

Phytochemical analysis of methanolic, aqueous extracts, and essential oil composition of *Alcea chrysantha* (Malvaceae)

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Abstract

Alcea chrysantha (Malvaceae) is a medicinal plant with limited phytochemical characterization. This study aimed to provide the first profiling of its methanolic and aqueous extracts by determining total phenolic and flavonoid contents, evaluating antioxidant activity, and analyzing the essential oil composition. Methanolic and aqueous extracts were analyzed for total phenolic and flavonoid contents using standard spectrophotometric methods. Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assays. Essential oils were analyzed by gas chromatography mass spectrometry (GC–MS). The methanolic extract exhibited higher total phenolic and flavonoid contents than the aqueous extract. The methanolic extract also demonstrated stronger antioxidant activity with an IC₅₀ value of 712.3 ± 6.46 µg/mL. The FRAP assay revealed that the methanolic extract had higher reducing power in comparison with the aqueous extract. GC–MS analysis of the essential oil identified more than 30 compounds, predominantly aliphatic hydrocarbons (91.01%) and minor diterpenes (0.68%). Results showed that *A. chrysantha* is a medicinal plant with high phenolic and flavonoid contents, good antioxidant activity, and that it is a promising source of bioactive compounds with potential applications in pharmaceutical, nutraceutical, and industrial sectors.

Keywords

Alcea chrysantha, essential oil, GC-MS, medicinal plants antioxidant

Introduction

The genus *Alcea* is classified within the Malvaceae family (AL-Eisawi 2013), encompassing over 40 identified subspecies. *Alcea* is indigenous to both Asia and Europe. This genus is characterized by attractive flowering plants, and blooms with large and very colorful flowers (Ozturk 2022). Numerous species within this genus have historically been employed in traditional medicine for the treatment of various ailments, including gastrointestinal disorders, dermatological inflammations, and infections of the respiratory tract (Abdullatif Azab 2016). These traditional applications imply the presence of potent bioactive compounds. *Alcea chrysantha* (Sam.) Zohary (syn. *Althaea chrysantha* Sam.) is a perennial species distinguished by its yellow flowers (Fig. 1). *A. chrysantha* is endemic to Jordan, with its geographical distribution in the Mafrqa-Safawi region (AL-Eisawi 1998) and East Amman (Zohary 1972). A comprehensive review of the literature indicates that *A. chrysantha* is comparatively under-investigated compared to other *Alcea* species, with a notable absence of documentation of the biological activities associated with this species. Several species of the genus *Alcea* have been extensively studied for their biological properties using aqueous and methanolic extracts, solvents commonly used in research on this genus to characterize bioactive compounds with different polarities (Taskin et al. 2020; Abodayak et al. 2022). Therefore, the current study evaluated the phytochemical profile, essential oil constituents, and associated antioxidant properties of aqueous and methanolic extracts of *A. chrysantha*. This research aims to enhance our understanding of this plant species and its potential applications in therapeutic applications.

Materials and methods

Plant material collection and identification

A total of 20 individual whole plants of *Alcea chrysantha* (Sam.) Zohary (syn. *Althaea chrysantha* Sam.) were collected during their flowering period from the Eastern



Figure 1. *Alcea chrysantha*.

Desert in Mafrq–Safawi, Jordan (GPS coordinates: 32.3159860, 37.1650000) in April 2024. The research and verification were undertaken by Professor Sawsan Oran, a plant taxonomist in the Department of Science at the University of Jordan. These specimens have long been archived in the Department of Science herbarium at the University of Jordan, under reference number 710.

Preparation of extracts

Methanolic and aqueous extract of the *Alcea chrysantha*

A total of 20 grams of the dried-powdered whole plant was subjected to maceration in 200 mL of 70% methanol along with 200 mL of distilled water at room temperature, accompanied by constant agitation over a period of 72 h. The resultant homogenate underwent filtration and was subsequently subjected to evaporation at 40 °C under vacuum until dryness. Prior to its subsequent utilization, the crude extract was stored at a temperature of 4 °C as stock extracts (Oran et al. 2022) (Azadeh et al. 2019). The extraction yields were determined as the ratio of the mass of the dried extract to the initial mass of the dried plant material, expressed as a percentage, according to the following equation: extraction ratio (%) = (mass of dried extract / mass of dried plant material) × 100 (Tourabi et al. 2024)

Determination of total phenolic and flavonoid content

Total phenolic and flavonoid content

The assessment of total phenolic compounds and flavonoid content was conducted utilizing the Folin-Ciocalteu's phenol reagent methodology and the aluminum chloride colorimetric technique, as delineated by Taskin et al. (2020) and Aryal et al. (2019) respectively. In these analytical approaches, the quantification of total phenolics was expressed in terms of gallic acid equivalents (GAE) per gram of extract, with gallic acid serving as the standard reference. Total flavonoid content was quantified as milligrams of quercetin equivalents per gram (mg QE/g) of dry extract.

Antioxidant activity

DPPH radical scavenging assay

The antioxidant capacity of the extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the methodology outlined by Althaher et al. (2024). A range of plant extract concentrations was prepared, with ascorbic acid as a positive control. Two mL of DPPH were added to each sample, and the absorbance was measured at a wavelength of 517 nm. The percentage of DPPH radical scavenging was calculated employing the subsequent equation: % scavenging of DPPH = [(A0 - A1) / A0] × 100, where A0 = absorbance of the control and A1 = absorbance of the test

extracts. The IC_{50} value, characterized as the concentration required to inhibit 50% of DPPH radicals, was estimated by plotting the percentage of inhibition against extract concentration (Oran et al. 2022; Althaher et al. 2024).

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of the extracts was determined according to the methodology developed by Benzi and Strain (1996), with minor modifications. Briefly, 100 μ L of each extract was mixed with 3 mL of freshly prepared FRAP reagent, and the samples were incubated at 37 °C for 30 min. Trolox was used as a positive control to provide a comparative standard for assessing the antioxidant capacity of the extracts. Absorbance was measured at a wavelength of 593 nm, and FRAP values were derived from a corresponding standard calibration curve. The results are expressed in mg of Trolox equivalents (TE) per gram of dry weight of the extract.

Essential oil extraction and GC-MS analysis

A 200-g sample of fresh plant material was subjected to 4 h of hydrodistillation using a Clevenger apparatus. The extract was then dried with anhydrous sodium sulfate and stored in sterile bottles at 4 °C until further analysis (Al-Taher et al. 2021; Shaheen et al. 2024). The hydrodistilled essential oil underwent analytical evaluation, and the relative peak area of each oil component was determined. The concentrations of the oil components were calculated as percentages based on these relative peak areas, assuming a uniform response from all components. Each oil sample was analyzed three times. The oil's components were determined by correlating its mass spectra with known mass spectrometry (MS) libraries (WILEY, NIST, and ADAMS-2007) and by comparing its linear retention indices with values documented in the literature, with reference mainly to Adam's library (Adams 2007).

Statistical analysis

Statistical analysis of the data was performed using one-way ANOVA, followed by Tukey's post-hoc test to determine statistical significance at a $p < 0.05$ level. Results from three repeated observations are presented as mean $\pm$ standard deviation. Graph-Pad Prism 8 software was used for statistical analysis.

Results

Extraction yield

The extraction yields derived from 20 g of dried plant material were determined to be 5% (w/w) for the aqueous extract and 10% (w/w) for the methanolic extract.

Total phenolic content

For the methanolic extract and aqueous extract, total phenolic content (TPC) was 39.1 ± 3.7 and 12 ± 0.3 mg GAE/g extract, respectively (Fig. 2), revealing significant differences between the two extracts.

Total flavonoid content

For the methanolic extract and aqueous extract total flavonoid content (TFC) was 29 ± 1.8 and 8.3 ± 0.8 mg QE/g extract, respectively (Fig. 2). These results showed that methanol is a better solvent to extract these bioactive compounds from *A. chrysantha*.

Antioxidant activity

DPPH radical scavenging assay

Antioxidant activity was assessed through the DPPH radical-scavenging assay and expressed in terms of IC_{50} which is the concentration of plant extract able to scavenge 50% of DPPH radicals. For the methanolic extract, IC_{50} was 712.3 ± 6.46 μ g/mL, while for the aqueous extract the IC_{50} was 810.6 ± 6.43 μ g/mL (Fig. 3). Ascorbic acid, utilized as a positive control, gave an IC_{50} value of about $4.0 \pm .13$ μ g/mL. Regarding ascorbic acid equivalents (AAE) at 50% inhibition (IC_{50}), the methanolic extract showed higher antioxidant activity (0.0055 μ g/mL AAE) compared to the aqueous extract (0.0048 μ g/mL AAE).

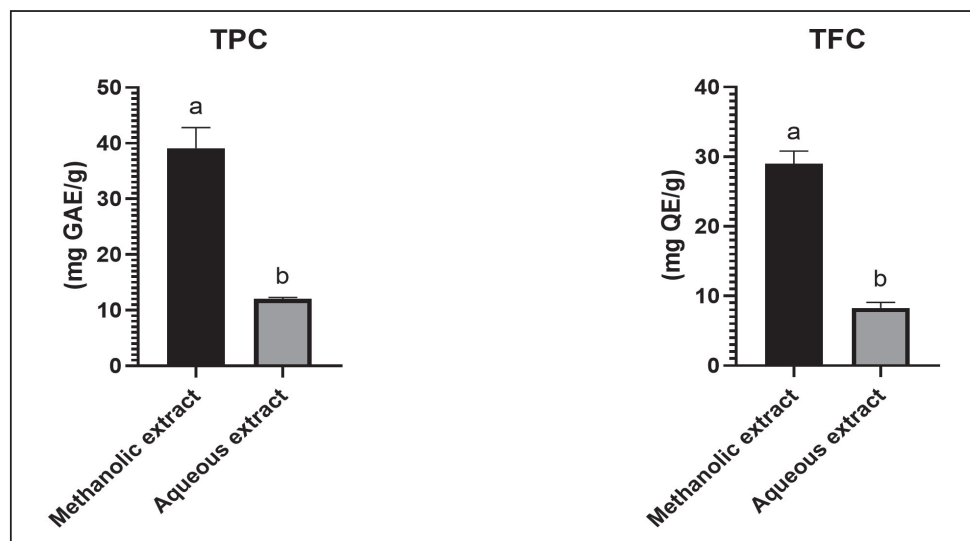


Figure 2. Total phenolic and flavonoid contents of the tested extracts. Values are reported as mean $\pm$ SD of three replicates, p value < 0.0001.

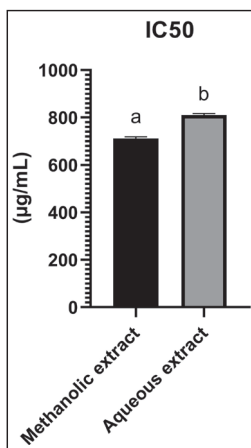


Figure 3. Antioxidant activity DPPH Assay. Values are reported as mean $\pm$ SD of three replicates, p value < 0.0001 .

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay revealed that the methanolic extract had higher reducing power (3.86 ± 0.06 TE mg/g DW), than the aqueous extract (1.23 ± 0.01 TE mg/g DW) (Fig. 4). These results correlated with the DPPH radical scavenging activities, confirming the presence of significant antioxidant compounds in both extracts.

GC-MS profiling of the essential oil

GC-MS analysis of the essential oil obtained from whole plants of *A. chrysanth* identified more than 30 compounds, dominated by hexadecenoic acid (palmitic acid; 21.67),

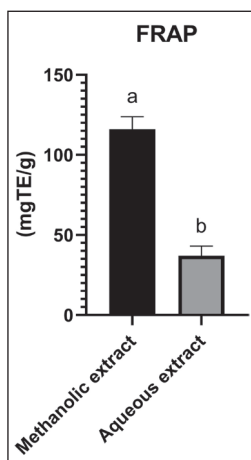


Figure 4. Ferric reducing antioxidant power FRAP Assay. Values are reported as mean $\pm$ SD of three replicates, p value < 0.0001 .

followed by (9Z)-octadec-9-enoic acid (oleic acid; 11.82), tricosane (10.77), tetradecanoic acid (5.27), and 1-docosene (4.88) (Fig. 5). The aliphatic hydrocarbons (HC), representing 91.01% of the extract, predominantly represent the volatile oil composition, whereas the least prevalent category is that of diterpenes, accounting for 0.68% (Fig. 6).

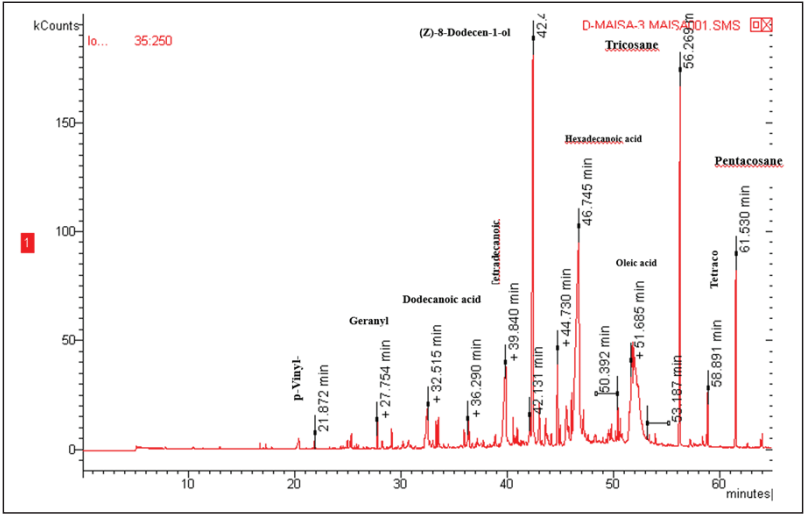


Figure 5. Chromatogram of major compounds in *Alcea chrysantha* essential oil.

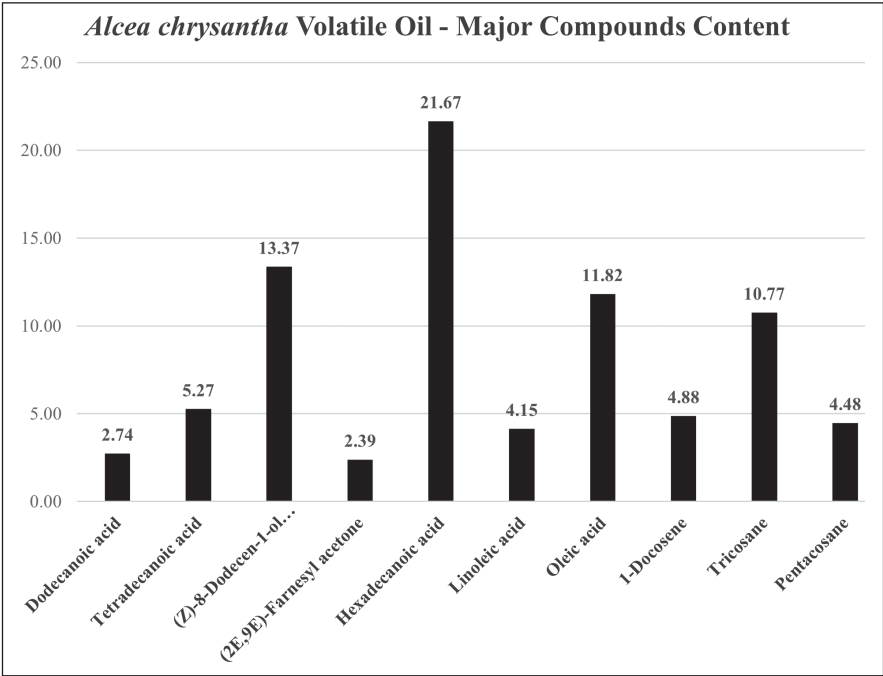


Figure 6. *Alcea chrysantha* essential oil – major compounds.

Discussion

The results of this study clearly showed that methanolic extracts were richer in phenolic compounds and flavonoids than aqueous extracts, confirming the effectiveness of organic solvents in extracting these valuable phytochemicals from *A. chrysantha*. This observation highlights the significant phytochemical potential of this plant, enhancing its value as a source of natural bioactive compounds. Results also align with those of Taskin et al. (2020) on *Alcea dissecta* (Baker f.) Zohary, showing higher phenolic content by using an organic solvent. In accord with the current study, research on *Alcea calvertii* Boiss. showed that the TPC of the methanolic extract was higher than that of the aqueous extract, with value of 82.92 ± 1.47 and 64.17 ± 0.92 GAE $\mu\text{g/g}$, respectively (Abudayyak et al. 2022). Furthermore, it was reported that organic extracts were more effective in extracting phenols and flavonoids of *A. pallida* (Willd.) Waldst. & Kit. and *A. apterocarpa* Boiss. (Ertas et al. 2016). Another study conducted by Zakizadeh and colleagues in their investigation of *Alcea hyrcana* (Grossh.) Grossh. methanolic extract reported the highest flavonoid content in leaves, followed by seeds and flowers (Zakizadeh et al. 2011).

A broader comparison of four *Alcea* species also revealed that flowers yielded the highest flavonoid concentrations when extracted using organic solvents, further demonstrating organ-specific differences (Azadeh et al. 2020). Interestingly, an exception was reported in *A. fasciculiflora* Zohary, where aqueous extracts showed higher phenol and flavonoid contents (Ozturk et al. 2022), suggesting that extraction efficiencies may vary across species. Taken together, these results reinforce the promising phytopharmacological potential of *Alcea* and position *A. chrysantha* as a valuable reservoir of natural compounds, with potential applications in the health and therapeutic sectors.

The antioxidant activity of *A. chrysantha* extracts was assessed via two methods, DPPH radical scavenging and FRAP assays. A strong correlation was observed between the results of the DPPH radical scavenging assay and the FRAP reducing power, indicating that both assays, despite being based on different mechanisms, reflect the antioxidant capacity of *A. chrysantha* extracts. The methanolic extract exhibited the lower IC_{50} value in the DPPH assay and the higher FRAP value, confirming its superior antioxidant potential. Therefore, the higher reducing power and stronger radical scavenging activity of the methanolic extract can be attributed to its higher content of phenolic and flavonoid compounds (Abeyrathne et al. 2022). Previous studies on *Alcea* species have demonstrated significant variation in antioxidant potential depending on the extraction solvent and plant part used. Our findings agree with previous reports in other *Alcea* species, where organic solvent extracts showed superior antioxidant capacity compared to aqueous extracts using the DPPH and FRAP assays (Taskin et al. 2020; Abudayyak et al. 2022).

Similarly, moderate DPPH-scavenging capabilities of *A. setosa* Alef. extracts have been reported in several studies (Jamous et al. 2015; Alhage and Elbitar 2019). These findings align with previous investigations by Abdulqader et al. (2024), whose results showed that the methanolic extracts of *A. kurdica* (Schltdl.) Alef. flower, leaf, and root exhibited a concentration-dependent increase in antioxidant activity as measured by the DPPH assay. Furthermore, higher activity of ethanolic extracts was observed in other

Alcea species measured by the DPPH assay and revealed higher activity in flowers than in leaves (Azadeh et al. 2020). Previous findings of antioxidant activity of the methanolic extracts of *Althaea rosea* Cav. revealed that polar extracts, particularly from flowers or aerial parts, showed higher free radical scavenging activity (Ammara et al. 2013).

The observed antioxidant activity can be attributed to the phenolic and flavonoid contents detected in the extracts, which are known to protect cells from oxidative stress through hydrogen atom transfer, electron transfer, and metal ion chelation mechanisms (Rice-Evans et al. 1996). Therefore, these results support a close correlation between total phenolic/flavonoid content and antioxidant capacity, consistent with previous reports in medicinal plants (Choudhary and Swarnkar 2011).

Essential oils, as a major class of plant secondary metabolites, play a crucial role in enhancing the biological activity and therapeutic potential of medicinal plants. In this study, the essential oil of *A. chrysantha* was characterized for the first time, revealing a diverse mixture of fatty acids, saturated and unsaturated fatty acid esters, and hydrocarbons (Table 1).

Previous studies have analyzed the essential oils of other *Alcea* species, such as *A. pallida*, *A. apterocarpa*, and *A. rosea*, and identified a range of fatty acids, terpenoids, and phenolic derivatives (Ertas et al. 2016; Hanif et al. 2019). *A. chrysantha* possesses a unique chemical composition that enriches the phytochemical diversity of this genus.

Table 1. Volatile oil composition of *Alcea chrysantha* extract analyzed by GC-MS.

#	Rt ^a	Cal-AI ^b	Lit-AI ^c	Compound	%Content ^d	Identification Method
1	32.374	1568	1565	Dodecanoic acid-(Lauric acid)	2.74	MS and RI
2	39.840	1772	NA	Tetradecanoic acid*)	5.27	Tentatively identified
3	42.416	1848	NA	(Z)-8-Dodecen-1-ol acetate*	13.37	Tentatively identified
4	43.023	1866	NA	Dibutyl phthalate*	1.22	Tentatively identified
5	45.304	1936	1933	Cyclohexadecanolide	1.97	MS and RI
6	46.155	1962	1959	Hexadecanoic acid	21.67	MS and RI
7	50.292	2097	2095	Methyl linoleate	1.24	MS and RI
8	51.395	2135	2132	Linoleic acid	4.15	MS and RI
9	51.674	2144	2141	Oleic acid*	11.82	Tentatively identified
10	52.992	2189	2189	1-Docosene	4.88	MS and RI
11	56.269	2305	2300	Tricosane	10.77	MS and RI
12	58.891	2401	2400	Tetracosane	1.21	MS and RI
13	61.530	2505	2500	Pentacosane	4.48	MS and RI
Total identified						98.92
Monoterpenes						0.76
Sesquiterpenes						4.18
Diterpenes						0.68
Aliphatic Hydrocarbons (HC)						91.01
Aromatics and Phenolics						2.30
Unknown						1.08
Total						100.00

^a Compounds listed in order of their elution from a DB-5 equivalent column.

^b Linear (arithmetic) retention index (modified Kovats index) calculated on DB-5 column.

^c Linear (arithmetic) retention index value based on literature (standard value) based on same column.

^d Average % content of 6 determinations (2 oil samples, 3 replicates each), for which the standard deviation (SD) values were within 2% ($\pm 2\%$) of the mean.

^e Compounds were tentatively identified based on mass spectral data only*.

The complex composition of these volatile oils, including terpenoids, hydrocarbons, alcohols, aldehydes, esters, and phenolic compounds, suggests their significant medicinal value. Volatile oils are known for their broad biological effects, such as antimicrobial, antifungal, antitumor, anti-inflammatory activities and antioxidants (Miguel 2010), in airway disorders like asthma and chronic bronchitis (Hanif et al. 2019), fungicidal, antiparasitic, insecticidal, medicinal and cosmetic applications (Bakkali et al. 2008). It is important to acknowledge that the identification of some components of the essential oil was based exclusively on mass spectrometry data and should, therefore, be considered preliminary, since structural isomers cannot be definitively distinguished in the absence of linear retention index (LRI) calculations (Table 1). Nonetheless, the unique composition of *A. chrysantha* essential oil not only adds to the chemical diversity of the *Alcea* genus but also highlights the plant's rich chemical diversity and its potential as a valuable source of bioactive compounds for pharmaceutical, nutraceutical, and industrial applications.

Conclusions

A. chrysantha is a rich source of bioactive compounds, with its methanolic extracts showing higher phenolic and flavonoid contents and stronger antioxidant activity compared to aqueous extracts. These results confirm the key role of these phytochemicals in free radical scavenging and highlight the importance of solvent selection for optimal extraction. The essential oils of *A. chrysantha*, described in this research for the first time, revealed a complex mixture of fatty acids, esters, hydrocarbons, and phenolic derivatives, demonstrating significant biological potential. Together, these results demonstrate that *A. chrysantha* is a promising medicinal plant with potential applications in the pharmaceutical, food, and industrial sectors that deserve further investigation into its potential uses.

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