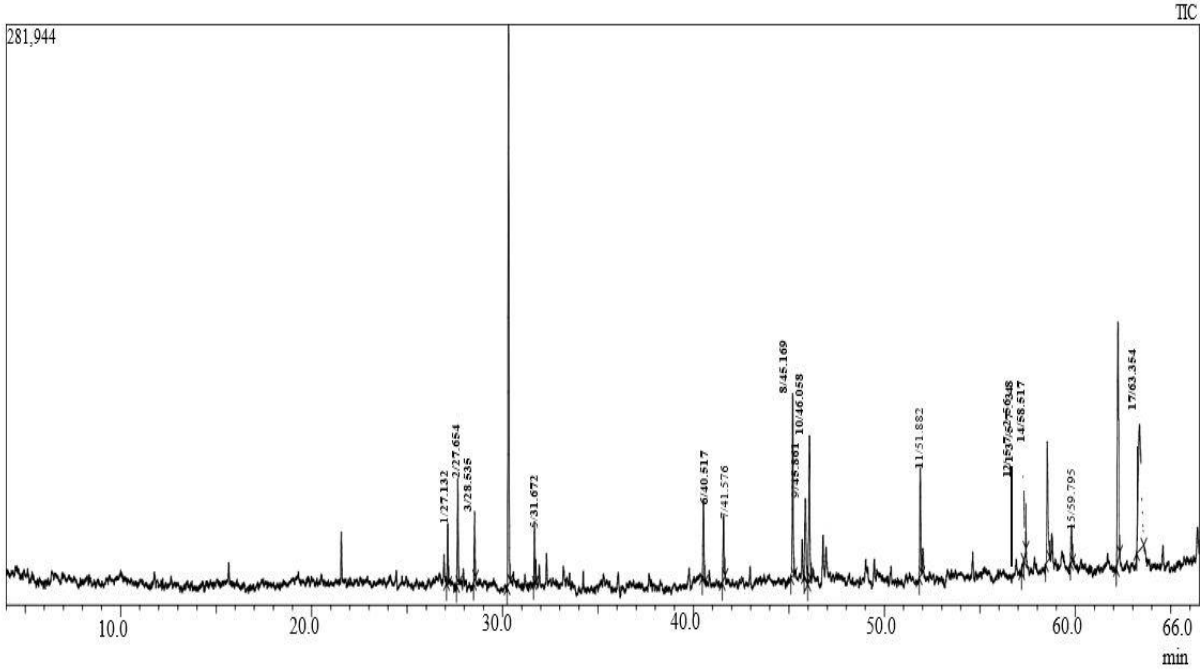

Fig 2. GCMS analysis of Calendula officinalis L.

Table 4. Chemical compounds of *Ammi visnaga* L. (AV) methanolic extract identified by GCMS.

	AV compounds	Rtention time (min)	Area	Peak area %	Height %	Chemical formula	Molecular weight
1	Visnagin	43.502	1742513	4.03	4.02	$C_{13}H_{10}O_4$	230.22
2	Humulane-1,6-dien-3-ol	44.370	1058832	2.45	2.22	$C_{15}H_{26}O$	222.37
3	Cembrene	46.557	22172440	51.23	52.93	$C_{20}H_{32}$	272.5
4	Farnesyl acetate	48 .048	1845738	4.26	4.47	$C_{17}H_{28}O_2$	264.4
5	Khellin	49.140	4019235	9.29	8.95	$C_{14}H_{12}O_5$	260.24
6	Suberosin	61.035	3446482	7.96	7.10	$C_{15}H_{16}O_3$	244.28
7	Edulisin III	61.235	8514592	19.67	19.10	$C_{21}H_{24}O_7$	388.4
8	(E)-Cnidimine	63.084	483535	1.12	1.22	$C_{21}H_{22}O_7$	386.4

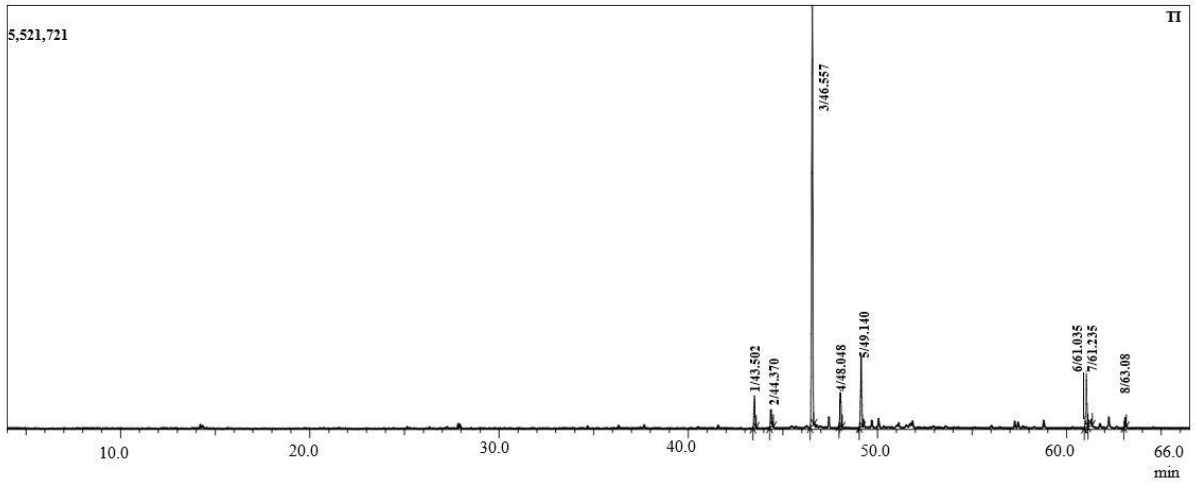

Fig 3. GCMS analysis of *Ammi visnaga* L

4. Conclusion

This study significantly contributes to our understanding of the phytochemical composition and potential therapeutic applications of *C. officinalis* and *A. visnaga*, two medicinal plants native to Morocco. Our research demonstrates that these species are rich in terpenoids, alkaloids, flavonoids, and coumarins, providing a scientific basis for their traditional medicinal uses.

Furthermore, this research highlights the importance of the extraction method in determining the concentration of active compounds, with infusions showing higher levels of phenols and flavonoids compared to decoctions and methanolic extracts. This finding not only emphasizes the need for careful consideration of extraction methods in future studies but also points toward the potential development of more effective extraction techniques for these medicinal plants.

The characterization of specific molecules through GC/MS analysis of the methanolic extracts enhances our understanding of the biochemical uniqueness of these species. The range of detected compounds confirms the remarkable diversity of bioactive constituents present in these plants, paving the way for future research into their individual and combined therapeutic effects.

Overall, these findings underscore the potential of *C. officinalis* and *A. visnaga* as valuable resources for natural remedies and pharmaceutical developments. However, further in-depth studies are essential to explore the full extent of their pharmacological properties, safety profiles, and efficacy in diverse therapeutic applications. This research represents a significant step forward in our understanding of these species, reinforcing their role in traditional medicine and providing a foundation for their potential utilization in modern medicine.

5. Conflicts of interest

The authors declare that there is no conflict of interest.

6. Formatting of funding sources

The data used to support the findings of this study are included within the article.

7. Acknowledgements

The authors would like to thank the Regional University Center of Interface (CURI) attached to the University Sidi Mohamed Ben Abdellah (USMBA) of Fez and especially Pr. Fath-Ellah Laghrib for the execution of the mass spectrometry by gas chromatography (GC/MS).

References:

[1] Satrani, B., Farah, A., Fechtal, M., Talbi, M., & Bouamrani, M. L. 2004. Composition chimique et activité antibactérienne et antifongique de l'huile essentielle d'Ammi visnaga (L.) Lam. du Maroc. Acta Bot. Gallica. 151(1): 65–71. Doi: 10.1080/12538078.2004.10516021

[2] Batista, A. L. A., Diógenes Alves UchÔa Lins, R., de Souza Coelho, R., do Nascimento Barbosa, D., Moura Belém, N., & Alves Celestino, F. J. 2014. Clinical efficacy analysis of the mouth rinsing with pomegranate and chamomile plant extracts in the gingival bleeding reduction. Complementary Therapies in Clinical Practice, 20(1): 93–98. Doi: 10.1016/j.ctcp.2013.08.002

[3] Jan, N., ANDRABI, K. I., & JOHN, R. 2017. Calendula Officinalis-An Important Medicinal Plant with Potential Biological Properties. Proc Indian Natn Sci Acad, 83(4): 769–787. Doi: 10.16943/ptinsa/2017/49126

[4] Kemper, K. J. 1999. Calendula (Calendula

officinalis). The Longwood Herbal Task Force, 1: 1–13.

[5] Khalil, N., Bishr, M., Desouky, S., & Salama, O. 2020. Ammi Visnaga L., a potential medicinal plant: A review. Molecules. 25(2): 301. Doi: 10.3390/molecules25020301

[6] Sellami, H. K., Napolitano, A., Masullo, M., Smiti, S., Piacente, S., & Pizza, C. 2013. Influence of growing conditions on metabolite profile of Ammi visnaga umbels with special reference to bioactive furanochromones and pyranocoumarins. Phytochemistry. 95: 197–206. Doi: 10.1016/j.phytochem.2013.08.001

[7] Thamkaew, G., Sjöholm, I., & Galindo, F. G. 2020. A review of drying methods for improving the quality of dried herbs. Critical Reviews in Food Science and Nutrition, 61(11): 1763–1786. Doi: 10.1080/10408398.2020.1765309

[8] Chian, J. R. C. 2006. Stratégies de séchage à haute température pour le bois à valeur ajoutée. Laval university.Quebec

[9] Fennane, M., IbnTattou, M., and El oualidi, J. 2014. Flore Pratique du Maroc, Travaux Institut scientifique. Sèr. botanique N° 40. Rabat.

[10] Wong, S. P., Leong, L. P., & William Koh, J. H. 2006. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry. 99(4): 775–783. Doi: 10.1016/j.foodchem.2005.07.058

[11] Jaradat, N., Hussen, F., & Al Ali, A. 2015. Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata decne. J. Mater. Environ. Sci. 6(6): 1771–1778.

[12] Lama-muñoz, A., Contreras, M. del M., Espínola, F., Moya, M., Romero, I., & Castro, E. 2020. Content of phenolic compounds and mannitol in olive leaves extracts from six Spanish cultivars : Extraction with the Soxhlet method and pressurized liquids. Food Chemistry, 320: 126626. Doi: 1016/j.foodchem.2020.126626

[13] Palmieri, S., Pellegrini, M., Ricci, A., Compagnone, D., & Lo Sterzo, C. 2020. Chemical composition and antioxidant activity of thyme, hemp and coriander extracts: A comparison study of maceration, soxhlet, UAE and RSLDE techniques. Foods, 9(9): 1221. Doi: 10.3390/foods9091221

[14] Adusei, S., Otchere, J. K., Oteng, P., Mensah, R. Q., & Tei-Mensah, E. 2019. Phytochemical analysis, antioxidant and metal chelating capacity of Tetrapleura tetraptera. Heliyon. 5(11): e02762. Doi:10.1016/j.heliyon.2019.e02762

[15] Fannang, V. S., Tankeu, S. E., Etame Loe, M. G., Yinyang, J., Ngouondjou Foze, T., Bamal, H. D., & Mbida Mvomo, B. D. 2021. Phytochemical screening and study of the acute oral toxicity of the aqueous extract of the leaves of Diospyros hoyleana F . white (Ebenaceae). Saudi J Med Pharm Sci, 7(5): 230–235. Doi: 10.36348/sjmps.2021.v07i05.006

[16] Jaadan, H., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S., & Belmalha, S. 2020. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of Marrubium vulgare L. from 2 different localities of Northeast of Morocco. Heliyon, 6(11): e05609. Doi: 10.1016/j.heliyon.2020.e05609

[17] Benzeggouta, N. 2015. Evaluation des Effets Biologiques des Extraits Aqueux de Plantes Médicinales Seules et Combinées. Mentouri-Constantine University. Algeria.

[18] Kim, D. O., Chun, O. K., Kim, Y. J., Moon, H. Y., & Lee, C. Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 51(22): 6509–6515. Doi: 10.1021/jf0343074

[19] Waterhouse A, L. 2002. Determination of total phenolics. Current Protocols in Food Analytical Chemistry, 6(1): I1.1.1-I1.1.8.

[20] Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. 2002. Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis, 10(3), 178–182. Doi: 10.38212/2224-6614.2748

[21] Nurhaslina, C. R., Bacho, S. A., & Mustapa, A. N. 2022. Review on drying methods for herbal plants. Materials Today: Proceedings, 63: S122–S139. Doi: 10.1016/j.matpr.2022.02.052

[22] Okoh, O. O., Sadimenko, A. P., Asekun, O. T., & Afolayan, A. J. 2008. The effects of drying on the chemical components of essential oils of Calendula officinalis L. Afr. J. Biotechnol. 7(10): 1500–1502.

[23] Zrira, S., Elamrani, A., Pellerin, P., Bessière, J. M., Menut, C., & Benjilali, B. 2008. Isolation of Moroccan Ammi visnaga oil: Comparison

between hydrodistillation, steam distillation and supercritical fluid extraction. Jeobp, 11(1): 30–35. Doi:10.1080/0972060X.2008.10643593

[24] Zaher, A., Boufellous, M., Ouhssine, M., & Bourkhiss, B. 2019. Phytochemical Screening Of An Umbelliferae : Ammi visnaga L . (Lam .) In The Region Of Sidi Slimane- North-West Of Morocco. J. Mater. Environ. Sci.10(10): 995–1002.

[25] El Karkouri, J., Drioiche, A., Soro, A., Ailli, A., Benhlima, N., Bouzoubaa, A., El Makhoukhi, F., Oulhaj, H., Elombo, F. K., & Zair, T. 2020. Identification and antioxidant activity of Ammi visnaga L. polyphenols from the Middle Atlas in Morocco. Mediterr.J.Chem. 10(7): 649. Doi: 10.13171/mjc10702007221459jek

[26] Ghaima, K. K., Rasheed, S. F., & Ahmed, E. F. 2013. Antibiofilm, antibacterial and antioxidant activities of water extract of Calendula officinalis flowers. International Journal of Biological & Pharmaceutical Research, 4(7): 465–470.

[27] Verma, P. K., Raina, R., Agarwal, S., & Kour, H. (2018). Phytochemical ingredients and pharmacological potential of Calendula officinalis Linn. Pharm Biomed Res. 4(2): 1–17.

[28] Aourabi, S., Driouch, M., Ammor, K., Sfaira, M., Touhami, M. E., & Mahjoubi1, F. 2018. Evaluation of anticorrosion and antioxidant activities of ethanolic extract of Ammi visnaga. Anal. Bioanal. Electrochem. 10(7): 912–929.

[29] Belkacem, I., Rebai, O., & Djibaoui, R. 2016. Antimicrobial and antioxidant activity of Ammi visnaga (L) phenolic extracts and their effects on planktonic and biofilm growth of food spoilage Bacillus cereus. Int. J. Biosci. 9(4), 32–47. Doi:10.12692/ijb/9.4.32-47

[30] Kamal, F. Z., Stanciu, G. D., Lefter, R., Cotea, V. V, Niculaua, M., Ababei, D. C., Ciobica, A., & Ech-chahad, A. 2022. Chemical composition and antioxidant activity of Ammi visnaga L . essential oil. Antioxidants, 11: 347. Doi: 10.3390/antiox11020347

[31] Rigane, G., Younes, B., & Salem, B. 2013. Investigation into the biological activities and chemical composition of Investigation into the biological activities and chemical composition of Calendula officinalis L . growing in Tunisia. IFRJ, 20(6): 3001–3007.

[32] Efstratiou, E., Hussain, A. I., Nigam, P. S., Moore, J. E., Ayub, M. A., & Rao, J. R. 2012. Complementary therapies in clinical practice antimicrobial activity of Calendula of fi cinalis petal extracts against fungi , as well as gram-negative and gram-positive clinical pathogens. Complementary Therapies in Clinical Practice, 18(3): 173–176. Doi: 10.1016/j.ctcp.2012.02.003

[33] Antony, A., & Farid, M. 2022. Applied sciences Effect of Temperatures on Polyphenols during Extraction. Appl. Sci. 12: 2107. Doi:10.3390/app12042107

[34] Biesaga, M. 2011. Influence of extraction methods on stability of flavonoids. J. Chromatogr. A. 1218(18): 2505–2512. Doi: 10.1016/j.chroma.2011.02.059

[35] Al-mussawi, Z. K., & Al-hussani, I. M. 2019. Phytochemical study of Calendula officinalis plant by used GC-MS and FTIR techniques. Plant Archives, 19(1): 845–851.

[36] Hasan, H. A., & Alnaqqash, Z. A. E. 2020. Pharmacognostical and phytochemical study of Calendula officinalis L. leaves cultivated in Baghdad. AIP Conference Proceedings, 2290: 020019–1–020019–9. Doi: 1063/5.0027577

[37] Zaher, A., Aslam, R., Lee, H., Khafouri, A., Boufellous, M., Alrashdi, A. A., El aoufir, Y., Lgaz, H., & Ouhssine, M. 2021. A combined computational & electrochemical exploration of the Ammi visnaga L . extract as a green corrosion inhibitor for carbon steel in HCl solution. Arabian Journal of Chemistry, 15(2): 103573. Doi: 10.1016/j.arabjc.2021.103573

[38] Khadhri, A., Mokni, R. El, Mguis, K., Ouerfelli, I., & Araújo, M. E. M. 2011. Variability of two essential oils of Ammi visnaga (L .) Lam . a traditional Tunisian medicinal plant. J. Med. Plant. Res. 5(20): 5079–5082.

[39] Khalfallah, A., Labed, A., Semra, Z., AI Kaki, B., Kabouche, A., Touzani, R., & Kabouche, Z. 2011. Antibacterial activity and chemical composition of the essential oil of Ammi visnaga L . (Apiaceae) from Constantine, Algeria. Int. J. Med. Arom. Plants. 1(3): 302–305.

[40] Alaatabi, R. M., Almousawi, U. M. N., Mosa, M. N., & Hamarashid, S. H. 2020. Phytochemical screening by using TLC and GC-MS methods for qualitative determination of compounds in Ammi visnaga L . extract. Plant Archives, 20(2): 4326–4330.