

Comparative Mineral Content, Total Phenolic and Flavonoid Levels and Chlorophyll, Carotenoid and DPPH Assays of Three Wild Moroccan Medicinal Plants from the Settat Region

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Marrubium vulgare L., *Calendula arvensis* (Vaill.) L. and *Ajuga iva* L. are Moroccan medicinal plants with diverse phytochemical profiles and established traditional uses. This study aimed to comparatively characterize the mineral composition, photosynthetic pigments, total phenolic, flavonoid contents, and antioxidant activity of these wild species collected from the Settat region of Morocco. Mineral ions were quantified by ion chromatography, while phenolic compounds, flavonoids, chlorophylls and carotenoids were determined using spectrophotometric methods. Antioxidant activity was assessed using the DPPH radical-scavenging assay. The species exhibited distinct mineral and phytochemical profiles. *C. arvensis* (Vaill.) L. had the highest chloride (155.57 ± 4.65 ppm), sulphate (53.02 ± 0.91 ppm), and carotenoid (0.0834 ± 0.0521 mg kg⁻¹ DW) contents, whereas *A. iva* L. showed the highest phosphate (58.33 ± 0.13 ppm), nitrite (49.15 ± 0.01 ppm), chlorophyll a (0.150 ± 0.0591 mg kg⁻¹ DW) and DPPH activity (135.87 ± 5.81 mg AAE kg⁻¹ FW). *M. vulgare* L. contained the highest total phenolic (992.02 ± 38.94 mg GAE kg⁻¹ FW) and flavonoid (2034.47 ± 81.00 mg QE kg⁻¹ FW) contents. These findings provide a descriptive comparative framework of the biochemical composition of these species, which may inform future studies into their pharmacological potential.

Keywords: Medicinal plants, Phytochemical profile, Mineral composition, Antioxidant activity, Bioactive compounds.

INTRODUCTION

Various cultures have long used medicinal and aromatic plants (MAPs) for their potential health-enhancing and therapeutic benefits [1]. These have been associated with the bioactive agents present in plant extracts. Of the over 500,000 plant species found worldwide, only a small proportion are said to be involved in herbal medicine [2]. With diverse soil and climatic conditions, the Mediterranean region is home to more than 10,000 MAP varieties, each with distinct attributes that warrant detailed study [3]. Morocco is one of the main producers of medicinal aromatic plants (MAPs), boasting a highly diverse and endemic flora. However, this heritage remains largely unexploited and uninvestigated to date [4].

Therefore, studies of such plants can provide much-needed data on their pharmacological potential for the development of functional foods and sources of nutraceutical compounds, including new antioxidants [5,6]. Among them, *Ajuga iva* L., *Marrubium vulgare* L. and *Calendula arvensis* (Vaill.) L. are well known in Moroccan traditional medicine for pharmaceutical use. These plants, belonging to the families Asteraceae and Lamiaceae, have characteristic phytochemical profiles that make them suitable candidates for study [7].

Ajuga iva L. is traditionally used to treat rheumatic pain, hypertension, cardiovascular disorders, ocular infections, allergies, cancer, renal diseases and digestive ailments [8,9]. Bioactive compounds, including flavonoids and terpenoids, have been reported in this representative of the Lamiaceae

family and may contribute to its pharmacological potential [10,11]. *Marrubium vulgare* L. (Lamiaceae). White horehound is rich in phenolic compounds and is generally used for respiratory conditions such as bronchitis and coughs [12]. Its bioactive profile includes flavonoids, diterpenoids and phenylethanoid glycosides [13,14]. *Calendula arvensis* (Vaill.) L., known for its healing and anti-inflammatory properties, is recognized for its therapeutic potential and diverse chemical composition, which includes terpenoids, flavonoids and carotenoids [15-18].

This study examines the mineral composition, antioxidant activity and levels of key bioactive compounds, with particular emphasis on chlorophyll, phenolics and flavonoids, in three plant species collected in their wild, uncultivated state from the Settât region. Although the phytochemical profiles of these species have been investigated to some extent in earlier studies, comparative assessments that consider mineral composition alongside major bioactive constituents are still limited. The present work seeks to provide a broader comparative profile of these species by relating their mineral characteristics to their antioxidant potential and phytochemical composition. The present work therefore aims to (i) determine and compare the mineral composition (cations and anions) and selected biochemical parameters (total phenolics, flavonoids, chlorophylls, carotenoids and antioxidant activity) of *Marrubium vulgare* L., *Calendula arvensis* (Vaill.) L. and *Ajuga iva* L. collected from the Settât region of Morocco and (ii) provide an updated characterization of their biochemical diversity under natural growing conditions. Rather than establishing direct relationships between mineral and bioactive traits, this study seeks to offer complementary information on the elemental and biochemical composition of these medicinal species, contributing to a broader understanding of their potential value in traditional and pharmaceutical applications. In this respect, the research integrates these analyses to characterize the chemical and bioactive compositions of these plants and their applications in traditional medicine and pharmaceutical development.

EXPERIMENTAL

The plants under study were collected in June 2025 from wild fields in Morocco, Settât Province, Chaouia-Ouardigha

region (latitude 32.992 and longitude -7.622). The collected plant samples were identified by Prof. Benkhniq Ouafae of the Department of Botany and Plant Ecology at the Scientific Institute of Mohammed V University, Rabat, Morocco.

Ajuga iva L. (RAB 114823) belongs to the family Lamiaceae (Fig. 1a) and is native to North Africa and Southern Europe [19]. It is a small herbaceous plant that generally reaches a height of 5-20 cm. In Morocco and Algeria, the species is commonly known as 'Chondgoura' or 'Chendghoura' [20].

Marrubium vulgare L. (RAB 114825), commonly known as white horehound, is a perennial herb of the family Lamiaceae (Fig. 1b). The plant generally reaches 30.0 cm in height and has stems densely covered with soft, woolly hairs. Its flowering period extends from June to September, during which it develops characteristic flowers [21]. The genus *Marrubium* comprises approximately 49 accepted species [22]. *Marrubium vulgare* L. is native to the Mediterranean region and Central Asia and has adapted to a wide range of environmental conditions, contributing to its distribution across many parts of the world [23].

Calendula arvensis (Vaill.) L. (RAB 114824) is a self-seeding annual herb belonging to the family Asteraceae (Fig. 1c). It is well adapted to warm and humid climatic conditions and generally grows to a height of 30-46 cm. The species is widely distributed across the Mediterranean region and occurs in several parts of Central Europe [24,25].

Extract preparation: For the determination of chlorophylls, total flavonoid content, total phenolic content and antioxidant activity, a methanolic extract was prepared from the lyophilized samples. Briefly, 50 mg of the lyophilized material was suspended in 1.5 mL of methanol in a 2 mL Eppendorf tube. The mixture was vortexed thoroughly and incubated overnight at 4 °C to facilitate the extraction of bioactive constituents. Following incubation, the samples were centrifuged at $16,000 \times g$ for 5 min at 4 °C. The clear supernatant obtained after centrifugation was collected and used as the methanolic extract for subsequent analyses.

Mineral analysis: For the extraction of cations and anions, three samples were analyzed for each treatment. Briefly, 0.2 g of dried plant leaves was weighed into a tube, followed by the addition of 50 mL of distilled water. The samples were agitated on an orbital shaker (Stuart SSL1, Stone, U.K.) at

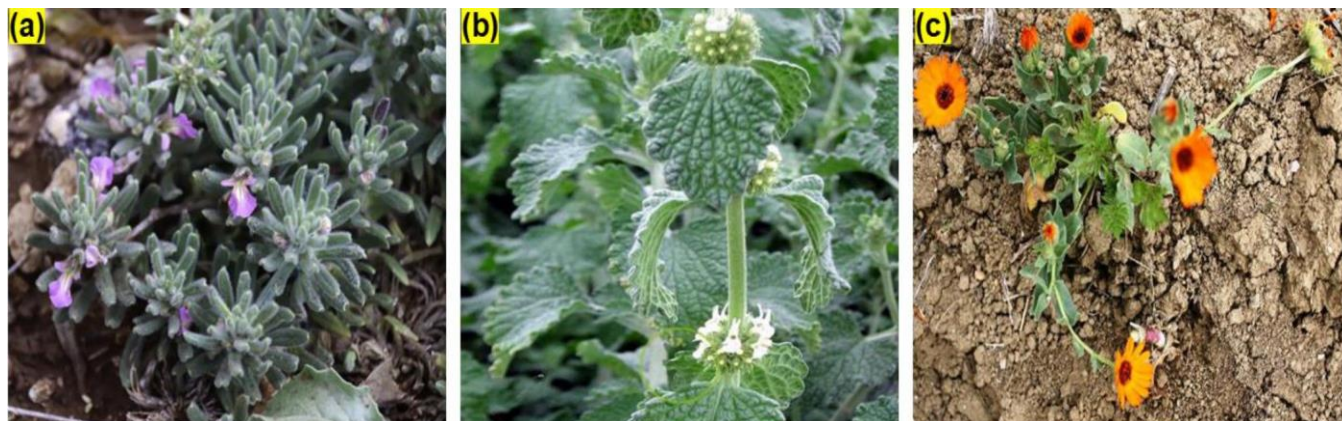


Fig. 1. Images of *Ajuga iva* L. (a), *Marrubium vulgare* L. (b) and *Calendula arvensis* (Vaill.) L. (c) plants

110 rpm and 50 °C for 45 min. The extracts were subsequently analyzed by ion chromatography. Anions were separated using a Metrosep A SUPP 5 column (Metrohm AG, Switzerland) at a flow rate of 0.7 mL min⁻¹, while cations were analyzed using a Metrosep C 2-250 column (Metrohm AG, Switzerland) at a flow rate of 1.0 mL min⁻¹ [26].

Chlorophylls and carotenoids: Chlorophyll and carotenoid concentrations were determined spectrophotometrically in 50 µL methanol extracts. Absorbance readings at 652, 665 and 470 nm were obtained using a UV-visible spectrophotometer (Hewlett-Packard 8453) to calculate chlorophyll a, chlorophyll b and total carotenoid levels according to reported method [27]. Results were expressed as mg/kg dry weight (DW) based on triplicate plant samples per treatment.

Total flavonoids content: Total flavonoid content was determined by mixing methanol extracts (50 µL), MeOH (300 µL) and AlCl₃ (2 %) (350 µL). The samples were mixed and then incubated at room temperature in dark for 20 min. The absorbance was measured at 430 nm. All three plants from each replicate were analyzed to ensure adequate representation of the experimental material [28].

Total phenolic content: Total phenolic content was quantified using the Folin-Ciocalteu colorimetric method, as described by Everette *et al.* [29]. In a 2 mL microcentrifuge tube, 50 µL of methanol extract, 790 µL of distilled water and 50 µL of Folin-Ciocalteu reagent were mixed and incubated for 5 min. Subsequently, 150 µL of 20 % sodium carbonate solution was added. After a 2 h incubation period in the dark at room temperature, the absorbance was measured at 765 nm using a UV-visible spectrophotometer.

DPPH antioxidant capacity: A modified DPPH assay [30] was used to assess the antioxidant capacity of the methanol extracts. Briefly, 25 µL of extract was mixed with 600 µL of 0.1 mM DPPH solution and incubated in dark at room temperature for 15-20 min. The absorbance was then measured at 517 nm.

Statistics: The data were statistically analyzed using Tukey's test, with each cultivar analyzed separately and significance was assessed at $p < 0.05$.

RESULTS AND DISCUSSION

Mineral contents: The major anions present in plant tissues, nitrate (NO₃⁻), chloride (Cl⁻), sulfate (SO₄²⁻) and

phosphate (PO₄³⁻), play crucial roles in mineral nutrition and metabolic processes [31]. Analysis of *M. vulgare* L., *C. arvensis* (Vaill.) L. and *A. iva* L. revealed significant variations in the anion concentrations (Table-1). *C. arvensis* (Vaill.) L. exhibited the highest chloride concentration (155.57 ± 4.65 ppm), followed by *A. iva* L. (146.21 ± 0.25 ppm) and *M. vulgare* L. (132.55 ± 2.68 ppm). Regarding nitrite ions (NO₂⁻), *A. iva* L. showed the highest concentration (49.15 ± 0.01 ppm), slightly surpassing *C. arvensis* (Vaill.) L. (48.39 ± 1.35 ppm), with *M. vulgare* L. displaying the lowest (44.36 ± 0.93 ppm). These variations in chloride and nitrite concentrations may reflect differences in nutrient uptake mechanisms and metabolic processes across the species. Nitrate concentrations were relatively uniform across all three species, with *M. vulgare* L. (12.87 ± 0.02 ppm) and *C. arvensis* (Vaill.) L. (12.90 ± 0.07 ppm) and *A. iva* L. (12.75 ± 0.01 ppm). The narrow range of values indicates only minor variation in nitrate levels among the species. However, the phosphate contents differed significantly, with the highest recorded for *A. iva* L. (58.33 ± 0.13 ppm), followed by *C. arvensis* (Vaill.) L. (50.64 ± 0.58 ppm) and *M. vulgare* L. (47.32 ± 0.74 ppm).

All the three plant species also exhibited distinct cation profiles (Table-1). Potassium (K⁺) was highest in *C. arvensis* (Vaill.) L. (145.78 ± 4.96 ppm) and *A. iva* L. (145.77 ± 1.68 ppm), while *M. vulgare* L. had the lowest concentration (126.92 ± 2.60 ppm). Calcium (Ca²⁺) was highest in *M. vulgare* L. (48.19 ± 2.59 ppm), followed by *A. iva* L. (37.55 ± 21.92 ppm) and *C. arvensis* (Vaill.) L. (32.19 ± 19.04 ppm). Magnesium (Mg²⁺) reached its highest level in *A. iva* L. (16.97 ± 0.14 ppm), followed by *M. vulgare* L. (15.69 ± 1.39 ppm) and *C. arvensis* (Vaill.) L. (9.69 ± 4.52 ppm). These differences point to species-specific patterns of mineral accumulation and nutrient uptake. The higher calcium content in *M. vulgare* L. is consistent with previous reports on its response to saline conditions [32]. The cation profiles provide useful compositional information on the three Moroccan medicinal species, while controlled studies are needed to establish relationships between mineral composition and secondary metabolite accumulation.

Although the present study does not establish a direct functional relationship between the mineral composition and secondary metabolite accumulation, differences in mineral profiles, particularly phosphate and sulfate levels, occurred alongside variations in the phytochemical composition of the

TABLE-1
QUANTITATIVE ANALYSIS OF ANIONS AND CATIONS IN
Marrubium vulgare L., *Calendula arvensis* (Vaill.) L. AND *Ajuga iva* L. in ppm

Plants	Anions (ppm)							
	F ⁻	Cl ⁻	NO ₂ ⁻	Br ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻	C ₂ O ₄ ²⁻
<i>M. vulgare</i> L.	1.12±0.09	132.55±2.68	44.36±0.93	6.03±0.02	12.87±0.02	47.32±0.74	44.3±0.69	5.99±0.09
<i>C. arvensis</i> (Vaill.) L.	1.39±0.01	155.57±4.65	48.39±1.35	6.05±0.01	12.9±0.07	50.64±0.58	53.02±0.91	6.69±0.02
<i>Ajuga iva</i> L.	1.20±0.09	146.21±0.25	49.15±0.01	6.03±0.01	12.75±0.01	58.33±0.13	50.41±0.09	3.92±0.16
Plants	Cations (ppm)							
	Li ⁺	Na ⁺	NH ₄ ⁺	K ⁺	Ca ²⁺	Mg ²⁺		
<i>M. vulgare</i> L.	LD < 0.03	55.42 ± 0.62	13.41 ± 1.03	126.92 ± 2.6	48.19 ± 2.59	15.69 ± 1.39		
<i>C. arvensis</i> (Vaill.) L.	LD < 0.03	68.95 ± 2.45	17.64 ± 0.88	145.78 ± 4.96	32.19 ± 19.04	9.69 ± 4.52		
<i>Ajuga iva</i> L.	LD < 0.03	62.38 ± 0.31	21.12 ± 0.56	145.77 ± 1.68	37.55 ± 21.92	16.97 ± 0.14		

studied species. Sulphate concentration was the highest in *C. arvensis* (Vaill.) L. (53.02 ± 0.91 ppm), followed by *A. iva* L. (50.41 ± 0.09 ppm) and *M. vulgare* L. (44.30 ± 0.69 ppm). The lowest oxalate concentration was recorded in *A. iva* L. (3.92 ± 0.16 ppm), while *C. arvensis* (Vaill.) L. and *M. vulgare* L. contained 6.69 ± 0.02 and 5.99 ± 0.09 ppm, respectively. Such differences may be associated with species-specific ecological adaptation and metabolic requirements related to nutrient utilization, storage and plant defense.

Mineral nutrients can influence metabolic processes involved in the formation and accumulation of secondary metabolites. Phosphate and sulfate availability, for instance, has been associated with pathways involved in the synthesis of nitrogen- and sulphur-containing compounds, several of which contribute to the biological and pharmacological properties of medicinal plants [33]. Nitrogen sources, including nitrates and nitrites, are similarly involved in primary metabolic processes that supply precursors for amino acid and alkaloid biosynthesis [34]. These relationships have been discussed in relation to the regulation of specialized metabolism and alkaloid formation by Ziegler & Facchini [35] and Taiz *et al.* [36]. The mineral-metabolite associations observed in the present study should be interpreted as potential relationships rather than evidence of a direct biosynthetic mechanism, which would require targeted physiological and molecular investigations.

Polyphenols, flavonoids and DPPH antioxidant capacity: The total polyphenol and flavonoid contents, along with antioxidant activity, were assessed in *M. vulgare* L., *C. arvensis* (Vaill.) L. and *A. iva* L. to compare their bioactive profiles and assess their potential medicinal relevance. The results (Table-2) indicate that *M. vulgare* L. contained the highest total polyphenol content (992.02 ± 38.94 mg GAE/kg FW), followed by *C. arvensis* (Vaill.) L. (769.14 ± 68.38 mg GAE/kg FW) and *A. iva* L. (707.79 ± 7.49 mg GAE/kg FW). A similar pattern was observed for total flavonoids, with *M. vulgare* L. recording the highest content (2034.47 ± 81.00 mg QE/kg FW), followed by *C. arvensis* (Vaill.) L. (1971.18 ± 129.91 mg QE/kg FW) and *A. iva* L. (1747.45 ± 35.42 mg QE/kg FW). The comparatively higher polyphenol and flavonoid contents of *M. vulgare* L. indicate a rich profile of phenolic constituents that may contribute to its antioxidant properties and reported medicinal relevance.

DPPH radical-scavenging activity varied only slightly among the three species. *A. iva* L. recorded the highest activity (135.87 ± 5.81 mg AAE/kg FW), followed closely by *C. arvensis* (Vaill.) L. (135.27 ± 12.17 mg AAE/kg FW), while *M. vulgare* L. exhibited a value of 128.04 ± 3.22 mg AAE/kg FW. The relatively similar antioxidant activities suggest that radical-scavenging capacity cannot be attributed solely to the total polyphenol or flavonoid content. Other

constituents, as well as differences in the composition and antioxidant efficiency of individual metabolites, may contribute to the observed activity.

The high polyphenol and flavonoid levels detected in *M. vulgare* L. support previous reports describing its rich bioactive profile under different environmental conditions, including salinity [32]. *C. arvensis* (Vaill.) L. exhibited intermediate levels of polyphenols and flavonoids together with strong DPPH activity, consistent with its recognized medicinal importance [37]. The antioxidant capacity of *A. iva* L. was comparable to that of the other species and is consistent with observations reported in earlier studies [38-40].

Chlorophylls and carotenoids: This study assessed the concentrations of carotenoids, chlorophyll a and chlorophyll b in *M. vulgare* L. and *C. arvensis* (Vaill.) L. and *A. iva* L. The results are shown in Table-3 showed significant differences among species. The highest carotenoid concentration was measured in *C. arvensis* (Vaill.) L. (0.0834 ± 0.0521 mg kg⁻¹ DW), followed by *M. vulgare* L. (0.0501 ± 0.019 mg kg⁻¹ DW) and *A. iva* L. (0.0323 ± 0.0162 mg kg⁻¹ DW). Regarding chlorophyll a content, *A. iva* L. had the highest level (0.150 ± 0.0591 mg kg⁻¹ DW), while *C. arvensis* (Vaill.) L. and *M. vulgare* L. showed slightly lower concentrations (0.144 ± 0.1244 mg kg⁻¹ DW and 0.123 ± 0.0424 mg kg⁻¹ DW, respectively). For chlorophyll b, *C. arvensis* (Vaill.) L. again led with the highest concentration (0.254 ± 0.134 mg kg⁻¹ DW), followed by *A. iva* L. (0.231 ± 0.0617 mg kg⁻¹ DW) and *M. vulgare* L. (0.199 ± 0.077 mg kg⁻¹ DW). The chlorophyll a/b ratio varied among the studied species, with *A. iva* L. exhibiting the highest value (0.65). This variation may reflect species-specific differences in pigment allocation and photosynthetic acclimation. *C. arvensis* (Vaill.) L. contained a higher carotenoid content and exhibited substantial DPPH radical-scavenging activity. A direct contribution of carotenoids to the measured antioxidant activity cannot be established from the present data and requires further investigation using approaches that distinguish the contribution of individual pigment classes. *A. iva* L. showed comparatively higher chlorophyll levels, which may be associated with its photosynthetic performance and capacity to adapt to variable environmental conditions. *M. vulgare* L. displayed inter-mediate pigment levels, indicating a distinct pigment profile relative to the other two species [32].

Seasonal variation in light exposure has similarly been associated with changes in chlorophyll levels in *A. iva* L., with higher levels reported during winter [38]. Carotenoids have functions beyond light harvesting and photoprotection and can participate in the quenching of reactive oxygen species (ROS), thereby contributing to the plant's defense against oxidative damage [41]. Such properties may be relevant to the biological and medicinal potential of pigment-rich plant materials. The observed pigment profiles are consistent with

TABLE-2
PHYTOCHEMICAL CONTENTS AND DPPH RADICAL-SCAVENGING ACTIVITY
RESULTS OF *Marrubium vulgare* L., *Calendula arvensis* (Vaill.) L. AND *Ajuga iva* L.

Plants	Polyphenols (mg GAE/kg FW)	Flavonoids (mg QE/kg FW)	DPPH (mg AAE/kg FW)
<i>Marrubium vulgare</i> L.	992.02 ± 38.94	2034.47 ± 81	128.04 ± 3.22
<i>Calendula arvensis</i> (Vaill.) L.	769.14 ± 68.38	1971.18 ± 129.91	135.27 ± 12.17
<i>Ajuga iva</i> L.	707.79 ± 7.49	1747.45 ± 35.42	135.87 ± 5.81

TABLE-3
COMPARATIVE CHLOROPHYLL AND CAROTENOID CONTENTS
OF *Marrubium vulgare* L., *Calendula arvensis* (Vaill.) L. AND *Ajuga iva* L.

Plants	Carotenoids (mg kg ⁻¹ DW)	Chlorophyll a (mg kg ⁻¹ DW)	Chlorophyll b (mg kg ⁻¹ DW)	Ch a/Ch b
<i>Marrubium vulgare</i> L.	0.0501 ± 0.019	0.123 ± 0.0424	0.199 ± 0.077	0.62
<i>Calendula Arvensis</i> (Vaill.) L.	0.0834 ± 0.0521	0.144 ± 0.1244	0.254 ± 0.134	0.57
<i>Ajuga iva</i> L.	0.0323 ± 0.0162	0.150 ± 0.0591	0.231 ± 0.0617	0.65

reports emphasizing the importance of growth and environmental conditions in modulating the accumulation of bioactive compounds [42].

The present study should be regarded as a preliminary characterization of the elemental and phytochemical profiles of the collected plant materials. Variations in the mineral and pigment concentrations were observed alongside differences in DPPH radical-scavenging activity, but these associations remain correlative. A larger sample size, controlled environmental conditions and targeted experiments will be necessary to determine whether mineral availability and pigment composition are functionally related to antioxidant capacity.

Conclusion

This study provides a comparative assessment of the mineral composition, bioactive constituents, pigment profiles and antioxidant activity of *Marrubium vulgare* L., *Calendula arvensis* (Vaill.) L. and *Ajuga iva* L. collected from the Settatt region of Morocco. The three species exhibited distinct compositional profiles, with differences in ion concentrations suggesting species-specific patterns of mineral accumulation. *M. vulgare* L. contained the highest total polyphenol and flavonoid contents, while *C. arvensis* (Vaill.) L. showed relatively higher carotenoid and chlorophyll b levels. *A. iva* L. exhibited comparatively higher chlorophyll content and the highest DPPH radical-scavenging activity, although antioxidant activity was broadly comparable among the species. The observed differences in mineral and phytochemical profiles may reflect species-specific, environmental and developmental factors. Further studies under controlled conditions with larger sample sizes and complementary antioxidant assays are required to clarify these relationships and support the nutraceutical potential of these Moroccan medicinal plants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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