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Phytochemical Profile and Antioxidant Properties of *Verbascum cheiranthifolium* Boiss. from Kolakchal, Alborz Mountains in northern Tehran: A GC-MS Based Study

Zahra Sadat Aghakhah Razlighi*, Abdolhossein Rustaiyan and Kambiz Larijani

Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran

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***Corresponding author**
z.aghakhah@yahoo.com

ABSTRACT

Verbascum cheiranthifolium, an endemic medicinal herb native to Iran, has long been employed in traditional medicine to treat rheumatism, earaches, menstrual irregularities, and inflammatory disorders. Nevertheless, its phytochemical composition and antioxidant potential remain insufficiently investigated. To fill this knowledge gap, aerial parts of *V. cheiranthifolium* were harvested from the Alborz Mountains (approx. 2500 m altitude) and subjected to comprehensive phytochemical and antioxidant analyses. Qualitative screening of the crude aerial parts demonstrated the occurrence of flavonoids, phenolics, tannins, saponins, and triterpenoids, whereas alkaloids and resins were absent. Among the extraction solvents tested, methanol provided the highest yield of compounds associated with antioxidant potential. The leaf-derived diethyl ether extract was analyzed using gas chromatography-mass spectrometry (GC–MS), which led to the identification of six major constituents, with n-eicosane (7.19%) being the most abundant, followed by n-pentacosane (3.31%). Spectrophotometric quantification revealed a total phenolic content of 4.90 ± 0.11 mg gallic acid equivalents (GAE) per gram of dry weight, which was low compared to previous reports. However, an exceptionally high total flavonoid content of 49.05 ± 0.94 mg quercetin equivalents (QE) per gram was observed, suggesting a chemotypic adaptation to high-altitude stress. The methanolic extract exhibited a concentration-dependent capacity to scavenge free radicals in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with an IC_{50} value of 79.7 µg/mL. In contrast, the synthetic antioxidant butylated hydroxytoluene (BHT) achieved an IC_{50} below 50 µg/mL. Collectively, these results offer compelling experimental evidence for the ethnomedicinal relevance of *V. cheiranthifolium* and underscore its promise as a natural source of antioxidant agents for prospective nutraceutical and pharmaceutical exploitation.

Keywords: *Verbascum cheiranthifolium*, Phytochemical Screening, Antioxidant Activity, Total Phenolic Content, Flavonoids

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INTRODUCTION

Natural products derived from medicinal plants remain an essential resource in pharmacological and phytochemical research, owing to their structural diversity, broad-spectrum bioactivities, and generally favorable safety margins. Polyphenolic compounds have received considerable attention for their pronounced free radical-scavenging capacity and their ability to modulate oxidative stress, a key biochemical process implicated in the etiology of numerous diseases [1]. Furthermore, a wide range of plant-derived secondary metabolites exhibit enzyme inhibitory activity and demonstrate therapeutic potential in the management of metabolic, neurodegenerative, and dermatological conditions [2].

The genus *Verbascum* (family Scrophulariaceae), commonly referred to as mullein, encompasses roughly 360 species distributed primarily across Eurasia and North Africa. The Middle East, and specifically Iran, Turkey, and Pakistan, is considered a hotspot for the genus's biodiversity [3, 4]. Iran harbors 42 species, 15 of which are endemic and adapted to ecological niches ranging from high-altitude mountainous zones to riparian habitats [3, 5]. Ethnobotanical records document the medicinal use of various *Verbascum* species for treating respiratory diseases, rheumatism, gastrointestinal disorders, and skin ailments [4]. *Verbascum cheiranthifolium* Boiss., in particular, has been traditionally utilized in Eastern Anatolia, Turkey, for the treatment of asthma, menstrual irregularities, eczema, joint pain, and hemorrhoids [6, 7], while in Iran it has been applied for skin disorders and, in some local contexts, as a fish poison.

Previous phytochemical investigations on *V. cheiranthifolium* have focused mainly on the aerial parts—particularly stems and flowers. These studies have documented a wide range of bioactive constituents, including glycosides such as iridoids (*e.g.*, aucubin and catalpol) and phenylpropanoids (*e.g.*, verbascoside), along with various flavonoids (*e.g.*, luteolin derivatives). These compounds are well-established for their significant antioxidant, anti-inflammatory, and antimicrobial activities [7, 8]. Analyses of leaf material from Eastern Anatolia have identified luteolin hexoside in high concentrations, together with smaller quantities of apigenin, chlorogenic acid, and rosmarinic acid. These compounds all demonstrate notable anti-inflammatory and cytoprotective effects, partially mediated through nitric oxide inhibition [6]. Crude extracts from this species have been found to contain elevated levels of total phenolics (up to 33.1 mg GAE/g dry weight) and to

exhibit potent antioxidant activity [6]. In addition to their antioxidative capacity, *V. cheiranthifolium* extracts have demonstrated cytotoxic effects toward cancer cell lines, specifically cervical (HeLa) and ovarian (Skov-3) carcinomas [9], and antifungal activity against *Candida* spp. [9], and insecticidal properties effective against stored-product pests [10, 11].

Geographic variation in the chemical profile of this species has also been observed. For example, studies in southern Iran have revealed differences in volatile composition and metabolite distribution across plant organs, indicating that environmental factors and organ-specific biosynthesis influence secondary metabolite production [12]. While recent comprehensive studies have profiled other species, such as *V. thapsus* [13], and explored phenolic compounds exclusively in the flowers of nine endemic *Verbascum* species from Iran [14], a critical knowledge gap remains. A detailed, comprehensive analysis of the leaf-derived chemical composition and antioxidant potential of *V. cheiranthifolium* — especially from populations subject to the unique ecological stressors of the high-altitude Kolakchal environment (approx. 2500 m altitude) and potential urban pollution—remains critically under-investigated. Considering that the leaves are the primary site for photosynthesis and the immediate response to abiotic stressors like UV radiation, a thorough analysis of their specific phytochemical expression is warranted in this context to facilitate the capturing of potential chemotypic adaptations.

Therefore, the present work represents the first comprehensive investigation of the phytochemical composition and antioxidant activity of *V. cheiranthifolium* aerial parts sourced from the Kolakchal area of the Alborz Mountains in northern Tehran, with a specific focus on the leaf volatile profile via GC-MS. Consequently, the objectives of this study were: (i) to analyze volatile and semi-volatile constituents using GC–MS; (ii) to determine the total phenolic and flavonoid concentrations by spectrophotometric techniques; and (iii) to evaluate their antioxidant activity by measuring their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. The results are anticipated to fill current knowledge gaps and strengthen the ethnopharmacological relevance of this species.

MATERIALS AND METHODS

Plant Material Collection and Taxonomic Verification

Aerial parts of *Verbascum cheiranthifolium*, an endemic medicinal plant of Iran, were gathered in July 2017 from the Kolakchal highlands of the Alborz Mountains at an elevation of roughly 2500 m above sea level. The Kolakchal area was specifically chosen for this study because its high altitude (approx. 2500 m) in combination with its proximity to urban pollution sources (Northern Tehran) induces specific ecological stressors hypothesized to influence secondary metabolite production, which differentiates this population from those in remote rural areas. According to the *Field Book for Describing and Sampling Soils* by the Natural Resources Conservation Service (NRCS), the geographical coordinates of the collection site at Kolakchal, Tehran, were approximately 35.792° N, 51.412° E. Based on reports from the Iran Meteorological Organization for 2017, the area exhibits a semi-arid temperate montane climate, characterized by cold, snowy winters with temperatures often dropping below -5 °C (average January low: -2.3 °C in Tehran, adjusted for elevation) and heavy snowfall (typical of high-altitude zones), and mild, dry summers with maximum temperatures of (significantly cooler than the Tehran urban average at 2500 m) and annual precipitation of approximately 230 mm (mostly in winter months). According to the Food and Agriculture Organization (FAO) and soil studies of Iran, the soil in the area is typically classified as a Calcaric Cambisol with a stony loam texture and low organic matter content (less than 2%), reflecting the semi-arid conditions of the Alborz Mountains [15-21]. Taxonomic authentication was carried out in Tehran, Iran, by the highly reputable authority on Iranian flora, Dr. Valiollah Mozaffarian, at the Research Institute of Forests and Rangelands (RIFR). Following authentication, the plant material was air-dried under ambient conditions (approximately 25 °C, away from direct sunlight) to preserve heat-sensitive compounds. The dried samples were subsequently ground into a homogeneous powder using a laboratory-grade mechanical grinder and stored in sealed containers at 4 °C in the dark for later use. Crucially, the key chemical and bioactivity analyses (TPC, TFC, DPPH, GC-MS) were performed on these samples between 2017 and 2018. The subsequent delay in publication has been due to the writing and submission processes.

Fig. 1 Map of the study area. Location of the *V. cheiranthifolium* collection site in the Kolakchal region of the Northern Alborz Mountains, Iran, 35.792° N, 51.412° E. The inset map shows the general geographical location of the study area within Iran.

Fig. 2 Herbarium-style documentation showing the dried roots, stems, leaves, and inflorescence of the *V. cheiranthifolium* specimen collected from the Kolakchal region.

Chemicals and Apparatus

The solvents employed in this study—methanol, ethyl acetate, chloroform, n-hexane, and hydrochloric acid (HCl)— were of analytical grade and procured from reputable commercial vendors. Instrumentation central to the experimental procedures was a Hewlett-Packard HP-6890 gas chromatograph coupled to an HP-5973 mass selective detector for GC–MS analyses, a Cary 300 Conc ultraviolet–visible spectrophotometer (Agilent Technologies), and a Heidolph rotary evaporator (Germany).

Additional laboratory resources included a UV inspection chamber, temperature-controlled drying oven, precision analytical balance, borosilicate glassware, and complete filtration setups. The entire experimental work was conducted at the laboratories of the Islamic Azad University Science and Research Branch.

Fig. 3 The live plant habit (rosette structure) of *V. cheiranthifolium* photographed at the collection site (Kolakchal).

Fig. 4 Dried and coarsely processed aerial parts (leaves and flowers) of the *V. cheiranthifolium* specimen prior to pulverization for extraction.

Extraction of Volatile Compounds for GC-MS

Powdered leaves of *V. cheiranthifolium* (5 g) were extracted with 50 mL of diethyl ether, maintaining a 1:10 (w/v) plant-to-solvent proportion, in an Erlenmeyer flask. The flask was wrapped in aluminum foil and sealed with Parafilm to minimize solvent loss, then maintained at ambient temperature for 24 hours with periodic agitation. Following extraction, the resulting mixture was filtered through Whatman No. 1 filter paper. The obtained filtrate was concentrated at 40 °C under reduced pressure with a rotary evaporator. The residue was then redissolved in n-hexane, filtered to eliminate particulates, and prepared for GC–MS examination.

GC-MS Analysis

Volatile profiling was carried out using a Hewlett-Packard HP-6890 gas chromatograph connected to an HP-5973 mass selective detector, which was equipped with an HP-5MS capillary column. The column specifications were as follows: length 30 m; internal diameter 0.25 mm; stationary phase coating 0.32 µm. The oven program was initiated at 60 °C (maintained for a duration of 3 min) and subsequently ramped up to 220 °C at a heating rate of 5 °C/min. The injector was operated in split/splitless mode at a temperature of 250 °C, with helium gas of 99.999% purity serving as the carrier at a constant flow of 1 mL/min. Electron ionization (EI) was performed at 70 eV, with the ion source and quadrupole temperatures adjusted to 230 °C and 150 °C, respectively. Compound identification was achieved by comparing their mass spectra with records in the NIST spectral database and verified with published literature [22]. Retention Indices (RI) were calculated based on the retention times of n-alkanes, according to the methodology of Van den Dool and Kratz.

Phytochemical Screening and Qualitative Analysis

Sequential solvent fractionation was applied to the powdered aerial parts, employing solvents with progressively higher polarity in the following order: n-hexane (non-polar), chloroform (low polarity), ethyl acetate (medium polarity), methanol (high polarity), and 1 N HCl (acidic). A constant ratio of plant material to solvent (1:10 w/v) was maintained. Each extraction step consisted of immersing a 10 g powdered sample in 100 mL of the selected solvent for a 48-hour period at ambient temperature, with occasional shaking, prior to filtration. The residue from each step was re-extracted with the next solvent under identical conditions. The extracts obtained from this process were then subjected to qualitative phytochemical screening to detect major secondary metabolite classes, including saponins, carbohydrates/glycosides, alkaloids, flavonoids, tannins, sterols, triterpenes, diterpenes, resins, coumarins, proteins/amino acids, phenolics, and anthraquinones. These assays were performed according to established protocols as detailed in previous studies [23–29]. The specific tests included the Froth test, which was performed for saponins (Froth test), reducing sugars (Fehling's test), alkaloids (Mayer's and Dragendorff's tests), flavonoids (Shinoda's and lead acetate tests), as well as phenolics and tannins (ferric chloride tests).

Total Phenolic Content (TPC) Measurement

Total phenolic content (TPC) was measured using the Folin–Ciocalteu (FC) colorimetric procedure, an electron-transfer reaction in which phenolic compounds reduce the phosphomolybdate–phosphotungstate complex to form a blue chromophore. For the assay, a 0.5 mL aliquot of the methanolic extract (1:10 w/v) was combined with 2.5 mL of 10% (v/v) FC reagent. After a 5-minute equilibration period at ambient temperature, 2 mL of 5% (w/v) sodium carbonate solution was added, and the resulting mixture was allowed to stand in the dark for 30 minutes to permit full color development. A reagent blank was prepared by substituting methanol for the extract.

Gallic-acid standards (10, 50, and 100 ppm) were prepared and processed identically to construct the calibration curve. Absorbance values were obtained at 760 nm using a UV–Visible spectrophotometer (Cary 300 Conc, Agilent). The TPC was calculated from the regression equation of the gallic acid calibration curve and is reported as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). All analyses were conducted in triplicate [30].

Total Flavonoid Content (TFC) Measurement

The total flavonoid content (TFC) was quantified using a colorimetric technique that involves aluminum chloride ($AlCl_3$). This method is predicated on the formation of stable flavonoid-$AlCl_3$ complexes, which exhibit absorbance at 415 nm. In brief, 0.5 mL of the plant extract (1:20, w/v) was combined with 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$ solution, and 0.1 mL of 1 M potassium acetate, followed by the addition of 2.8 mL of distilled water. A corresponding blank was prepared by replacing the extract with methanol.

Quercetin standard solutions (50, 75, 100, and 250 ppm) were prepared identically to generate a calibration curve after a 30-

minute dark incubation period at ambient temperature. The absorbance was measured at 415 nm using a UV–Visible spectrophotometer. The final results are presented as milligrams of quercetin equivalents per gram of dry sample (mg QE/g). All experiments were performed in triplicate [30].

DPPH Radical Scavenging Activity (Antioxidant Activity)

The antioxidant potential of the methanolic extract was determined via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. This method quantifies the capacity of antioxidants to neutralize the stable DPPH radical through electron or hydrogen atom transfer. A 0.1 mM DPPH solution in methanol was freshly prepared. The dried methanolic extract (obtained from a 1:10 w/v extraction) was reconstituted to yield a 500 µg/mL stock solution, which was then sequentially diluted to produce final concentrations of 250, 100, 75, and 50 µg/mL.

Butylated hydroxytoluene (BHT) served as a reference standard antioxidant at the same concentration range. For each test, equal volumes (1 mL) of the sample solution and 1 mL of the DPPH reagent were combined and kept in the dark at room temperature for 30 minutes. A control solution containing only methanol and DPPH was used for comparison. Absorbance values were recorded at 517 nm, and the percentage of radical inhibition was calculated as:

$$\% \text{ Inhibition} = \left[\frac{(\text{A control} - \text{A sample})}{\text{A control}}\right] \times 100 \quad (1)$$

where $A_{control}$ represents the control's absorbance value, and A_{sample} corresponds to the absorbance measured for the sample. The half-maximal inhibitory concentration (IC_{50}) was determined by plotting inhibition percentage against concentration and applying regression analysis. All experiments were carried out in triplicate, and data are presented as mean ± standard deviation (SD).

RESULTS

Qualitative Phytochemical Profiling

The stepwise solvent fractionation successfully separated secondary metabolites based on their polarity, with the consolidated qualitative results summarized in Table 1. As expected, highly polar constituents (including flavonoids, phenolic compounds, tannins, proteins, and amino acids) showed the most intense positive reactions in the methanol extract. In contrast, extremely polar compounds, such as carbohydrates and saponins, were exclusively detected in the final aqueous (distilled water) fraction. Compounds with lower to medium polarity, such as triterpenes and diterpenes, also exhibited strong responses in methanol, suggesting that methanol was the most comprehensive solvent among those tested for these particular classes. The absence of detectable alkaloids and resins across all fractions, including the acidic extract, confirms their negligible contribution to the plant's overall phytochemical profile.

Table 1 Phytochemical constitute screening of the extracts of *V. cheiranthifolium* Boiss.

Chemical constituents	Tests	The extracts of *V. cheiranthifolium* Boiss.	Results
Carbohydrates and/or glycosides	Fehling	Water	+
alkaloids	Mayer	Hydrochloric acid	-
alkaloids	Dragendroff	Hydrochloric acid	-
alkaloids	Hager	Hydrochloric acid	-
flavonoids	Shinoda	Methanol	+
flavonoids	Alkaline test	Methanol	+
flavonoids	Lead acetate	Methanol	+
Saponins	The froth test	Water	+
Tannins	Ferric chloride	Methanol	+
Triterpenes and/or unsaturated sterols	Liebermann-Burchard	Methanol	+
Triterpenes and/or unsaturated sterols	Salkowski	Methanol	+
Diterpene	Copper acetate	Water	+
Resins		Methanol	-
Coumarins	UV test	NaOH	+
Proteins and/ or amino acids	Xanthoproteic	Methanol	+
Phenols	Ferric Chloride	Methanol	+
Glycosides	Borntrager	Hydrochloric acid	+
Anthraquinones		Hydrochloric acid	+

Note: (+) indicates the identification of secondary plant compounds; (-) indicates non-detection of secondary plant compounds.

GC-MS Analysis of the Leaf Ether Extract

Analysis of the diethyl ether extract via GC–MS revealed six prominent volatile and semi-volatile constituents. Compounds were identified through retention time comparison, spectral interpretation, and NIST library matching. The major detected substances were predominantly long-chain alkanes and fatty acids, including *n*-eicosane (7.19%), *n*-pentacosane (3.31%), *n*-tricosane (1.08%), *n*-octacosane (1.39%), palmitic acid (0.69%), and linoleic acid (0.43%).

Table 2 Chemical composition of the ether extract of *Verbascum cheiranthifolium* as determined by GC–MS.

Identified compound	Molecular formula	Chemical class	MW (g/mol)	RT(min)	RI (Experimental)	RI (Reference) [22]	Peak area %
Acids							
Palmitic acid a	$C_{16}H_{32}O_2$	Fatty Acid	256.42	29.92	1897	1959	0.69
Linoleic acid a	$C_{18}H_{32}O_2$	Fatty Acid	280.45	32.19	2011	2132	0.43
Alkane							
n-Eicosane	$C_{20}H_{42}$	Alkane	282.55	32.09	2000	2000	7.19
n-Tricosane	$C_{23}H_{48}$	Alkane	324.637	34.89	2300	2300	1.08
n-Pentacosane	$C_{25}H_{52}$	Alkane	352.69	34.79	2500	2500	3.31
n-Octacosane	$C_{28}H_{58}$	Alkane	394.772	36.62	2800	2800	1.39

[a] Experimental RI values for all compounds were calculated relative to the retention times of n-alkanes. The RI values for Palmitic acid (1897) and Linoleic acid (2011) show agreement with the literature RI of their respective methyl ester derivatives [22] (e.g., Methyl Palmitate, 1959; Methyl Linoleate, 2132), confirming identification and suggesting esterification during analysis.

It is noteworthy that the retention indices for the fatty acids (palmitic acid and linoleic acid) showed a strong agreement with their respective methyl ester derivatives (Table 2), suggesting that transesterification of these compounds occurred during the GC-MS analysis or sample preparation [22]. Among these, n-eicosane constituted the highest proportion of total peak area. The detailed chemical composition is listed in Table 2.

Antioxidant Activities

Quantification of Total Phenolic Content (TPC)

Phenolic levels in the methanol extract were quantified employing the Folin–Ciocalteu colorimetric method, using gallic acid (10–100 ppm) as the calibration reference (Fig. 5). The resulting calibration curve followed the equation $Y = 0.0107x - 0.1261$, with strong linearity ($R^2 = 0.9816$). Based on the absorbance value for the extract (0.136), the phenolic content was calculated as 4.90 ± 0.11 mg gallic acid equivalents (GAE) per gram of dry extract. Values are expressed as mean values accompanied by standard deviations from three separate replicates.

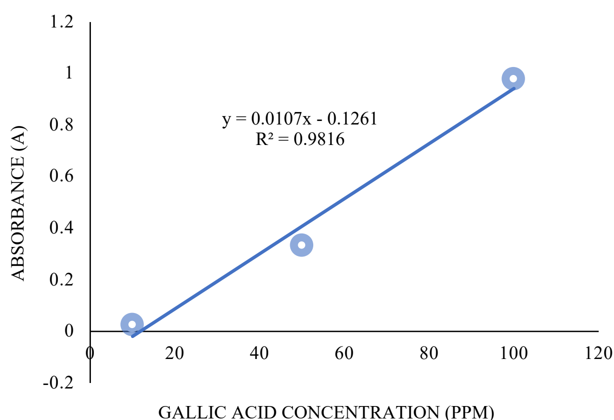

Fig. 5 Standard calibration curve of gallic acid used for total phenolic content (TPC) measurement.

Quantification of Total Flavonoid Content (TFC)

Flavonoid levels in the methanol extract were quantified employing the aluminum chloride-based colorimetric method, using quercetin standards (50–250 ppm) for curve construction (Fig. 6). The derived calibration curve followed the equation $Y = 0.0151x - 0.2278$, showing strong linearity ($R^2 = 0.9963$). The extract absorbance (0.513) corresponded to a TFC of 49.05 ± 0.94 mg expressed as quercetin equivalents (QE) per gram of dried sample. All measurements are given as mean values with corresponding standard deviations from three parallel determinations.

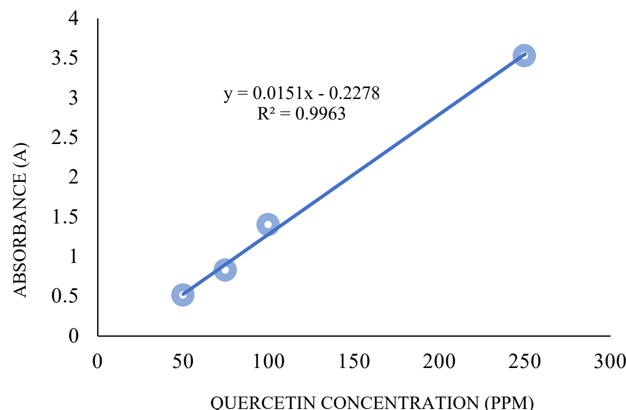

Fig. 6 Standard calibration curve of quercetin used for total flavonoid content (TFC) measurement.

Free Radical Scavenging Capacity (DPPH Assay)

The antioxidant capability, assessed through free radical quenching, of the methanolic extract and butylated hydroxytoluene (BHT) was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method within the concentration range of 50–500 µg/mL. Percentage inhibition, calculated from absorbance readings at 517 nm, demonstrated a clear dose-dependent trend. The extract achieved 41.6% inhibition at 50 µg/mL, increasing to 94.8% at 500 µg/mL, with an estimated IC_{50} value of approximately 79.7 µg/mL. In comparison, BHT exhibited higher activity, surpassing 60% inhibition even at the lowest concentration tested, suggesting an IC_{50} below 50 µg/mL. Regression analysis produced high correlation coefficients for both the extract ($R^2 = 0.9618$) and BHT ($R^2 = 0.9579$), confirming strong dose–response relationships. A comprehensive summary of the results is given in Table 3.

Table 3 Radical scavenging performance of *V. cheiranthifolium* extract in the DPPH assay and BHT at various concentrations. (values expressed as mean ± standard deviation, n = 3)

Concentration (µg/mL)	Absorbance (A) – extract	% Inhibition – extract	Absorbance (A) – BHT	% Inhibition – BHT
50	0.236 ± 0.003	41.6 ± 0.15	0.154 ± 0.002	61.9 ± 0.21
75	0.197 ± 0.005	51.2 ± 0.22	0.141 ± 0.001	65.1 ± 0.12
100	0.189 ± 0.002	53.2 ± 0.18	0.116 ± 0.004	71.3 ± 0.28
250	0.097 ± 0.004	75.1 ± 0.31	0.068 ± 0.002	83.2 ± 0.14
500	0.021 ± 0.001	94.8 ± 0.12	0.014 ± 0.001	96.5 ± 0.09

Note: A_0 (absorbance of control) = 0.404
Linear regression (extract): $y = 0.0011x + 0.4123$, $R^2 = 0.9618$
Linear regression (BHT): $y = 0.0007x + 0.611$, $R^2 = 0.9579$
Estimated IC_{50} (extract): ≈ 79.7 µg/mL
Estimated IC_{50} (BHT): < 50 µg/Ml

DISCUSSION

The phytochemical and antioxidant profiling of *Verbascum cheiranthifolium* aerial parts collected from the Kolakchal region of the Alborz Mountains (northern Iran) revealed a distinct chemical signature plausibly influenced by high-altitude environmental conditions. Elevations exceeding 2500 m, intensified UV radiation, and marked diurnal temperature variation are abiotic stressors known to affect secondary metabolite biosynthesis and contribute to chemotypic diversity within *Verbascum*.

Qualitative screening confirmed the occurrence of multiple biologically active phytoconstituent classes—including flavonoids, phenolic compounds, tannins, saponins, and triterpenoids—consistent with previous reports for other *Verbascum* species [1, 12,

13]. The quantification results, however, revealed a unique pattern: the total phenolic content (TPC = 4.90 ± 0.11 mg GAE/g) was relatively low compared to some reported populations [6]. Crucially, the total flavonoid content (TFC = 49.05 ± 0.94 mg QE/g) was exceptionally high. This suggests a specific chemotypic adaptation in the Kolakchal population, possibly driven by high-altitude stressors (such as intense UV exposure). Studies on other species, such as *Artemisia* and *Asarum* growing at high elevations, consistently demonstrate that increased UV radiation enhances the synthesis of flavonoids for photoprotection and defense, often leading to a trade-off where non-flavonoid phenolic content decreases [31, 32]. This phenomenon is further supported by recent phytochemical work on other *Verbascum* species, which links environmental stress to increased biosynthesis of protective flavonoids [13, 33]. This high concentration of flavonoids is therefore likely the primary driver of the potent antioxidant activity observed in the DPPH assay.

GC–MS profiling of the diethyl ether fraction indicated a relatively chemical profile dominated by long-chain alkanes such as n-eicosane (7.19%) and n-pentacosane (3.31%), together with fatty acids including palmitic acid (0.69%) and linoleic acid (0.43%). Although these constituents are recognized for exhibiting antioxidant and anti-inflammatory activities [6, 13], the limited volatility spectrum observed here differs from the richer volatile assemblages reported in some other *Verbascum* taxa. Differences in solvent selectivity, the plant organ analyzed, and site-specific microclimatic stressors likely explain these compositional discrepancies. The relatively weak correlation between volatile composition and antioxidant performance in this study underscores the predominant role of non-volatile phenolic constituents, especially flavonoids, in radical-scavenging activity. Moderate DPPH radical scavenging activity was observed for the methanolic leaf extract ($IC_{50} \approx 79.7$ µg/mL), which, although less potent than BHT ($IC_{50} < 50$ µg/mL), is comparable to values reported for extracts of other *Verbascum* species [6]. The high flavonoid content measured here likely contributes substantially to the observed antioxidant capacity. Interpopulation differences in antioxidant potency are plausibly attributable to environmental pressures, genetic background, harvest timing, and phenological stage.

Beyond antioxidant effects, *V. cheiranthifolium* has been reported to display anti-inflammatory, cytoprotective, antifungal (anti-Candida), and insecticidal activities [6, 9, 11]. Detection of phenolic acids and flavonoids in the present samples supports these pharmacological prospects; however, the lack of bioactivity-guided fractionation prevents definitive assignment of activity to individual constituents. Given organ-dependent accumulation patterns reported for phenylpropanoid glycosides and flavonoids [7, 12], comprehensive phytochemical surveys across stems, flowers, and leaves are recommended to fully define the species' pharmacognostic profile.

Limitations and Research Outlook

This work is subject to limitations that merit further examination in upcoming studies. Single-site, single-season sampling constrains the assessment of spatial and temporal chemotypic variability. The application of a single extraction solvent for GC–MS may have limited detection of certain volatile classes (*e.g.*, terpenoids). Future studies should employ multi-site and multi-season sampling, bioactivity-guided fractionation, and additional antioxidant/anti-inflammatory assays (*e.g.*, FRAP, ABTS, nitric oxide inhibition), and include within *in vitro* assays and *in vivo* experimental models. Comparative analyses of populations from urban-adjacent versus pristine habitats could further elucidate the influence of anthropogenic stressors (such as air pollution) on secondary metabolite expression.

CONCLUSION

This work provides the first comprehensive phytochemical and antioxidant evaluation of *Verbascum cheiranthifolium* collected from the high-altitude Kolakchal region. The key finding is the distinct chemotypic profile characterized by an exceptionally high total flavonoid content (49.05 mg QE/g), which strongly correlates with its notable DPPH radical scavenging capacity (IC50 approx. 79.7 µg/mL). This robust antioxidant activity, likely a result of the high-altitude environment, offers direct scientific validation for the traditional use of the plant in treating inflammatory and dermatological disorders. Thus, *V. cheiranthifolium* sourced from the Alborz mountains is highly promising as a readily available, natural source for the development of antioxidant-rich nutraceuticals or standardized herbal supplements targeting oxidative stress-related conditions.

Preliminary screening confirmed the presence of diverse bioactive secondary metabolites (flavonoids, tannins, saponins, triterpenoids) while definitively ruling out alkaloids and resins. GC–MS analysis identified long-chain alkanes n-eicosane and n-pentacosane as the dominant volatile constituents. These findings establish a baseline for understanding the species' chemotypic adaptation to its unique ecological niche.

For future research, a multidisciplinary approach is highly recommended, focusing on bioactivity-guided fractionation to isolate and structurally elucidate the specific flavonoid compounds responsible for the observed antioxidant potency. Extended pharmacological assays (e.g., ABTS, FRAP, anti-inflammatory models) and comparative studies of plants from different habitats should be conducted to fully realize the species' pharmaceutical potential.

Funding and Competing Interests

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