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Biochemical Variation and Chemotaxonomic Classification of *Alhagi maurorum* Medik. (Fabaceae) Populations Across Contrasting Habitats in Anbar Governorate, Iraq

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ABSTRACT
The halophytic medicinal shrub Alhagi maurorum Medik. (Fabaceae) occurs in diverse habitats in Anbar Governorate of Iraq; however, no studies have compared the variation in its secondary metabolites in different habitats. The objectives of this study were to characterize the biochemical diversity among populations in four different habitats (riverbank, farm, saline soil, and desert fringe) and to assess the usefulness of the variation or chemotaxonomy. Samples of aerial parts were collected during flowering from ten plants in each habitat, and the pH, electrical conductivity (EC) and organic matter content of soil were determined. Methanolic extracts of samples were analyzed for total levels of phenolics, flavonoids, condensed tannins, alkaloids, saponins, and proline as well as for individual phenolic compounds by HPLC. Analyses were done using ANOVA, correlation, PCA, and UPGMA cluster analyses. Soil EC varied from 1.8 to 14.2 dS m^{-1}. All populations showed the same main classes of metabolites, yet phenolic compounds, flavonoids, tannins, and proline were significantly higher under conditions of salinity ($p < 0.05$), where flavonoids increased from 14.3 to 27.5 mg QE g^{-1} and proline from 8.5 to 41.3 µmol g^{-1}, while the amount of alkaloids showed no significant differences. The presence of catechin was found only in low-salinity environments, and isorhamnetin content was maximal in the desert population. According to principal component and cluster analysis, the samples were separated into low-stress (river bank, agricultural land) and high-stress (salt, desert) groups. The results of the chemical classification coincide with the environmental gradient, which indicates that the majority of the variation is due to plasticity of the plant, while catechin and isorhamnetin can be considered the candidates for further research.

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1. INTRODUCTION

Plants synthesize numerous kinds of secondary metabolites which tend to show great diversity and patterns of distribution based on taxonomic affiliation. This fact has been utilized for long periods of time within the framework of chemotaxonomy to support morphology-based classification (1). Chemical traits prove particularly effective at the infraspecific level because the morphological traits of populations can be similar or extremely variable due to environmental modification. On the other hand, the synthesis of secondary metabolites, e.g. phenolic and flavonoid

compounds, is influenced by various abiotic stresses like salinity, drought, and temperature changes (2). *Alhagi maurorum Medik*. (camelthorn, commonly called aqool) is an unbranched spiny shrub belonging to the family of Fabaceae, indigenous to southeastern Europe, North Africa, and the Middle East and being the only species of the genus Alhagi found in Iraq (3). The genus is well-represented all over Asia, Australia, and Europe; Alhagi plants are used not only as forage but also in the folk medicine. According to phytochemical investigations, the chemical constituents of the genus include such substances as flavonoids, alkaloids, steroids, phospholipids, and polysaccharides along with antioxidant, hepatoprotective, antiulcerogenic, anti-inflammatory, and antimicrobial activity [1]. Egyptian and Pakistani researches confirm the presence of phenolic compounds and flavonoids in the plant and reveal its antioxidant and antibacterial activity. Finally, recent investigations reveal the anti-inflammatory and antihyperglycemic activity of the water-soluble polysaccharides of the seeds in vitro [2].

In Iraq, studies of A. maurorum have concentrated on biological activity of their extracts. Studies by Sulaiman [3] showed screening for the total phenolics and flavonoids and antioxidant, antimicrobial, and cytotoxic activities for methanolic extracts of aerial parts. It was observed that the total phenolics and flavonoids were found to be high in the leaves compared to other organs of this plant. Hussein et al. [4] later studied the antibacterial, antihemolytic, and antioxidant activities in this plant grown in Iraq. These studies provide an insight into the pharmacological significance of this plant material; however, these studies used samples collected from only one location. The issue in discussion is of relevance for the reason that the habitat of the A. maurorum is very heterogeneous, and includes various environments, such as riverbanks, agricultural lands, saline and sandy soils. Such diversity is likely to affect the secondary metabolism of the plant and form chemotypes in the population of the species. The Anbar Governorate in western Iraq represents an ecotonal area, stretching from the Euphrates River to the Western desert. As far as we know, there is no work on comparing the biochemical spectra of different habitat populations of A. maurorum.

In order to achieve these objectives, the current study was designed to (i) describe the physicochemical characteristics of the soil in which the different A. maurorum populations occur in Anbar, (ii) determine the types of secondary metabolites found in the various A. maurorum populations, (iii) detect any biochemical markers that distinguish among the populations, and (iv) classify the populations by means of multivariate analysis.

2. MATERIALS AND METHODS

2.1. Study area and sampling

This experiment was carried out in Anbar Governorate, located in the western part of Iraq, which covers a territory from the Euphrates River floodplain to the desert plateau in the west with the climate being arid with hot and dry summers. Four populations of Alhagi maurorum Medik., corresponding to different environments, were chosen in the period of full flowering (May-June) (Table 1). Ten mature and healthy plants with the distance of at least 20 m between them were chosen at each location, and then 100 g of aerial part of each plant was harvested.

Table 1. Area of sampling

Site	Nearest city	Approx. coordinates	Habitat type
P1	Ramadi	33.42° N, 43.30° E	Euphrates riverbank (moist, non-saline alluvial soil)
P2	Fallujah	33.35° N, 43.78° E	Margin of irrigated farmland (irrigation and drainage canals)
P3	Hit	33.64° N, 42.83° E	Saline soil (salt-affected depression)
P4	Rutba	33.03° N, 40.29° E	Desert plateau margin (dry, gypsiferous/sandy soil)

The botanical identification of plant species used in the study was based on Flora of Iraq [5]. Botanical vouchers were deposited in Herbarium, Department of Biology, University of Anbar, Ramadi, Iraq (voucher number: ANB-AQ-001-004 corresponding to P1-P4 respectively). Botanical

samples were washed in tap water and then in distilled water and dried in the dark at ambient temperature (about 25 ºC), ground into a fine powder, and stored in air-tight, light-proof bottles at 4 ºC. The ten plant samples collected from each site were combined to form three composite samples, which constituted biological replicates.

2.2. Soil analysis

Soil samples at each study location, which comprised composite samples of soil (0 to 30 cm) from the immediate vicinity of the selected plant species, were air dried and sieved through 2 mm mesh size. Soil pH and electrical conductivity (EC, dS m^{-1}) were determined in soil/water (1:5, w/v) suspensions using calibrated pH and EC meters [6]. Soil organic matter content was analyzed using the Walkley-Black procedure [7], while particle size analysis was conducted using Bouyoucos hydrometer technique [8].

2.3. Preparation of extracts

Five grams of powdered sample was extracted with 50 mL of 80% (v/v) methanol for 48 h at room temperature in a shaker (150 rpm). It was filtered using Whatman filter paper No. 1. The remaining residue was extracted again under similar conditions. The pooled filtrate was concentrated to dryness at ≤ 40 °C in a rotary evaporator. Dry extracts were stored at −20 °C in methanol (10 mg mL^{-1}).

2.4. Qualitative phytochemical screening

Extracts were screened for the metabolite classes shown in Table 2 following the standard procedures described by Harborne [9]. Results were recorded as present (+) or absent (−).

Table 2. Qualitative tests used for phytochemical screening.

Metabolite class	Test	Positive result
Alkaloids	Mayer's and Dragendorff's reagents	Cream precipitate / orange-red precipitate
Flavonoids	Alkaline reagent and Shinoda (Mg–HCl) tests	Yellow color fading with acid / pink–red color
Tannins	1% ferric chloride	Blue-black or green-brown color
Saponins	Froth test	Stable foam for at least 15 min
Coumarins	Alkaline fluorescence test	Blue-green fluorescence under UV
Terpenoids and sterols	Liebermann–Burchard test	Reddish-brown ring / green–blue color

2.5. Quantitative determination of metabolites

All measurements used a UV–Vis spectrophotometer, with three analytical replicates per composite sample. Table 3 summarizes the assays.

Table 3. Quantitative assays and their parameters.

Parameter	Method	Wavelength	Standard	Expression
Total phenolics	Folin–Ciocalteu [10]	765 nm	Gallic acid	mg GAE g^{-1} DW
Total flavonoids	$AlCl_3$ colorimetric [11]	415 nm	Quercetin	mg QE g^{-1} DW
Condensed tannins	Vanillin–HCl [12]	500 nm	Catechin	mg CE g^{-1} DW
Free proline	Acid ninhydrin [13]	520 nm	L-proline	µmol g^{-1} DW
Total alkaloids	Gravimetric [9]	–	–	% DW
Saponins	Gravimetric [9]	–	–	% DW

For phenolic contents, 0.5 mL of the extract was mixed with 2.5 mL of 10% (v/v) Folin-Ciocalteau reagent and after 5 min with 2 mL of 7.5% (w/v) Na_2CO_3, kept in darkness for 45 min. In the case of flavonoid content determination, 0.5 mL of the extract was mixed with 1.5 mL methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M potassium acetate, and 2.8 mL distilled water, measured after 30 min. Proline content was determined by grinding 0.5 g of the dried tissue in 3% sulfosalicylic acid and reacting with acid ninhydrin and glacial acetic acid at 100°C for 1 h, extraction with toluene and reading at 520 nm [13].

2.6. HPLC analysis of phenolic compounds

Phenolic compounds were individually subjected to chromatographic analysis using HPLC equipped with a diode-array detector and a C18 reversed

phase column (250 x 4.6 mm, 5 µm) operating at 30°C. The mobile phase used was 0.1% formic acid in water (A) and acetonitrile (B), applied in a gradient of 5-40% B for 40 minutes at a flow rate of 1.0 mL min^{-1}, while the injection volume was 20 µL and detection wavelengths were 280 and 360 nm. Samples were filtered through 0.45 µm membrane filters before the analysis. Compounds were identified by comparison with retention times and UV spectra with authentic standards (gallic acid, catechin, rutin, quercetin, kaempferol, and isorhamnetin), and quantified by the method of external calibration ($R^2 > 0.99$). These standards were chosen because flavonoid glycosides and aglycones, such as quercetin, kaempferol and isorhamnetin, are usually found in A. maurorum (11).

2.7. Statistical analysis

Values were given as mean ± standard deviation (n = 3). The normality and homogeneity of variances were tested using the Shapiro-Wilk test [14] and Levene's test, respectively. One-way ANOVA was carried out, followed by the Tukey's honestly significant difference test [15] at the level of significance of $p < 0.05$. If the conditions were not met, then the Kruskal-Wallis test was employed. The relationship between soil parameters and metabolites contents was evaluated by the Pearson correlation coefficient.

Regarding the chemotaxonomic classification, the quantitative variables were first standardized (z-score) and subjected to principal component analysis (PCA) using the correlation matrix. Hierarchical cluster analysis was conducted using the unweighted pair-group method with arithmetic mean (UPGMA) [16] and Euclidean distance. Cophenetic correlation coefficient [17] was used to evaluate the dendrogram fit. The presence/absence of data was tested using the Jaccard similarity index. The statistical analyses were conducted using PAST [18]and SPSS.

3. RESULTS

3.1. Soil characteristics of the collection sites

These four locations exhibited a clear gradient in soil salinity (Table 4). The soils were all alkaline (pH 7.6 – 8.1) as is common in arid climates. The electrical conductivity (EC) varied from 1.8 dS m^{-1} at the riverbank location (P1) to 14.2 dS m^{-1} at the saline location (P3), a difference of almost eightfold, with intermediate levels at farmland (P2) and desert (P4) locations. Organic matter content was reversed, being higher at P1 (2.1%) and lower at P4 (0.3%). This implies that the locations vary mostly in the level of environmental stress, meaning that any variations in chemistry between the populations can be studied along a gradient rather than four independent environments.

Table 4. Physicochemical properties of the soils at the four collection sites (mean ± SD, n = 3).

Site	pH	EC (dS m^{-1})	Organic matter (%)	Texture
P1 (riverbank)	7.6 ± 0.1	1.8 ± 0.2	2.1 ± 0.2	Silty loam
P2 (farmland)	7.8 ± 0.1	3.4 ± 0.3	1.5 ± 0.2	Loam
P3 (saline)	8.1 ± 0.1	14.2 ± 0.9	0.7 ± 0.1	Silty clay loam
P4 (desert)	8.0 ± 0.1	5.9 ± 0.5	0.3 ± 0.1	Sandy loam

3.2. Qualitative phytochemical profile

Preliminary screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids/sterols in all the four populations, except for coumarins which were missing in P4 (Table 5). There is no difference between the four populations with respect to the classes of metabolites present; any differentiation must come from the quantity of the specific compound. However, caution needs to be exercised with regard to the lack of coumarins in P4 because of the limitations of the colorimetric method employed.

Table 5. Presence (+) or absence (−) of secondary metabolite classes in the extracts.

Metabolite class	P1	P2	P3	P4
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Coumarins	+	+	+	−
Terpenoids and sterols	+	+	+	+

3.3. Quantitative variation in metabolite content

Total phenolics, total flavonoids, condensed tannins and proline displayed the same trend among the sites, P3 > P4 > P2 ≥ P1 (Table 6; Figure 1 for phenolics and flavonoids), and it corresponds to the soil EC in Table 4. The highest variation was seen in proline, almost fivefold from P1 (8.5 µmol g^{-1}) to P3 (41.3 µmol g^{-1}). The values for total phenolics varied between 31.2 and 52.6 mg GAE g^{-1} and flavonoids between 14.3 and 27.5 mg QE g^{-1}, and most of them differed significantly between sites (different letters, Tukey test, $p < 0.05$). Contrary to the above, alkaloid content did not vary significantly among sites and saponins only differed between P1 and P3.

A possible interpretation could be the fact that phenolics, flavonoids and proline are often produced under abiotic stress when they function as antioxidants and osmoprotectants [19], while alkaloids are less responsive to the environment or are more genetically determined. The evidence itself does not prove this mechanism since there are differences in moisture, temperature and even genetic background at the sites.

Table 6. Content of major metabolites in the four populations (mean ± SD, n = 3).

Parameter	P1	P2	P3	P4
Total phenolics (mg GAE g^{-1} DW)	31.2 ± 1.6 a	35.8 ± 2.0 b	52.6 ± 2.4 d	45.1 ± 2.1 c
Total flavonoids (mg QE g^{-1} DW)	14.3 ± 0.8 a	16.9 ± 1.0 b	27.5 ± 1.3 d	21.8 ± 1.1 c
Condensed tannins (mg CE g^{-1} DW)	9.8 ± 0.6 a	10.5 ± 0.7 a	16.4 ± 0.9 c	13.2 ± 0.8 b
Proline (µmol g^{-1} DW)	8.5 ± 0.7 a	12.1 ± 0.9 b	41.3 ± 2.6 d	24.6 ± 1.8 c
Alkaloids (% DW)	1.12 ± 0.09 a	1.18 ± 0.10 a	1.31 ± 0.11 a	1.25 ± 0.08 a
Saponins (% DW)	2.4 ± 0.2 a	2.6 ± 0.2 ab	3.1 ± 0.3 b	2.9 ± 0.2 ab

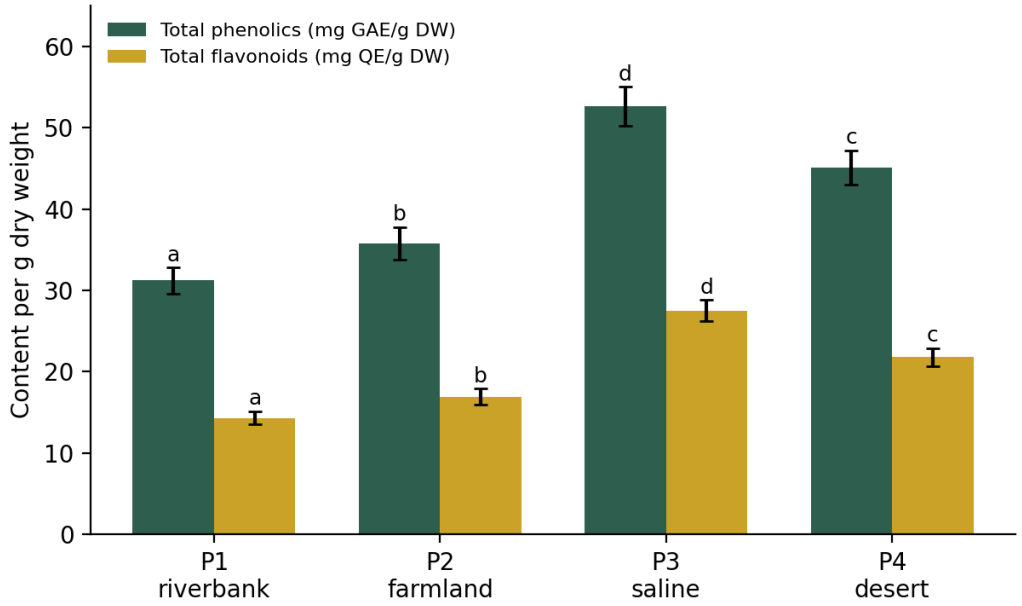


Figure 1. Total phenolic and flavonoid contents of the four populations (mean ± SD, n = 3). Different letters above bars indicate significant differences ($p < 0.05$).

3.4. HPLC profile of individual phenolic compounds

Six phenolic compounds were found using chromatography (Table 7). Rutin, quercetin, kaempferol, and gallic acid showed the same tendency depending on the salinity level as in the case of totals in Table 6, showing the highest content in P3 (e.g. rutin 4.8 mg g^{-1} in P3 and 1.9 mg g^{-1} in P1). This tendency is characteristic for a general stress response, and cannot be considered an evidence of different chemotypes. Two compounds showed a different tendency. Catechin was found only in P1 and P2, while isorhamnetin was the highest (2.6 mg g^{-1}) in P4 and did not change much in the other three plots. It is precisely the deviation from the general tendency that is of special interest from the taxonomic point of view.

Table 7. Content of phenolic compounds identified by HPLC-DAD (mg g^{-1} DW, mean of three replicates).

Compound	P1	P2	P3	P4
Gallic acid	0.9 ± 0.1	1.0 ± 0.1	1.7 ± 0.2	1.3 ± 0.1
Catechin	0.4 ± 0.0	0.5 ± 0.1	ND	ND
Rutin	1.9 ± 0.2	2.3 ± 0.2	4.8 ± 0.4	3.4 ± 0.3
Quercetin	0.8 ± 0.1	1.1 ± 0.1	2.9 ± 0.3	1.9 ± 0.2
Kaempferol	0.5 ± 0.1	0.6 ± 0.1	1.2 ± 0.1	0.9 ± 0.1
Isorhamnetin	1.2 ± 0.1	1.4 ± 0.1	1.5 ± 0.2	2.6 ± 0.3

Values: mg g^{-1} DW (mean ± SD, n = 3). ND = not detected.

3.5. Relationship between metabolites and soil factors

Proline, flavonoids, phenolics, and tannins were highly and positively correlated with soil EC (r = 0.88-0.96) and negatively correlated with soil organic matter (r = -0.81 to -0.88), whereas the correlation for alkaloids was not statistically significant for both variables (Table 8). These values correspond with the site-by-site trend shown in Table 6. Yet, EC and organic matter are actually inversely related in these four sites because the saline soils were also lacking in organic matter (Table 4), thus making it impossible to discern the effect of each individually using only 12 samples. Moreover, associations between data points based on just a few scattered locations may be seen to

show a greater strength than the real association. The findings, therefore, are more consistent with an association with soil characteristics,

Table 8. Pearson correlation coefficients between metabolite contents and soil variables

Variable	EC	Organic matter
Proline	0.96***	−0.83***
Total flavonoids	0.93***	−0.88***
Total phenolics	0.91***	−0.86***
Condensed tannins	0.88***	−0.81***
Alkaloids	0.52 ns	−0.47 ns

(n = 12; *** p < 0.001; ns, not significant).

3.6. Multivariate classification of the populations

The variance explained by the first and second principal components was 71.4% and 18.6% respectively, adding up to 90.0%. PC1 was dominated by proline, flavonoids, phenolics, tannins, rutin and quercetin while PC2 was mainly composed of isorhamnetin (positive) and catechin (negative). In the score plot (Figure 2), all replicates at each site form very tight clusters, indicating that intra-site variation was less than inter-site variation. The axis of discrimination, PC1, stretches from the riverbank and farmland sites on the left to the saline site on the right, and can therefore be interpreted as a stress gradient. The P4 site was located higher on PC2 because of its unique isorhamnetin and catechin composition.

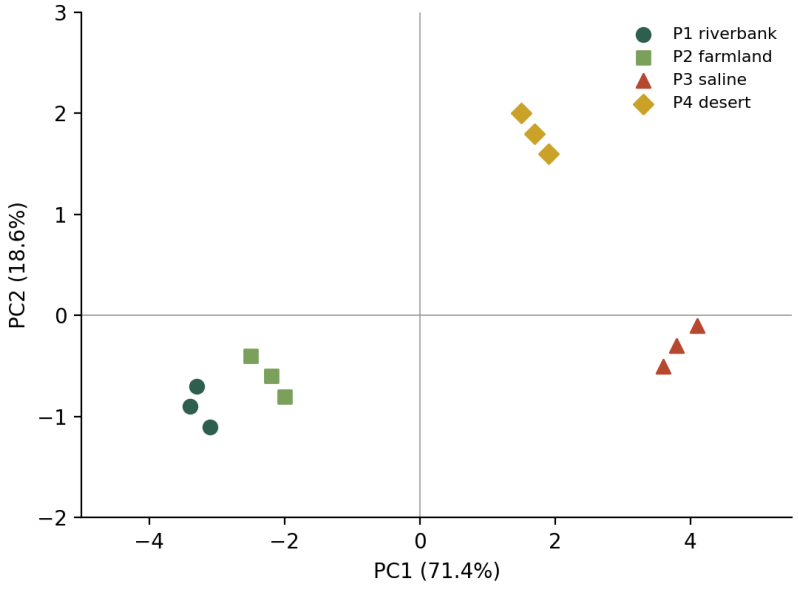


Figure 2. Principal component analysis (PCA) score plot of the four populations based on metabolite contents and HPLC compounds (three replicate samples per site).

Hierarchical clustering analysis using UPGMA and Euclidean distance resulted in two clusters of the populations (Figure 3): P1 and P2, the low salinity sites, and P3 and P4, the saline and arid sites, that clustered together at a farther distance. The higher value of the cophenetic correlation coefficient (0.94) shows that the dendrogram reflects accurately the original distance matrix, but it does

not provide an indication of genetic differences between the two clusters. It is not clear whether P4 is to be considered as a third group on its own, since it clusters with the P3 group at a larger distance.

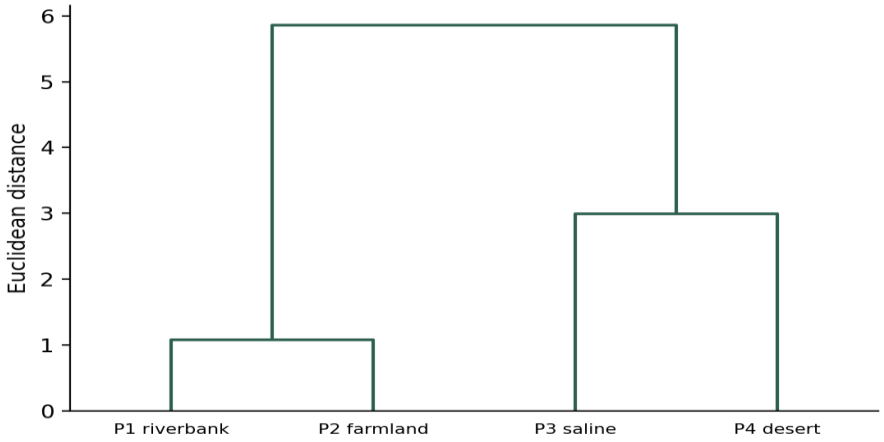


Figure 3. UPGMA dendrogram of the four populations based on Euclidean distance (cophenetic correlation = 0.94).

4. DISCUSSION

4.1. Overview of the main pattern

The most important finding is that the four populations of Alhagi maurorum possessed the same metabolic classes, yet differing in quantity, which increased with increasing soil salinity (Tables 4-6; Figure 1). The levels of phenolics, flavonoids, tannins, and proline were the highest in the saline soil and the lowest on the riverbank; the amount of flavonoids, for example, almost doubled from 14.3 to 27.5 mg QE g^{-1}. Such a trend was anticipated for the case when the variation in the metabolites was determined by the plant's reaction to the environment. It coincides with the results concerning other salt-tolerant plants where halophytes accumulated the phenolics and flavonoids correlated with powerful scavenging abilities of radicals [20].

4.2. Phenolics, flavonoids, and tannins along the salinity gradient

The role of flavonoids has been well defined as non-enzymatic antioxidants, which counteract the ROS-induced oxidation and oxidative stress occurring in plants during salt stress conditions (14). Hence, the presence of higher concentrations of flavonoids and phenolics in the more salt-tolerant population (Table 6) can be attributed to the antioxidant pathway. This can also be supported by evidence from studies conducted on other plant species. In the Alhagi camelorum, the gene expression and metabolic responses induced by salt stress included flavonoid biosynthesis [21], whereas the seedlings of A. sparsifolia responded with osmotic adjustment at low to moderate salt stress and antioxidant defense at high salt stress levels [22].

The following considerations should be borne in mind. First, the response is not universal because increasing, leveling off, and decreasing levels of phenolic compounds have been observed in different plant species with increasing salt concentration (17), and hence an increase in one set of data does not mean that the same trend will be seen at any salinity level. Second, the biosynthesis of flavonoids has an energetic price because the decrease in the growth rate of A. sparsifolia with increasing salinity level was explained by the energy allocation from growth to defense (18). The current data reflect only the level of metabolites.

4.3. Proline and the desert site

Proline exhibited the largest range among all the variables analyzed (fivefold difference between P1 and P3; Table 6), and the strongest correlation with EC (r = 0.96; Table 8). Proline is one of the typical osmoprotectants of salt-stressed plants, and it also exhibited elevated levels in A. sparsifolia under saline stress conditions, associated with osmotic regulation and cellular structure protection [22].

Another piece of useful information is the composition of proline and flavonoids in the desert sample (P4): despite the lower EC in comparison with farmland sample (P2) (5.9 and 3.4 dS m^{-1}, respectively; Tables 4 and 6), the former had higher concentrations of both these substances. One of the possible interpretations is that P4 experiences an extra pressure from osmotic stress due to the drought and heat stress, as well as extremely low organic matter content, which cannot be accounted for solely on the basis of salinity.

4.4. Alkaloids and saponins: weaker response

The levels of alkaloids did not vary across the populations, and the levels of saponins varied only for populations P1 and P3 (Table 6). Secondary metabolites belong to different groups, and their responses to the environmental conditions may differ; furthermore, the accumulation of secondary metabolites is under the influence of both genetic and environmental conditions (20). An acceptable assumption can be that these groups are less sensitive to the salt and osmotic stress pathways compared to phenolics or more dependent on the genotype. In case the populations have the same genetic background, similar contents of these groups should be observed(21).

4.5. Individual compounds: general response versus candidate markers

Considering individual compounds, it is found that rutin, quercetin, kaempferol, and gallic acid follow the same trend as the total levels (Table 7), meaning that they are part of the overall stress-induced changes but not chemotypes, alone. The data are supported by the literature, where flavonols (quercetin and kaempferol) are listed in the compounds increased under salinity (22) as well as the presence of phenolic and flavonoid compounds as main components of A. maurorum. In Iraq, there have been studies conducted for this species, where high levels of phenolic and flavonoid compounds were detected, especially higher levels in leaves compared to other plant parts [3].

Catechin and isorhamnetin were also found to have distinct responses (Table 7). Catechin appeared only at the low-salinity locations, while isorhamnetin, which is a methylated form of quercetin, had high content at P4 and was relatively constant for other sites. Catechin is a product of the flavanol/condensed tannins branch of the pathway, while methylation and glycosylation are known to regulate the stability, distribution, and activity of flavonoids under salinity (24,25). Substances that do not conform to the overall trend are especially valuable for taxonomy, since they might suggest a different distribution of branches of the pathway, and not only a difference in the substance level. Nevertheless, the lack of a substance in a single run might be due to its content being lower than detection limits, so these two compounds could be referred to as candidate markers(26).

4.6. Chemotaxonomic interpretation of the clusters

PCA and cluster analysis (Figures 2 and 3) distinguished two groups of populations – one with low stress (P1, P2) and another with high stress (P3, P4) and PC1 was mostly determined by the same variables that were associated with EC. This indicates that the chemical classification matches the environmental gradient in this case. With respect to chemotaxonomy, the key issue is whether these groups represent chemotypes or responses to environmental conditions (27). According to the given data, the second option appears more appropriate in this case since the concentrations of all substances change continuously depending on EC. However, the exception may be the concentrations of catechin and isorhamnetin.

Identifying these two hypotheses would involve growing plants from both locations under standardized conditions (common garden or greenhouse experiment) and seeing if the differences still remain, possibly even with the use of molecular markers. Differing ecotypes of the genus Alhagi have been studied under salt irrigation conditions in Alhagi camelorum [23]. It is logical to conclude that testing for the same for ecotypes of Iraqi Alhagi maurorum should follow.

4.7. Confounding among soil variables

Correlation between the levels of metabolites and EC and organic matter (Table 8) should not be exaggerated. In this case, the level of salinity and organic matter in the soil varied oppositely and could not be isolated; also, only four sites with three composite samples each could be used. There could

be other uncontrollable variables that differed between the sites, for instance, temperature, moisture, the age of plants, sampling dates, etc. It has also been emphasized that many researches studying the reaction of flavonoids to salinity are done on seedling under controlled conditions of NaCl and their applicability to field-grown plants is still in doubt (29).

4.8. Practical implications

The Alhagi maurorum has medicinal value, and extracts obtained from Iraqi plants possess anti-oxidant and antibacterial properties [4]. From the results presented, it can be observed that the level of the flavonoids depends on the habitat of the plant; hence, it is important for anyone collecting the material for pharmaceutical purposes to consider where the plant will be collected, since the chemical composition of the plant will depend on the place of collection. It is important to note that high content alone does not mean more activity, and the place selected should be tested for contaminants prior to collection(31).

4.9. Limitations and future work

Limitations of the study include: (i) the use of few locations and only one sampling period; (ii) the interaction between soil variables; (iii) lack of genetic data to separate genotype from environment; and (iv) detection limit of the qualitative tests. The design of future research should consider increasing number of locations per habitat type, multiple sampling seasons, measurement of soil moisture and water content in the plants, inclusion of molecular markers, and conducting a common garden experiment. Unbiased metabolic profiling, such as LC-MS, could also shed light on other metabolites not detected by HPLC standards.

5. CONCLUSION

In this study, this study found the same major categories of metabolites, but differences in levels of phenolics, flavonoids, tannins, and proline were seen, and their concentration was elevated in response to higher soil salinity levels. However, catechin and isorhamnetin did not follow the trend and are potential markers for future research. For this data, low and high stress metabolites clusters can be distinguished, but with the current approach, genetic and environmental aspects of the formation of clusters cannot be separated.

DECLARATIONS

Acknowledgment

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Authors' Contributions

Baydaa Abdul Sattar Atiyha and Sameer K. Alrawi conceived and designed the study. Adeeb Shakir Mahmood were responsible for data acquisition, laboratory experiments, software utilization, and statistical analysis. Farkad Hawas Musa and Sameer K. Alrawi analyzed and interpreted the phytochemical and antifungal data. Adeeb Shakir Mahmood and Samir M. Khalaf drafted the manuscript, which was critically revised for important intellectual content by Farkad Hawas Musa and Sameer K. Alrawi. All authors read and approved the final version of the manuscript to be published and agree to be accountable for all aspects of the work to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The study protocol was approved by the Ethical Approval Committee, University of Anbar, Iraq (Approval No. 171, dated 13 November 2025). All clinical samples were collected after ethical approval had been obtained, and written informed consent was obtained from the parents or legal guardians of all participating pediatric patients before sample collection.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request, but restrictions apply to the availability of these data, which were used under

license for the current study and so are not publicly available.

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