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Original Research Article



Phenolic content and antioxidant activity of wild and cultivated *Origanum compactum* (Benth) leaf extract

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ARTICLE INFO	ABSTRACT				
Article history: Received 20 November 2024 Revised 23 November 2024 Accepted 09 January 2025 Published online 01 April 2025	Medicinal and aromatic plants, as natural resources, are threatened by over-exploitation. Domestication is considered one of the approaches adopted to preserve these plants. The effectiveness of this approach depends on knowledge of the effect of domestication on the chemical composition and biological activities of the plants. The present work aimed to study the phenolic content and antioxidant capacities of wild and cultivated <i>Origanum compactum</i> , an endemic and overexploited Moroccan plant. It consists of monitoring phenolic compounds and				
Copyright: © 2025Salhi <i>et al.</i> This is an open-access article distributed under the terms of the <u>Creative</u> <u>Commons</u> Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.	endemic and overexploted Moleccan plant. It consists of infomoring plenome components and antioxidant activity in aqueous extracts obtained from the leaves of wild and cultivated plants. The plant was cultivated after acclimatisation using a vegetative technique. The polyphenol and flavonoid contents of the aqueous <i>Origanum compactum</i> extracts were monitored during two years of cultivation. Antioxidant activity, assessed by DPPH radical scavenging activity, ABTS radical scavenging assay, and Ferric Reducing Power Assay (FRAP), was also monitored. Results showed that polyphenol content was around 109.70±0.22 and 218.67±10.19 mgGAE/g dw, with the wild type showing the highest content, while cultivated plants showed a high flavonoid content (95.76±0.31 mgQE/g dw). Antioxidant activity revealed that both wild and cultivated plant extracts showed high activity. However, wild plant extracts were the most potent (IC ₅₀ = 11.16±0.19 µg/mL with DPPH, IC ₅₀ = 7.50±0.89 µg/mL with ABTS, and IC ₅₀ = 33.17±0.8 µg/mL with FRAP). In contrast, the antioxidant activity of the cultivated plant extracts, assessed over two years, remained unchanged or decreased significantly compared to the first year of cultivation. Domestication affects the chemical composition and, consequently, the biological activity of <i>Origanum compactum</i> .				

Keywords: Antioxidant activity, Origanum compactum, Phenolic compounds, Flavonoid content.

Introduction

Lamiaceae family, one of the most important plant families, contains about 365 genera and 6900 to 7200 species.¹ In Morocco, It is represented by 30 genera and 225 species.² According to the percentage of endemism, the Lamiaceae are in first place with 40%, while they are in 6th place in terms of specific richness.3Origanum compactum (Benth), commonly called Zaatar or Sahtar depending on the region, an endemic species of Morocco, is considered one of this family's most used medicinal species.⁴ It is used mainly in infusions or decoction to treat diabetes, high blood pressure,⁵ broncho-pulmonary diseases, gastric acidity, Genito-urinary pain, dermatological and gastrointestinal diseases.^{6,7} It is also used as a spasmolytic and sedative.⁸ Numerous scientific studies have been carried out to examine the range of activities of the Origanum compactum (Benth). However, the majority of them are focused on the study of essential oils with few studies on the organics extracts,9 and more particularly on the aqueous extracts, whereas aqueous extracts (infusion or decoction) are the most used in traditional medicine by the population.Considering the economic and industrial value of Origanum compactum (Benth), it has been identified as an endangered species due to overexploitation.

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The intense pressure of human activities, represented mainly by the deterioration of habitat, the destructive harvesting by uprooting or in the flowering stage before seeds germination, associated with the environmental factors (climatic change, drought) have induced a decline in the population of Origanum compactum (Benth) making it in a critical state.¹⁰ Faced with this situation and based on the evidence that the endemic, rare, or threatened flora constitutes the most important fraction of biodiversity, an additional effort must be undertaken to protect and preserve this plant. Various measures can be taken to conserve threatened species and ensure their sustainable use. Domestication is considered one of the approaches many countries adopt, given its advantages, especially in the industrial field.¹¹ The performance of this approach depends on the knowledge of the effect of domestication on the chemical composition and biological activities of plants. In this context, domestication of Origanum compactum may provide an additional source of this species, reducing pressure on the wild plant and potentially preventing its extinction. The present study aimed to investigate the phenolic composition and antioxidant activity of wild and cultivated Origanum compactum over one and two years, and to explore the influence of cultivation on the bioactive potential of Origanum compactum by comparing phytochemical content and antioxidant activity between wild and cultivated plants. Our results could be used to guide agricultural practices for the sustainable cultivation of Origanum compactum by highlighting the impact of domestication on its bioactive properties.

Material and Methods

Chemicals and Reagents

The chemicals and reagents used were of analytical grade. Methanol and DPPH (2,2-Diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (USA). ABTS (2,2-azinobis-3-ethylbenzothiazoline-6sulfonate), Gallic acid, Quercetin, and Folin-Ciocalteu reagent were obtained from Merck (Germany). Potassium persulphate was obtained from PanRec & AppliChem (Germany). Potassium acetate and Aluminium chloride were obtained from Solvapur. Potassium Ferricyanide was obtained from Farco Chemical Supplies (Netherlands).

Plant collection, identification and cultivation

The wild *Origanum compactum* (Benth)plant was collected in its natural habitat in the Ksar El Kebir region (North of Morocco) in March (2021) at an altitude of 134 m (34°06'03,11''N 03°56'35,94''W). After the taxonomic identification, a specimen was deposited in the herbarium of the Scientific Institute Rabat, Morocco, under the number RAB114273.

The plants were cultivated asexually after acclimatisation. The cultivation was carried out in a shade house. It was transplanted to experimental plots (National Agency of Medicinal and Aromatic Plants, Taounate, Morocco) to be grown on soil with periodic drip-irrigation and without input (without any treatment). After the cultivation, the plants were allowed to grow for two years. Crop and wild samples were collected yearly during the flowering season (spring). The plants were dried in the shade and under the temperature of the laboratory. The leaves were ground into a fine powder using an electric grinder.

Preparation of aqueous extracts

Extraction was performed using three traditional methods: maceration, infusion, and decoction. The decoction extract was obtained by boiling 30 g of the plant leaf powder in 300 mL of distilled water for 15 min. The maceration extract was obtained using 30g of the plant leaf powder in 300 mL of cold distilled water under low agitation for 24 hours. The infusion extract was prepared by mixing 30g of plant leaf powder with 300 mL of boiling distilled water, and the mixture was left to infuse for 15 min at room temperature.¹² All the extracts were filtered with Whatman paper No. 1 filter paper, and the filtrates obtained were dried using a rotary evaporator at 40°C. Dry extracts were recovered and stored at 4°C in a refrigerator.

Total polyphenolic content determination

The total phenolic content in the plant samples was measured using the Folin-Ciocalteu reagent method.¹³ Tested extracts were prepared at suitable concentrations, 100 μ L of each extract was mixed with 500 μ L of Folin–Ciocalteu reagent (diluted 10 times with deionized water). 400 μ L of aqueous sodium carbonate solution (7.5% w/v) was added. The mixture was incubated for 60 min at room temperature, and then the absorbance was measured at 765 nm. The standard calibration curve was constructed using Gallic acid, and total phenol contents were expressed as milligrams of gallic acid equivalents per gram of dry residue (mg GAE/g extract).

Flavonoid content determination

The extracts' total flavonoid content was determined using the AlCl₃ method.¹² 1.5 mL of methanol was added to 500 μ L of extracts at suitable concentrations and mixed. Then, 100 μ L of 10% aluminum chloride, 100 μ L of 1 M potassium acetate, and 2.8 mL of distilled water were added to the mixture to reach a total volume of 5 mL. Test tubes were incubated in the dark for 30 min at room temperature and the absorbance was measured at 415 nm. A standard curve of quercetin solution was constructed, and the results were expressed as milligrams of quercetin equivalents per gram of dry residue (mgQE/g extract)

Antioxidant Activity

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was evaluated using 1,1-diphenyl-2- picrylhydrazyl (DPPH).¹⁴ The reaction mixture contained 0.5 mL of sample extracts at different concentrations (5-140 μ g/mL) and 3 mL of a freshly DPPH (0.1 mM) methanolic solution. The result mixtures were incubated for 20 min in the dark and at room temperature. Then, the absorbance was measured at 517 nm (UV-1601 Spectrophotometer, Shimadzu Corporation, Japan). DPPH radical scavenging activity of each sample was then calculated as percent inhibition according to the following Equation (1):

$$PI(\%) = \frac{A (blank) - A (sample)}{A(blank)} \times 100$$
(Eq.1)

Where A (blank) is the absorbance of the control reaction (without plant extract) and A (sample) is the absorbance of the extract or standard. Ascorbic acid was used as positive control under the same test conditions. Concentrations providing 50% inhibition (IC_{50}) were calculated by plotting inhibition percentages against concentrations of the extracts.

ABTS radical scavenging assay

ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical scavenging assay was determined as previously described with slight modifications.¹⁵ The ABTS radical cations (ABTS++) were generated by reacting 7mM of the aqueous solution of ABTS with 2.45 mM of the aqueous solution of potassium persulphate. The obtained solution was allowed for 12-16 hours in the dark at room temperature before use. Then it was diluted with deoneside water to obtain an absorbance of 0.700 \pm 0.005 at 734 nm. Extracts were prepared at different concentrations (2-20 µg/mL), 10 µL of each concentration was added to 990 µL of ABTS++ solution and incubated for 6 min at room temperature. Then the absorbance was measured at 734 nm. Inhibition percentages of ABTS++ radical were calculated as follows:

$$PI(\%) = \frac{A (blank) - A (sample)}{A (blank)} \times 100$$
(Eq.2)

Where A (blank) is the absorbance of the control reaction, and A (sample) is the absorbance of the extract or standard (Ascorbic acid). The results were expressed as IC_{50} , the concentration providing the scavenging of 50% of ABTS++ radicals.

Ferric-reducing power assay

This method measures the reducing power of antioxidants in a mixture by their ability to reduce Fe^{3+} to Fe^{2+} . The reducing power of plant extracts was determined by a slightly modified previous method.¹⁶ Different concentrations (20-200 µg/µL) were prepared in distilled water. 0.5 mL of each dilution was mixed with 2.5 mL of phosphate buffer pH=6.6 and 2.5 mL of potassium ferricyanide solution (1%). The mixture was then incubated at 50 °C for 20 min in a water bath. 2.5 mL of (10%) trichloroacetic acid was added to stop the chemical reaction. The mixture was centrifuged for 10 min at 3000 rpm. Then, 2.5 mL of the upper layer was mixed with an equal volume of distilled water and 0.5 ml of (1%) ferric chloride solution. The absorbance was measured at 700 nm. The blank was prepared using the same procedure: replacing the extract with the phosphate buffer. Increased reducing power was assessed by an increase in the absorbance of the mixture.

Statistical analysis

The Data analysis was performed using GraphPad Prism 8 (GraphPad Software, 8, 2019). All the tests were performed in triplicate, and the results were expressed as the Mean+SD. The differences between cultivated and wild plants were compared using the One-way ANOVA test, followed by Tukey's test. A p-value less than 0.05 was considered significant. A correlation study was performed using XLstat (Addinsoft, 2016).

Results and Discussion

Many previous studies have analysed spontaneous *Origanum compactum*'s chemical composition and antioxidant activity, particularly its essential oil and organic extracts.^{9,17,18} However, there is a lack of data concerning the cultivated plant, the only study found in the literature compared the chemical composition and the antibacterial activity of essential oils of this species.¹⁹ Furthermore, this study is the first to compare the phenolic composition and antioxidant activity of aqueous extracts of *Origanum compactum* from wild and the cultivated plants for two consecutive years. Table 1 shows the total phenolic content of the different extracts of the wild and cultivated plants.The compound contents varied greatly in the three extracts from wild and cultivated plants. The values of polyphenols in the extracts obtained by decoction were around 114.87±1.66 and 216.89±9.41 mgGAE/g dw. The wild plant showed the highest content (P < 0.05), while the cultivated plant for the first year showed the lowest value.

		Phenolic conter	nt (mg GAE/g dw)		Flavonoid con	tent (mgQE/g dv	v)
Plant		Decoction	Infusion	Maceration	Decoction	Infusion	Maceration
Wild		216.89±9.41ª	218.67±10.19 ^a	178.04±6.63ª	54.02±1.44 ^b	54.28±2.14 ^b	47.84±1.15 ^t
Cultivated one year	for	114.87±1.66°	139.84±0.58 ^b	110.29±1.98 ^b	93.69±0.43ª	93.77±0.14ª	84.78±0.81 ²
Cultivated two years	for	144.94±0.22 ^b	127.36±0.25°	109.70±0.22 ^b	93.03±0.07 ^a	95.76±0.31ª	85.44±0.25 ^a

Data in the same column having different lower cases indicates a significant difference (p < 0.05) using an ANOVA test. Results are ranked in ascending order: a > b > c. dw: dry weight; mgGAE/g dw: mg gallic acid equivalents/g dry weight; mgQE/g dw: milligrams of quercetin equivalents per gram of dry weight. Each value is represented as mean \pm SEM (n = 3).

The extracts obtained by infusion vary from 127.36±0.25 to 218.67±10.19 mg GAE/g dw. The highest value was always obtained in the wild plant extract with significantly decreased values in cultivated plants. The extract obtained by maceration showed lower values, with a high content of polyphenols (178.04±6.63 mg GAE/g dw), particularly observed in the wild plant. A previous study reported that the total phenolic content of leaves of wild Origanum compactum (Benth) obtained by decoction was 125.4±3.2 g AGE/Kg dw, which was lower than the results obtained in this study.²⁰ However, the phenolic content of ethanol, petroleum ether, and ethylacetate extract ranged from 106.9±3.1, 707.8±13.4, and 355.2±6.4 g AGE/Kg dry weight. Maceration of the flowering tops of Origanum compactum (Benth) using ethanol, methanol, and hexane as solvents showed a phenolic amount ranging from 153.27 \pm 0.68 to 105.54 \pm 0.35 mg EAG/g extract. These results showed the impact of the extraction solvents on the total phenolic contents.²¹ The harvest period and storage time before use may also be included. The polyphenolic constituents of wild plant extracts of Origanum compactum (Benth) were higher than those of the cultivated plant. Cultivation conditions may explain the concentration differences of bioactive components between the wild and cultivated plants. The variations observed may be due to environmental conditions such as temperature, UV radiation, humidity, precipitation, and nutrients in the soil that influence the plant's synthesis of secondary metabolites.²² A previous study on Origanum compactum essential oil, collected from different areas revealed that essential oil did not preserve the major constituents between the wild and domesticated plants.¹⁹ This variation is significant according to the regions studied and within the same region between the wild and domesticated plants. Besides genetic factors, environmental factors also played an essential role in this chemical polymorphism.

The results of total flavonoid contents in the different extracts showed a significant difference between wild and cultivated plants. The cultivated plant samples had significantly (P < 0.05) higher flavonoid contents than the wild plant samples, ranging between 47.84 ± 1.15 and 54.28 ± 2.14 mg QE/g dw. However, flavonoid content showed no significant difference between cultivated plants over two years. They were between 84.78 \pm 0.81 and 95.76 \pm 0.31 mg QE/g dw. The flavonoid content of wild Origanum compactum extract obtained by decoction reported by El Babili et al. was 52.9±1.6 g QE/Kg dw, similar to this study.²⁰ Another study showed a comparative study of wild and cultivated Euphorbiasupina, in which cultivated plants showed higher levels of flavonoids and derivatives, which may play directly as protection molecules against stress or as precursors for the synthesising other compounds.23 Generally, decoction and infusion were the most effective methods to extract polyphenolic and flavonoid components, and in most cases, they do not show a significant difference.

Oxidative processes are very complex and depend on the components and the various nature of the antioxidants. Therefore, there is no standard method to measure antioxidant activity in an exact way quantitatively. For this reason, it is necessary to combine different and complementary methods to obtain an indication of the antioxidant capacity of the test sample. The antioxidant activity of aqueous extracts of wild and cultivated *Origanum compactum* leaves was evaluated using three complementary methods : DPPH, ABTS, and FRAP.

1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical used to assess the radical-scavenging activity of antioxidants. In this method, the antioxidant donates hydrogen to reduce the stable DPPH radical to a non-radical diphenyl picrylhydrazine (DPPH-H), decreasing its absorbance. The extent of the reduction of DPPH absorbance measures the antioxidant capacity of the antioxidant tested.²⁴ All extracts of both plants showed a dose-dependent DPPH radical scavenging (Figure 1).

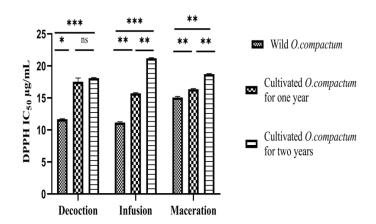


Figure 1: Antioxidant activity of wild, Cultivated *Origanum compactum* for one year, and cultivated *Origanum compactum* for two years, using different extraction methods. IC₅₀: concentration providing 50% inhibition of radicals. DPPH: 1,1-diphenyl-2- picrylhydrazyl free radical scavenging activity. Data are presented as mean±SD. * indicates P < 0.05, **indicates P < 0.01, *** indicates P < 0.001.

A low IC₅₀ value represents high activity, while a high IC₅₀ value indicates low antioxidant activity. The IC₅₀ values of the extracts obtained from leaves of wild, cultivated during one year, and cultivated plants for two years were respectively 11.66±0.14, 17.52±1.02, and 18.09±0.13 μ g/mL in the extract obtained by decoction. These values were respectively 11.16±0.19, 15.72±0.15, and 21.19±0.15 μ g/mL in the extracts obtained by infusion. In contrast, these values were respectively 15.18±0.41, 16.37±0.14, and 18.71±0.16 μ g/mL in the extract obtained by maceration. IC₅₀ values of all the extracts were

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significantly higher than ascorbic acid (IC₅₀= $3.30 \pm 0.46 \,\mu$ g/mL). The activity varied markedly between plants, it decreased significantly (P<0.05) according to the years of cultivation. The wild plant showed the most potent radical scavenging activity among the three types of extracts. Our results agree with El Babili *et al.* who reported that *Origanum compactum* organic extracts show high antioxidant activity, ²⁰ decoction was the most potent scavenging radical with an IC₅₀ of 4.8 \pm 0.2 mg/L. While others have reported that n-hexane extract exhibited the highest antiradical activity (IC₅₀ = 39.83 μ g/mL).^{9,18} However, EtOH and ethyl acetate extract expressed the lowest antiradical activity.

ABTS radical scavenging assay showed the same results as the DPPH assay, where all extracts displayed significant radical scavenging potential (Figure 2). The highest activity was obtained by aqueous extracts of wild plants, showing a lower value of IC50 than cultivated plants (p<0.05). Decoction and infusion extracts showed no significant difference between IC50 values of 7.50±0.89 and 7.56±0.30 µg/mL, respectively. The extract obtained by maceration exhibited the lowest activity (10.14±0.39 $\mu\text{g/mL}).$ There was no statistically significant change (p>0.05) between cultivated Origanum compactum for one year and two years from the aqueous leaf extracts obtained by decoction and infusion. El Babili et al.found that the IC50 value of the decoction extract was about 7.9 \pm 0.3 mg/L, which was similar to our results.²⁰ Ethylacetate was the most potent for organic extracts, and ethanol extract was the lowest. Martins et al.also showed that infusion and decoction extracts exhibited similar reducing power and free radical scavenging activity, but decoction exhibited the highest activity in other antioxidant assays.²⁵ In addition, Hmidani et al. also reported that decoction is the most efficient method either in extracting phenolic compounds or antioxidant activity.27

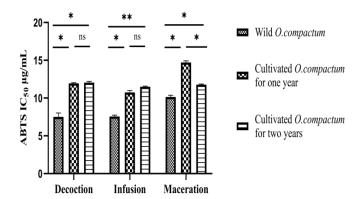


Figure 2: Antioxidant activity of wild, Cultivated *Origanum compactum* for one year, and cultivated *Origanum compactum* for two years, using different extraction methods. IC₅₀: concentration providing 50% inhibition of radicals. ABTS: 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging assay. Data are presented as mean±SD. * indicates P < 0.05, **indicates P < 0.01.

The effectiveness of the wild plant extracts was also confirmed by the FRAP test (Figure 3), showing a strong capacity to chelate ferric ions compared to the cultivated plant extracts, where the antioxidant activity decreases significantly from one year to the next. Infusion, in this case, was found to be a more potent extraction method that exhibited the highest antioxidant activity. IC_{50} values were found to be respectively about 33.17±0.8, 62.99±1.58, and 61.31±1.03 µg/mL in the wild, cultivated plant during one and cultivated plant during two years. Many studies conducted on different plant species have also revealed that wild plants are more powerful than cultivated plants. *Foeniculum vulgare* essential oil extracted from the wild plant had the highest content of phenolic compounds and the most potent antioxidant activity has been shown by several studies. This activity is mainly due to their redox

properties, which allow them the ability to reduce free radicals by donating hydrogen atoms and chelate metals.²

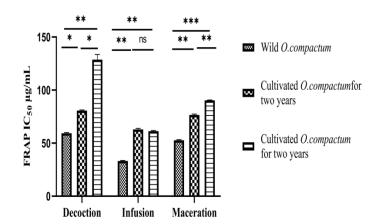


Figure 3: Antioxidant activity of wild, Cultivated *Origanum compactum* for one year, and cultivated *Origanum compactum* for two years, using different extraction methods. IC₅₀: concentration providing 50% inhibition of radicals. FRAP: Ferric reducing power assay. Data are presented as mean±SD. * indicates P < 0.05, **indicates P < 0.01, *** indicates P < 0.001.

Phenolics and flavonoids have been reported as the main types of phenolic compounds present in oregano. They are characterised by the presence of at least one aromatic ring with one or more hydroxyl groups attached which allows them antioxidant capabilities. Rosmarinic acid, apigenin, luteolin, quercetin, scutellarein, and their derivatives have been identified as the major phenolic acids and flavonoids in oregano species.²⁹ These secondary metabolites are used by plants as defense molecules under challenging conditions influenced by numerous environmental factors. Jordán et al. have reported that watering levels can affect the yield,30 chemical compositions, and biological activities of cultivated Thymus zygis. In this study, the differences observed between cultivated and wild plants may be due to cultivation conditions, which include irrigation, humidity, control of weeds, and soil nutrients. Considering the difference in the duration of cultivation, it appears that the plant adapts gradually to the conditions. Mangoale and Afolayan have shown that wild Alepidea amatymbica presents more phenolic components and antioxidant activities in most of the assessed trials.31

Correlation analysis of data showed significant relationships between phenolic compounds and antioxidant activity (Table 2). Polyphenols present a significant negative correlation with DPPH (r=0.846) and ABTS (r=0.911), which means that high concentrations of polyphenols are accompanied by high antioxidant activities because of the ability of phenolics to reduce DPPH and ABTS radicals by Hydrogen transfer. However, they present a non-significant correlation with FRAP (r=0.557). This may be due to the difference in its antioxidant process, which is based on transferring electrons to reduce Fe³⁺ to Fe^{2+,32} Flavonoids positively correlate with DPPH (r=0.709) and ABTS (r=0.709). In turn, DPPH and ABTS correlate positively, indicating a similar antioxidant process. Thus, phenolic components in aqueous leaf extracts of *Origanum compactum* (Benth)exhibited antioxidant activity by reducing radicals via hydrogen transfer.

Conclusion

The results of the present study revealed that domestication affects the phenolic content and antioxidant activity of *Origanum compactum* (Benth) aqueous extracts. This technique can potentially preserve *Origanum compactum* from extinction due to overexploitation, but further studies are needed to optimise cultivation conditions to improve its polyphenol content and antioxidant activity. Research into the

Table 2:Correlation coefficients between phenolic content, flavonoid content, and antioxidant activity of wild and cultivated *Origanum* compactum.

Variables	Polyphenols	Flavonoids	DPPH	ABTS	FRAP
Polyphenols	1				
Flavonoids	-0.8435**	1			
DPPH	-0.8462**	0.7920*	1		
ABTS	-0.9113**	0.7092*	0.7211*	1	
FRAP	-0.5570 ^{ns}	0.6086 ^{ns}	0.5548 ^{ns}	0.5882 ^{ns}	1

DPPH (IC50): DPPH radical scavenging activity expressed as IC₅₀; ABTS: 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging assay; FRAP: Ferric reducing power assay. ns indicate non-significant correlation p > 0.05, *indicate significant correlation at p < 0.01.

impact of different domestication times and environmental factors on the plant's chemical composition is crucial to improving its quality.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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