International Food Research Journal 31(6): 1494 - 1506 (December 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Phytochemical identification and antioxidant activities of wild Algerian plant *Atriplex halimus* L.

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Article history

Received:
1 August 2023
Received in revised form:
15 October 2024
Accepted:
16 October 2024

Keywords

Atriplex halimus L., phenolic compounds, flavonoid compounds, antioxidant activity

Abstract

The secondary metabolites of cultivated and wild plants offer numerous advantages for foods, medicines, and cosmetics. In this context, an evaluation to investigate the impact of plant parts and plant origins on the biological capacities of *Atriplex halimus* L. were conducted, with the aim of exploring its potential as a natural alternative for antioxidants. In the present work, phenolics and flavonoids from the aerial parts (leaves and stems) of *A. halimus* of three provinces (Mazagran, Biskra, and Sig) in Algeria were quantified and identified using spectrophotometry and chromatography (HPLC-DAD). Complementary assays such as DPPH, ABTS, and FRAP were performed to assess the antioxidant capacities. Results exhibited greater quantities of phenolics in leaf extracts. Significant variation was found between plant origins; leaf extract from Mazagran was richer in TPC and TFC. Qualitative analysis revealed 25 phenolic compounds, including five phenolic acids (four hydroxybenzoic acid and one hydroxycinamic acid) and 20 flavonoids from the leaf extracts. Leaf extracts also exhibited higher antioxidant powers than stem extracts. Generally, antioxidant tests demonstrated higher antiradical capacity of leaf extract from Mazagran. Therefore, *A. halimus* from Mazagran can be used as a source of natural antioxidant, and in different fields, particularly medicines and foods.

DOI
https://doi.org/10.47836/ifrj.31.6.10

Introduction

Biological active compounds of higher plants have become the main subject of scientific research. Root, stem, leaf, flower, or any other plant part can be used as a source of medicines due to their ability to combat many diseases (Huynh *et al*., 2022).

Outdoor cultivated plants from marginal and saline lands have drawn much attention for their potentials in phytotherapeutic applications (Ramakrishna *et al.*, 2020), and a strong economic potentials for nutrition and animal feed (Belal *et al.*, 2016), due to their potent bioactivity and relatively little toxicity (Kontogianni *et al.*, 2013).

In Algeria, researchers have started to promote plant species, including halophyte species (xero- and hydro-halophytes). These species, persisting to harsh environmental conditions such as instable salinity, temperatures, light, nutrient, and water (Medini and Ksouri, 2018), have been used as coal, human food, animal feed, medicines, and chemicals (Qasim *et al.*, 2011).

Atriplex halimus is one of the 100 *Atriplex* species that exist (Lamchouri *et al*., 2012); this xero-halophyte and perennial shrubs, commonly known as "Guettaf", is characterised by various biological and agronomic properties (Gattouche *et al*., 2020). It is used as livestock forage, as it could provide essential nutrients and innumerable therapeutic applications (Ounaissia *et al.*, 2020).

In this context, the present work focussed on the quantitative characterisation of both total phenolics and total flavonoids in the hydro-ethanolic extract of two aerial parts (leaves and stems) of *A. halimus* from three Algerian provinces (Mazagran, Biskra, and Sig) by colorimetric methods, followed by phytochemical screening by chromatographic method (HPLC-DAD). In addition, *in vitro*

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evaluation of the antioxidant activities was estimated by radical scavenging by DPPH, ABTS, and FRAP assays. The correlation between bioactive compounds and antioxidant activities of *A. halimus* was also studied. Finally, the evaluation of the antioxidant activities of the six hydro-ethanolic extracts was done in order to select the most promising plant part and plant origin. The lack of information of the chemical and/or biological properties of *A. halimus* from Algeria was the driving force of the present work.

Materials and methods

Plant material

The aerial parts (leaves and stems) of *A. halimus* were collected at random from various Algerian localities: Sig (35°31'14'30″N 0°10'09'97″W, 144 m altitude), Mazagran (35°53'17'19″N 0°03'14'76″E, 58 m altitude), and Biskra (34°55'42″N 5°38'58″E, 198 m altitude), in October 2022. The plant was identified by Prof. Brahim Lotmani. A voucher specimen was deposited under number MA 23/1 at the herbarium of the University of Mostagnem, Algeria. Then, the samples were dried separately (T° ≤ 40°C) until weight stabilisation, and preserved for further research.

Extract preparation

Atriplex halimus leaves and stems (100 g) were combined with 70% (v/v) aqueous ethanol, and left at room temperature for 24 h (Bourgou *et al*., 2016). Macerates were homogenised, decanted, and filtered through Whatman filter paper no.1 following three extractions. Using a rotating evaporator, the extracted was concentrated at 40°C under vacuum (Buchi Rotavapor R210). Three successive washes with petroleum ether (v/v) were carried out in order to eliminate all non-phenolic compounds (carotenoids, chlorophyll pigments, and fats) (Kebièche *et al*., 2011). The resulting aqueous extracts were lyophilised and stored at -20°C while being analysed.

Yield determination

Yields of ethanolic extracts were represented as a percentage of obtained extract weight relative to sample dried matter, using Eq. 1 (Drosoua *et al*., 2015):

$$\text{Yield \%} = (\text{mass of extract} / \text{mass of dry matter}) \times 100 \quad \text{(Eq. 1)}$$

Bioactive compound quantification

Total phenolic contents (TPC)

Total phenolic contents were determined spectrophotometrically according to Bouzouina *et al*. (2016). Briefly, 1 mL of ethanolic extract was mixed with 0.5 mL of Folin-Ciocalteu's reagent (2 M, diluted ten times) and 0.4 mL of sodium bicarbonate 7.5% ($NaHCO_3$), and incubated for 1 h in the dark at room temperature. Finally, absorbance was determined using spectrophotometer at λ = 765 nm. The same protocol was applied for the standard solution of gallic acid (0 - 100 µg/mL), and calibration curve was constructed. The extracts' TPC was expressed in terms of gallic acid equivalent per gram of lyophilised extract (mg GAE/g LE).

Total flavonoid contents (TFC)

Total flavonoid contents were determined colorimetrically. Reaction mixture was prepared by mixing 0.5 mL of quercetin (0 - 200 µg/mL) or ethanolic extracts, with 0.5 mL of aluminium trichloride ($AlCl_3$) at 2%. Absorbances were then measured at 430 nm against the blank after 10 min of incubation at room temperature (Ahn *et al.*, 2007). Results were expressed as microgram of quercetin equivalent per gram of lyophilised extract (µg QE/g LE).

Qualitative analysis of bioactive molecules by HPLC-DAD

The qualitative analysis of phenolic compounds present in the extracts was carried out by high performance reverse phase liquid chromatography system, coupled to a diode array detector (HPLC-DAD), Shimadzu LC-2030C 3D), using RP C_{18} column (110Å, 150 × 2 mm, ID) and 5 µm porosity. Compounds were separated based on the elution gradient A of water/acetic acid (solvent A), which were both acidified with 0.075% formic acid (Gourguillon *et al*., 2016) in order to avoid the ionisation of many hydroxyl groups of phenolic compounds during analysis, and at the same time improved the resolution and reproducibility (Stalikas, 2007), and the methanol/acetic acid mixture formed the solvent B. They were eluted during the first 5 min in the following gradient: 2% of solvent B, then a linear gradient of B from 2 to 100% over the next 69 min, with an elution rate of 0.8 mL/min (Buer *et al*., 2007), and an injection volume of 5 µL. Detection was performed using DAD detector (190 - 900 nm).

For all peaks, spectral data were collected in the range of 200 - 800 nm. The peak areas of extracts were measured at 280 nm, while their identification was made by comparing the retention times and UV spectra of peaks compared to those in the literature.

Antioxidant activities of A. halimus

DPPH assay

The antioxidant activities of *A. halimus* leaf and stem extracts were determined *in vitro* by scavenging the free 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Following the method of Jahromi *et al*. (2014) with slight revision, 5 mL of each extract concentration (20 - 100 mg/mL) were homogenised with 5 mL of methanolic DPPH solution 0.004% (m/v). The reaction mixture was well shaken and incubated for 30 min at room temperature in the dark. Absorbance was measured at 517 nm to determine the percentage of inhibition. Trolox at different concentrations (200 - 1,000 µg/mL) was used as a positive control, and to construct the calibration curve. Results were expressed as µg TE/mL of ethanolic extracts. The inhibition of DPPH radical (I%) was calculated using Eq. 2:

DPPH scavenging activity (%) = (Ablank – Asample / Ablank) × 100 (Eq. 2)

where, Ablank = absorbance of blank; and Asample = absorbance of sample.

The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

ABTS assay

ABTS test was performed following the method developed by El-Hallouty *et al*. (2020). Briefly, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) 7 mM and 2.45 mM of potassium persulphate ($K_2S_2O_8$) were mixed to stock solution, and incubated in the dark for 12 - 16 h. Previous solution was diluted by mixing 1 mL of $ABTS^{++}$ solution with 60 mL of ethanol to obtain an absorbance of 0.700 ± 0.05 units at 734 nm using a spectrophotometer. The reaction mixture comprised 0.9 mL $ABTS^{++}$ and 0.1 mL of extracts (20 - 100 mg/mL). After 15 min, absorbance was read at 734 nm. Trolox was used as standard (10 - 100 µmol/mL), and to construct the calibration curve. The inhibition of ABTS radical (I %) was determined using Eq. 3:

$ABTS^{+}$-scavenging activity (%) = ABTS (Ac – At)/Ac) × 100 (Eq. 3)

where, At and Ac = absorbance of tested samples and $ABTS^{+}$, respectively, and was expressed as µmol Trolox equivalents (TE) per gram of lyophilised extract. The concentration necessary for 50% reduction of ABTS was expressed as IC_{50} (TE µmol/mL).

FRAP assay

The method of Prahadeesh *et al.* (2018) with some modifications was used for evaluating the ferric ion reducing power of samples. Briefly, 0.1 mL of each tested extract (0 - 50 mg/mL) was added to 3.0 mL of freshly prepared reagent (FRAP) with a solution mixture of 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM) and ferric chloride (20 mM) in acetate buffer (pH = 3.6) (1/1/10, respectively). Absorbance was read at 593 nm after 30 min of incubation in the dark at 37°C. The ability of the sample to donate electrons was measured by the colour change related to the complex formation (Fe^{2+} TPTZ). Trolox (0 - 300 µmol/mL) was used as reference molecule, and tested samples were expressed as TE µmol/mL.

Statistical analysis

All tests were performed in triplicate, and expressed as mean ± SD. Statistically significant data were compared using ANOVA and Student's *t*-test, at the significance level $p < 0.05$. Pearson's correlation coefficient was used to detect relationships between parameters.

Results

Yields of extracts, total phenolic contents, and total flavonoid contents

Yields

The yields of lyophilised crude samples of *A. halimus* are summarised in Figure 1. The percentages of extractable compounds varied from 4.91 to 17.47%. Generally, extraction yield of leaves was higher compared to stems. Mazagran leaves showed the highest yield (17.44%), followed by Biskra leaves (13.78%), and Sig leaves (11.47%). For stems, Biskra yielded 10.23%, Sig yielded 7.27%, and Mazagran yielded 4.91%.

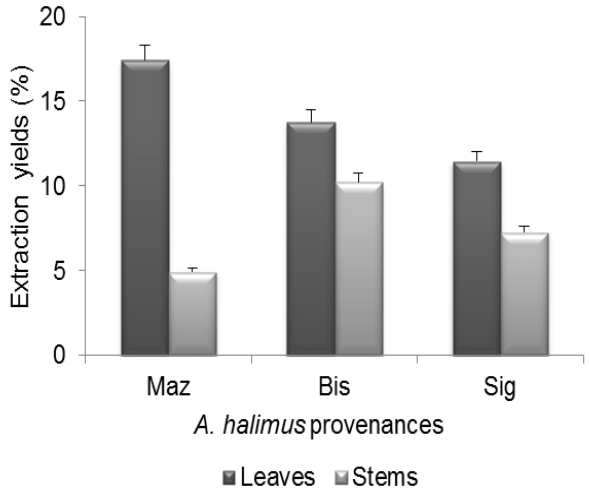

Figure 1. Yields (%) of dry leaf and stem extracts of *A. halimus* from three Algerian provinces of origin; Mazagran, Biskra, and Sig.

Total phenolic contents and total flavonoid contents

The TPC of *A. halimus* aerial parts differed significantly (Figure 2A). Leaves seem to be richer than stems ($F_{1,2}$ = 90.565, p = 0.). Mazagran leaves showed the highest concentration (45.46 ± 0.8 mg GAE/g LE) followed by Biskra leaves (35.31 ± 0.9 mg GAE/g LE), and Sig leaves (23.66 mg GAE/g LE). For stems, Biskra (29.79 ± 0.6 mg GAE/g LE) and Sig (31.65 ± 0.36 mg GAE/g LE) were lower than Mazagran (33.51 ± 1.36 mg GAE/g LE).

Figure 2B shows significant differences between *A. halimus* aerial parts' TFC ($F_{1.2}$ = 483.909, p = 0.). Higher amounts in Mazagran leaves (11.29 ± 0.05 mg EQ/g LE) and Biskra leaves (6.69 ± 0.5 mg EQ/g LE) were recorded. However, stems from the different harvesting regions showed lower concentrations of TFC which did not exceed 2.36 ± 0.01 mg EQ/g LE.

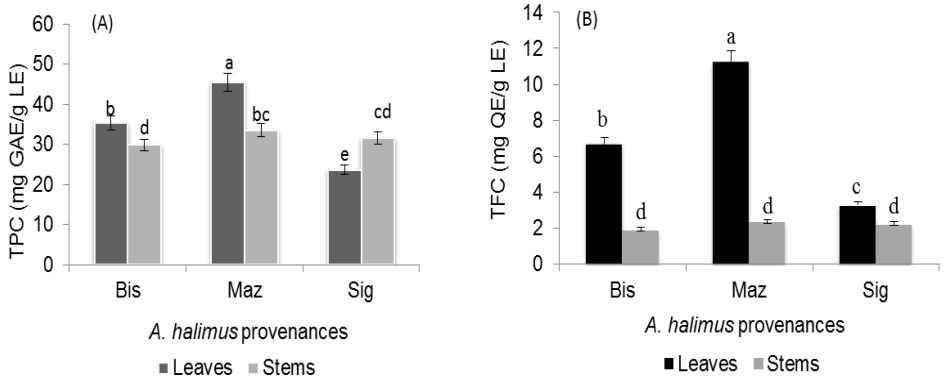

Figure 2. Total phenolics, mg EAG/g LE **(A)** and total flavonoids, mg QE/g LE **(B)** in leaf and stem extracts of *A. halimus* from three Algerian provinces of origin; Mazagran, Biskra, and Sig.

Antioxidant activities of A. halimus

DPPH assay

Table 1 shows the radical scavenging powers of *A. halimus* leaf and stem extracts using DPPH assay. All extracts showed dose-dependent activities against DPPH radical. At concentrations of 80 and 100 mg/mL, leaf extracts of Biskra and Mazagran revealed maximum radical scavenging power of 85.77 and 74.76% inhibition, followed by moderate activities recorded for Sig leaves (55.64%), Mazagran stems (52.31%), Biskra stems (51.75%), and Sig stems (41.17%). In addition, IC_{50} of Biskra stems and Mazagran leaves showed scavenging capacities similar to the Trolox activity of 623.684 ± 3.90 and 654.165 ± 1.32 µg TE/mL, respectively. The scavenging capacity of Sig stems, however, was less than 50% (Figure 3a).

ABTS assay

The evaluation of the radical cation scavenging ability by ABTS test revealed a dose-dependent antioxidant capacity, where the ethanolic extracts of both aerial parts of *A. halimus* zones noted inhibition rates ranging from 64.49 to 88.23 ± 0.14% at maximum concentrations of 15 and 20 mg/mL (Table 1). However, at minimum concentration (10 mg/mL), remarkable inhibitory power of Biskra leaves (73.95 ± 0.50%) was detected. Significant differences between tested extracts and standard (Trolox) were observed (F = 9.248; p = 0). Extracts of Biskra leaves (355.94 ± 1.59 µg TE/mL), Mazagran leaves (356.58 ± 3.20µg TE/mL), and Sig leaves (349.75 ± 10.00 µg TE/mL) and stems (358.96 ± 0.34 µg TE/mL) showed comparable activities to that of Trolox (IC_{50} = 338.60 ± 0.13 µg/mL). Meanwhile, stems of Biskra (391.56

Table 1. DPPH, ABTS scavenging activities, and reducing ability by FRAP assay of hydro-ethanolic extracts.

Antioxidant test	Concentration (mg/mL)	Leaf			Stem		
		Sig	Biskra	Mazagran	Sig	Biskra	Mazagran
DPPH (%)	20	04.60 ± 0.16	25.16 ± 0.28	22.03 ± 00	9.26 ± 0.55	19.26 ± 0.39	13.74 ± 2.65
	40	17.50 ± 0.48	43.57 ± 0.09	37.04 ± 0.00	13.67 ± 2.00	31.12 ± 2.24	28.53 ± 1.16
	60	27.67 ± 0.53	58.07 ± 3.64	53.70 ± 0.00	23.16 ± 7.04	39.87 ± 1.91	44.15 ± 0.00
	80	35.00 ± 0.00	72.75 ± 0.00	64.26 ± 2.52	31.54 ± 2.84	48.04 ± 0.68	45.52 ± 1.06
	100	55.647 ± 2.64	85.77 ± 4.01	74.76 ± 0.00	41.17 ± 2.10	51.75 ± 0.05	52.31 ± 0.86
ABTS (%)	5	30.08 ± 0.15	39.88 ± 0.28	34.34 ± 0.85	28.70 ± 0.22	22.84 ± 1.41	21.48 ± 1.84
	10	33.66 ± 0.37	73.95 ± 0.50	52.05 ± 0.07	41.74 ± 0.22	42.85 ± 2.53	58.77 ± 0.75
	15	75.35 ± 1.10	81.593 ± 0.43	69.61 ± 1.22	64.49 ± 00	67.30 ± 0.30	78.08 ± 0.30
	20	80.26 ± 0.80	88.23 ± 0.14	75.91 ± 0.50	79.61 ± 0.07	76.66 ± 0.08	86.22 ± 0.15
FRAP (Absorbance at 593 nm)	10	0.386 ± 00	0.260 ± 00	0.103 ± 00	0.420 ± 00	0.248 ± 00	0.150 ± 00
	20	0.541 ± 00	0.413 ± 00	0.205 ± 00	0.538 ± 00	0.439 ± 00	0.439 ± 00
	30	0.714 ± 00	0.532 ± 00	0.233 ± 00	0.657 ± 00	0.559 ± 00	0.347 ± 00
	40	0.755 ± 00	0.647 ± 00	0.333 ± 00	0.871 ± 00	0.874 ± 00	0.448 ± 00
	50	0.803 ± 00	0.693 ± 00	0.500 ± 00	1.001 ± 00	1.107 ± 00	0.602 ± 00

Data are mean ± SD.

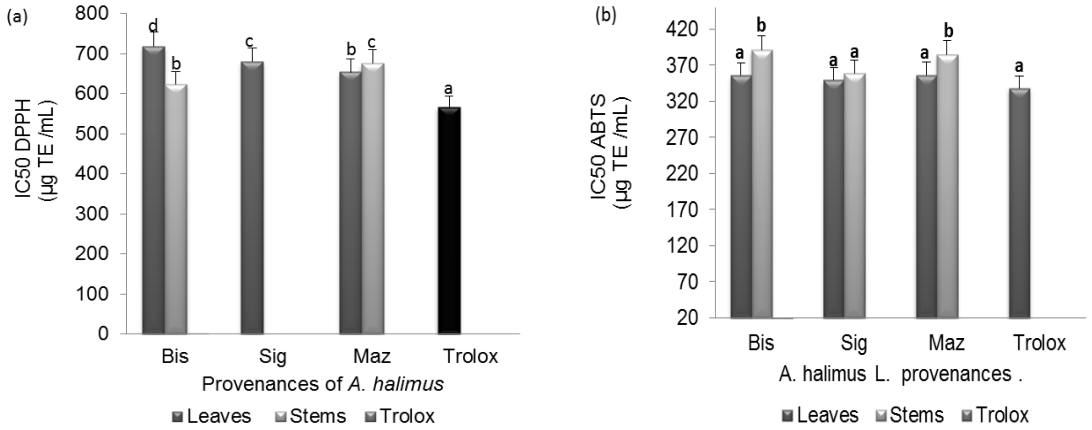

Figure 3. Antioxidant activities by DPPH **(a)** and ABTS **(b)** in leaf and stem extracts of *A. halimus* from three Algerian provinces of origin; Mazagran, Biskra, and Sig, expressed as IC_{50} (µgTE/mL).

± 26.30 µg TE/mL) and Mazagran (385.22 ± 4.75 µg TE/mL) showed less antioxidant potentials than standard (338.60 ± 0.13 µg/mL) (Figure 3b).

FRAP assay

Figure 4 shows that the reducing power of ferric ion exhibited variation based on the plant part and plant origin of *A. halimus*, and results were dose-dependent. Results indicated that among the three studied ecotypes, the leaf and stem extracts of Sig exhibited lower potency at a dose of 50 mg/mL with corresponding absorbance densities of 0.5 = 312.33 ± 3.22 and 0.602 = 376.042 ± 1.61 µmol TE/mL, respectively. However, significant reducing capacity was observed in stem extracts of Mazagran (1.00 equivalents to 625.62 ± 0.96 µmol TE/mL) and Biskra (1.1 equivalent to 612.037 ± 2.29 µmol TE/mL), followed by leaf extracts in order of Mazagran > Biskra (0.803 = 502.08 ± 3.22 and 0.693 = 433.33 ± 4.4 µmol TE/mL, respectively). Furthermore, Trolox showed higher reducing power compared to the extracts, with an absorbance value of 0.5 at a concentration of 300 µmol TE/mL (Table 1).

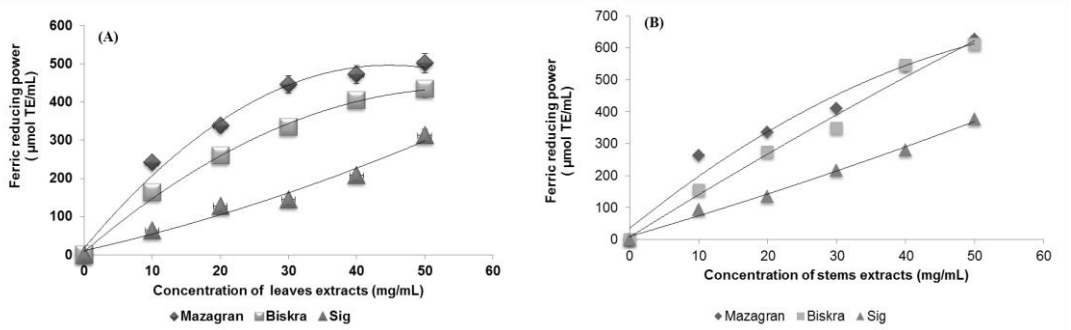

Figure 4. Ferric-reducing power from leaf extract **(A)** and stem extract **(B)** of *A. halimus* from three Algerian provinces of origin; Mazagran, Biskra, and Sig, expressed at µmol TE/mL.

Pearson's correlation coefficients (r) between antioxidant activities and phenolic compounds

Data shown in Table 2 indicate positive correlation between total phenolic compounds from all extracts and FRAP, DPPH, and ABTS assays with Pearson's coefficient values of 0.928, 0.885, and 0.750. Moderate correlations were revealed among TFC and FRAP methods (r = 0.651) shadowed by DPPH antiradical scavenging (r = 0.428), while weak correlation appeared between ABTS and flavonoids (r = 0.245).

Table 2. Correlation between antioxidant activities and secondary metabolites content of *A. halimus* extracts.

Antioxidant test	Secondary metabolite	
	TPC	TFC
DPPH	0.885	0.428
ABTS	0.750	0.245
FRAP	0.928	0.651

Identification and qualitative analysis of phenolic molecules by HPLC-DAD

The identification of phenolic compounds in *A. halimus* extracts by HPLC-DAD is summarised in Table 3 and Figure 5, revealing 25 bioactive molecules (Table 3). The comparison of their retention times (RT) as well as their lambda max (λ max) with those reported in bibliography revealed the existence of a qualitative difference in the profiles of these phenolic compounds (Figure 5). The identity and the proposed molecular structures are reported in Table 3. This analysis revealed diverse classes of phenolic compounds, including five phenolic acids and four hydroxybenzoic acids (gallic, derivative vanillic, *p*-hydroxybenzoic, and derivative benzoic acid), and one hydroxycinnamic acid (derivative quinic acid), in addition to 20 flavonoids identified and grouped into three distinct classes:

i. Flavones, in particular the O- and C-glucosides represented by apigenin and luteolin.
ii. Flavonols, derived from aglycones; quercetin, isorhamnetin, and kaempferol.
iii. Flavanones which are composed of naringenin and hesperidin, with their glucosides.

Leaf extracts showed more complex polyphenolic profiles with 25 compounds, including 20 flavonoids and five phenolic acids compared to stem extracts, which showed the presence of only nine different flavonoids, including two flavanones (peaks 17 and 21), four flavonols (peaks 18, 19, 22, and 23), and two flavones (peaks 20 and 24). This distribution varied based on the ecotype tested, while some peaks like 19, 21, 22, and 23 were common for all extracts (leaves and stems). Compared to leaves of Biskra, the results obtained showed a qualitative dominance of leaf extracts from Sig and Mazagran by the presence of the following phenolic acids: gallic, derivative vanillic, *p*-hydroxybenzoic, and derivative benzoic acid, represented respectively by peaks 1, 2, 4, and 5, and eluted at RT of 3.57, 3.7, 5.54, and 6.79 min, respectively, with the exception of hydroxycinnamic acid (quinic acid derivative), which was identified at 4.15 min in leaf extracts from Biskra, Sig, and Mazagran provinces. Moreover, the abundance of glycosylated flavonols such as quercetin-7-O-neohesperidoside (peak 19), kaempferol-3, 7-*O*-diglucoside, and kaempferol-3-glucoside (peaks 22 and 23) in the different samples was observed. In addition, other flavonols were identified only in the leaves of Mazagran and Sig: isorhamnetin glucoside (isorhamnetin-3-*O*-(2″,6″ dirhamnosyl)-glucoside), quercetin glucosides (quercetin-7-O-glucoside-3-O-rutinoside) (rutin), and quercitin-3-glucoside (isoquercetin), in peaks 12, 13, and 14 at 25.14, 25.54, and 27.55 min, respectively. Biskra leaves were characterised by the presence of quercetin 3-*O*-(2″,6″ dirhamnosyl)-glucoside and isorhamnetin-3-*O*-rutinoside (narcissin) in peaks 8 and 9, and detected at 19.39 and 20.56 min, respectively. Sig, Mazagran, and Biskra ecotypes were also characterised by the presence of flavones of different structures. *O*-glycosyl flavone, luteolin-7-*O*-rutinoside (veronicastroside) (peaks 7 and 10; RT = 18.08 - 23.51; λmax = 221 - 272 and 350, respectively), and *O*-C-glycosyl flavone (apigenin-6,8-di-C-glucoside) (vicenin-2) (peak 20; RT = 45.49; λmax = 227-326) characterised the leaves. In addition, apigenin-7-*O*-neohesperidoside (rhoifolin) (peak 6), luteolin-6,8-di-C-glucoside (Lucenin-2) (peak 15), and apigenin (peak 25) were also detected in Sig leaves at 17.34 - 31.34 and 52.02 min, respectively. Apigenin-7-O-glucoside (peak 24) was detected at 51.28 min in stems samples, and not in Mazagran and Sig leaves. Apigenin (peaks 6 and 25) at variable RT (17.34 and 52.02 min) were detected in Biskra stems. In addition, three flavanones were detected: hesperidin (peak 21; λmax = 281 - 327 at 46.55 min), naringenin-7-*O*-glucoside (prunin) (peak 11; λmax = 291 - 332 at 23.84 min), and naringenin (peak 17 at 44.28 min) in Mazagran leaves, Sig stems and leaves, and Biskra stems. Hesperidin (peak 21) was also identified in Biskra leaves at 46.55 min.

Discussion

Halophile plants' abilities, such *A. halimus*, to withstand various environmental conditions are attributed to an adaptive phytochemical response. This last one is influenced by geographical and temporal factors, resulting in the production of secondary metabolites that have demonstrated biological significance, as supported by prior research. As a result, this species has the ability to modulate the biosynthesis levels of bioactive compounds, and potentially generate novel compounds.

Table 3. Different classes of secondary metabolites identified in *A. halimus* extracts by HPLC-DAD.

No.	Molecule class	RT	Max λ	Leaf			Stem		
				Mazagran	Biskra	Sig	Mazagran	Biskra	Sig
	Phenolic compound								
1	Gallic acid	3.57	277	+	-	+	-	-	-
2	Derivative vanillic acid	3.7	260	+	-	+	-	-	-
3	Derivative quinic acid	4.15	260	+	+	+	-	-	-
4	*p*-Hydroxybenzoic acid	4.54	261	+		+	-	-	-
5	Derivative benzoic acids	6.79	254	-	-	+	-	-	-
	Flavone								
1	Rhoifolin	17.34	230 338	-	+	+	-	-	-
2	Veronicastroside	18.08	221 272	+	+	+	-	-	-
3	Apigenin	33.71	228 336	+	-	+	-	+	-
4	Vicenin-2	45.49	227 326	+	+	+	-	-	-
5	Lucenin-2	31.34	227 264	-	+	-	-	-	-
6	Apigenin-7-*O*-glucoside	51.28	271 366	+	-	+	+	+	+
	Flavonol								
1	Quercetin 3-*O*-(2″,6″ dirhamnosyl)-glucoside	19.39	254 355	+	-	-	-	-	-
2	Quercetin-7-*O*-neohesperidoside	45.38	274 326	+	+	+	+	+	+
3	Isorhamnetin 3-*O*-(2″,6″ dirhamnosyl) glucoside	25.14	255 353						
4	Kaempferol 3-*O* acyl-diglycoside	45.2	201 286 326	-	+	+	+	+	+
5	Kaempferol-3,7-*O* diglucoside	48.25	228 274 369	+	+	+	+	+	+
6	Kaempferol-3-glucoside	50.63	229 286 366	+	+	+	+	+	+
7	Isorhamnetin 3-*O*-(2″,6″ dirhamnosyl) glucoside	25.14	255 353	+	-	+	-	-	-
8	Rutin	25.54	253 354	+	-	+	-	-	-
9	Isoquercetin	27.55	296 353	+	+	+	-	-	-
10	Narcissin	20.56	254 354	+	-	-	-	-	-
	Flavonone								
1	Naringenin	44.28	228 283	+	+	+	-	+	-
2	Hesperidin	46.55	281,327	+	+	+	+	+	+
3	Prunin	23.84	291 332	+	-	-	-	-	-

RT: Retention times; max λ: Max lambda; +: detected, -: not detected.

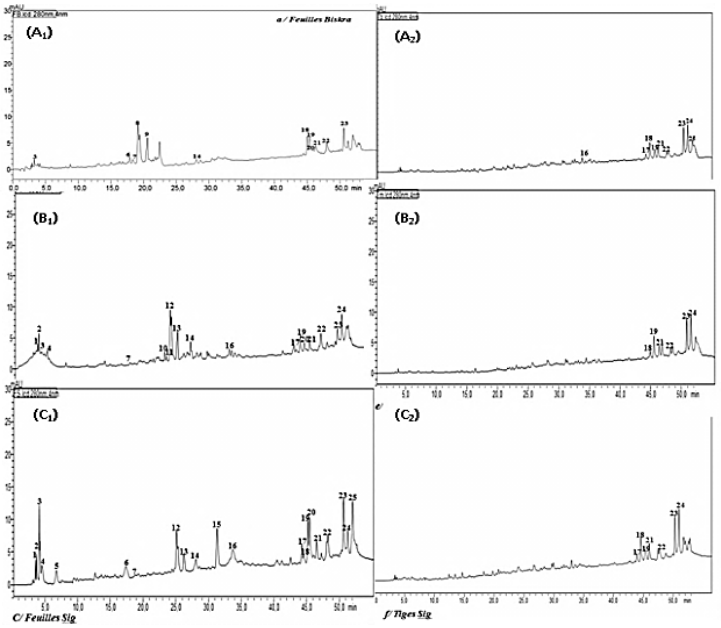

Figure 5. Bioactive compounds identified in *A. halimus* samples by HPLC-DAD at 280 nm. A_1, B_1, and C_1: leaves of Biskra, Mazagran, and Sig provinces, respectively; A_2, B_2, and C_2: stems of Biskra, Mazagran, and Sig provinces, respectively.

The extract yields appeared to be influenced by several factors such as locality, the solvent used, and the extraction method (Ould Kaddour *et al.*, 2019). Indeed, the ethanolic extract yields of *A. halimus* from Mazagran were found to be higher compared to M'sila (Dehimi *et al.*, 2020), Biskra (Ounaissia *et al*., 2020), and Tlemcen's methanolic extract contrary to El-Oued (Chaouche *et al.*, 2021). However, these yields were slightly lower than those observed from Ouregla (Gattouche *et al*., 2020). Biskra leaves showed similar results to Tlemcen samples but lower compared to El-Oued ones (Chaouche *et al*., 2021) and Biskra methanolic extract (Ounaissia *et al.*, 2020). Nevertheless, the data presented by Ould Kaddour *et al.* (2019) surpassed these findings.

The present work demonstrated that *A. halimus* could be a significant source of phytoconstituents, including phenolic, flavonoids, flavonols, and carotenoids, which are widely distributed throughout the different parts of the plant. Phenolics have the potential to provide benefits in terms of preventing various human diseases and preserving foods (Benhammou *et al*., 2009). The TPC and TFC vary significantly depending on the plant origins (Mazagran, Biskra, and Sig) and the plant parts (leaves and stems). The leaf extracts had higher concentrations, which agreed with Basharat *et al*. (2021).

Furthermore, both Mazagran and Biskra leaves and stems appeared to be rich in TPC and TFC. The TPC and TFC of Mazagran leaves observed in the present work were higher compared to values reported by Yahia *et al*. (2023) and Hasanain *et al.* (2023). In contrast, Mazagran leaves exhibited similar levels to Tlemcen samples (Chaouche *et al*., 2021).

Qualitative analysis performed by HPLC-DAD method on *A. halimus* extracts (leaf and stem) from Egypt (Wadi-surd) and Iraq (West of Al-Samaya) confirmed the works of Emam (2011) and Hasanain *et al*. (2022), respectively. These studies demonstrated the abundance of flavonoids, particularly flavonols, flavanones, flavones, and isoflavone glycosides. However, all the characterised molecules were different from those detected in the present work, except vicenin-2, hesperidin, and gallic acid. Interestingly, research conducted by Al-Jaber *et al*. (1991) on *A. farinosa* identified the same molecules. Additionally, Bylka *et al.* (2001) highlighted the presence of aglycone flavonols such kaempferol, quercetin, isorhamnetin, and occasionally patuletin, spinacetin, and tricin. Furthermore, kampferol-3-*O*-sulphate-7-*O*-arabinopyranoside and quercetin-3-*O*-sulphate-7-*O*-arabinopyranoside were isolated from the leaves of *A. hortensi*, as reported by Bylka *et al.* (2001). Based on these findings, variations in phenolic compounds, particularly flavonoids and phenolic acids, may be attributed to ecosystem factors such as climate, soil type, and rainfall. The characteristics of the geographical areas and sample origins can also impact the contents of these phytoconstituents

(Hasmida *et al*., 2014; Hasanain *et al.*, 2022). Furthermore, the contents differ depending on the plant parts (Oh *et al.*, 2009). Other factors, such as drought, salinity extraction method, and the polarity of solvents have been observed to increase the concentration of phytoconstituents in various plant species (Hasmida *et al*., 2014; Hasanain *et al.*, 2022.

Given the extensive range of phenolic compounds identified through qualitative analysis of the investigated extracts, three *in vitro* antioxidant activity tests, namely DPPH, ABTS, and FRAP, were employed to assess their free radical scavenging capability and metal ion reducing capacity. Saumya and Basha (2010) suggested the utilisation of multiple methods to evaluate the antioxidant potential of a plant due to diverse oxidative processes, and the intricate interplay between radical sources and antioxidants within a complex plant system. Analysis of results with previous studies revealed that the free radical scavenging capacity, as determined by the DPPH test, was comparatively lower compared to *A. halimus* leaves methanolic extracts of Bechar (Benhammou *et al*., 2009), and ethanolic extracts of Morocco (Elbouzidi *et al*., 2022), as well mixture extracts (acetone/methanol) from El Oued and Tlemcen (Chaouche *et al*., 2021). Remarkably, Dehimi *et al.* (2020) and Gattouche *et al.* (2020) demonstrated a notable antiradical potential in both aqueous and methanolic samples of *A. halimus* from M'sila, and methylene chloride extract of Ouargla. However, the findings surpassed those obtained by Hasanain *et al.* (2022). Additionally, the antioxidant activities of our extracts exceeded those of Orache *A. littoralis*. However, ethanolic extracts leaves of Morocco exhibited higher free radical scavenging than our findings (Elbouzidi *et al*., 2022).

The data obtained from the FRAP method were inconsistent with those reported by Dehimi *et al*. (2020), where the acetone fraction of *A. halimus* (leaves and stems) exhibited a high ferric ion reduction ability. Furthermore, the reducing activity of *A. halimus* from the three Algerian sources was significantly lower than the standard, which aligned with the findings of Chaouche *et al.* (2021). This reduced activity can be attributed not only to the content of TPC and TFC, but also to the phytochemical composition determined through qualitative analysis (Wannes and Marzouk, 2016). A strong correlation between the TPC and the antioxidant test was observed, while a moderate correlation was observed between the TFC and DPPH and FRAP assays, in contrast to the ABTS test. This discrepancy suggested that the antioxidant power was influenced by factors like concentration, method, solvent, and the structural characteristics of the active compounds (Zine *et al*., 2021).

To this effect, the observed results in Mazagran and Biskra leaves were likely attributed to the presence of hydroxyl groups in phenolic compounds, which act as electron donors. Antioxidants are reductants that neutralise oxidants. This has been supported by previous studies conducted by Bougandoura and Bendimerad (2012).

Likewise, it has been demonstrated in previous research (Kumaran and Karunakaran, 2007; Bougandoura and Bendimerad, 2012) that the reducing power of a compound serves as a significant indicator of its potential antioxidant activity. Among prooxidants, Fe^{2+} ions exhibit the highest reactivity. Consequently, effective chelators of ferrous ions can provide protection against oxidative damage by forming complexes with these ions, as highlighted by Gulcin *et al.* (2010). Sahreen *et al*. (2010) indicated that the chelating effects induced by plant extracts were directly influenced by the polarity of the solvents used. Phenolic and flavonoid compounds are excellent electron or hydrogen donors, and they possess the ability to chelate metal ions (Ladaniya, 2008).

Conclusion

The present work demonstrated the impact of different plant parts and origins of *A. halimus* on the quantity and quality of phenolic compounds, as well as their biological activities, including antioxidant properties. Specifically, the leaves were found to be rich in total phenolics and total flavonoids, resulting in high extract yields. The antioxidant potential of the extracts from Mazagran leaves, as demonstrated by DPPH, ABTS, and FFRAP tests, was influenced by the concentration of these secondary metabolites, and also by their nature, structure, extraction method, and solvents used. A. *halimus* from Mazagran showed promise as an alternative source of natural antioxidants in food and pharmaceutical applications. To further enhance its potential, future research could focus on developing new extraction methods, exploring different collection areas and seasons, and considering the controlled cultivation of *A. halimus* seeds.

Acknowledgement

The authors are grateful to the general directorate for scientific research and technological development (DGRSDT) for supporting scientific research in Algeria.

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