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Biochemical Study of the Medicinal Plant *Artemisia herba-alba* Collected in the Batna Region (Ain Touta and Forage Park, Algeria): Effects of Collection Site and Extraction Solvent

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Abstract

This study evaluated the effects of collection site and extraction solvent on the qualitative phytochemical profile, extraction yield, and antioxidant activity of Artemisia herba-alba collected from Ain Touta and Forage Park in the Batna region of Algeria. Qualitative phytochemical screening revealed no statistically significant differences between the two collection sites, and the resulting profiles were nearly identical. Proteins were not detected, whereas lipids, sterols, coumarins, and saponins were consistently detected in samples from both sites. Only minor between-replicate variations in glycosides, steroids, and tannins were observed at Forage Park. For antioxidant activity, neither the collection site nor the interaction between the collection site and the extract type had a statistically significant effect. In contrast, extract type had a pronounced effect, with a consistent ranking of methanolic extract > ethanolic extract > aqueous extract. Extraction yield and antioxidant activity were closely associated, although the strength of this relationship varied by solvent and remained similar between collection sites. These findings indicate that the antioxidant activity of A. herba-alba is influenced more strongly by the extraction solvent than by the plant material's geographic origin.

Keywords: *Artemisia herba-alba, antioxidant activity, collection site, extraction solvent, phytochemical screening*

INTRODUCTION

Since antiquity, medicinal plants have been used to alleviate and treat numerous ailments. Their therapeutic value is associated with a remarkable diversity of natural bioactive compounds, which may number in the hundreds or thousands (Wink, 2015). Medicinal plants are used in both traditional and modern phytotherapy, either as whole plants or as selected plant parts, depending on the active constituents responsible for their pharmacological effects (El-Saadony et al., 2025). They are also used in human and animal health, cosmetics, and the food industry in crude, dried, powdered, or formulated forms (El-Saadony et al., 2025). Beyond their direct use, medicinal plants remain central to pharmacological research because they provide leads for the discovery and development of new therapeutic agents (Verpoorte, 1998). Despite advances in modern medicine, medicinal plants continue to provide accessible and cost-effective health care options, particularly in Africa and other developing regions (Hadjadj et al., 2019).

Medicinal plants are important sources of primary metabolites, including carbohydrates, lipids, organic acids, and nucleic acids, as well as secondary metabolites such as alkaloids, phenolic compounds, terpenoids, sterols, and saponins. Many of these compounds exhibit biological and pharmacological activities (El-Saadony et al., 2025). Algeria has exceptionally diverse flora because of its biogeographic position between the Mediterranean region and sub-Saharan Africa (Abdelguerfi & Ramdane, 2003; Quézel & Santa, 1962–1963). The genus *Artemisia* is prominent within this medicinal flora and includes nearly 400 species, ranging from widespread to comparatively rare taxa (Jiao et al., 2023). Species within this genus have also been extensively investigated for the composition and biological properties of their essential oils (Akrout et al., 2001).

Plants of the genus *Artemisia* (Asteraceae) have long been recognized for their medicinal properties and occur mainly in arid and semi-arid regions of Europe, the Americas, North Africa, and Asia. *Artemisia herba-alba* has traditionally been used for several conditions, including digestive disorders, burns, ulcers, and diarrhea (Nigam et al., 2019). In Algeria, the species is abundant across a steppe belt approximately 1,200 km long, extending from the Tunisian to the Moroccan border and including the High Steppe Plains, the Hodna Basin, and the Constantine Plateau (Bougoutaia, 2018). Recent studies have further demonstrated the pharmacological potential of this species. Essential oils of *A. herba-alba* have shown antioxidant activity (Beniaich et al., 2025), and its phenolic extracts have demonstrated antioxidant and antibacterial properties (Lemmadi et al., 2025).

Studies of Moroccan *A. herba-alba* essential oils have reported geographic variation in chemical composition, as well as in physicochemical, toxicological, and bioactive properties (Saidi et al., 2025). These findings suggest that the collection site may influence the yield and biological activity of *A. herba-alba* extracts. Although the species is widespread throughout parts of the Aurès region and has attracted sustained research interest across Algeria (Bougoutaia, 2018), the Batna region remains insufficiently characterized from a phytochemical perspective.

Accordingly, this study aimed to (a) characterize *A. herba-alba* collected from Ain Touta and Forage Park in the Batna region; (b) identify primary and secondary metabolites through qualitative phytochemical screening; (c) compare the phytochemical profiles of aqueous, ethanolic, and methanolic extracts from each site; (d) evaluate extraction yields across solvents and sites; and (e) determine how extraction solvent and collection site influence antioxidant activity.

MATERIALS AND METHODS

Plant Material

Artemisia herba-alba (Asteraceae) is a medicinal and aromatic plant distributed across arid regions of the Mediterranean Basin and extending toward the northwestern Himalayas. Plant material was collected from February through April 2025 at two sites in Batna Province, Algeria: Forage Park and Ain Touta. Forage Park has a semi-arid climate according to the Köppen–Geiger classification, a mean temperature of 13.5 °C, and approximately 50 frost days per year. Ain Touta has a cold semi-arid climate (BSk), with summer temperatures exceeding 35 °C, winter temperatures below 2 °C, and annual precipitation ranging from 250 to 350 mm.

Preparation of Plant Material

The leaves were separated, dried for 15 days in a light-protected location, ground, and sieved to obtain a fine powder. The powder was stored in airtight glass containers labeled by collection site. All analyses were conducted separately for the Forage Park and Ain Touta samples.

Preparation of Extracts

Extraction was performed by maceration at room temperature, protected from light, using three solvent systems.

Aqueous extract. Ten grams of plant powder were macerated in 100 mL of distilled water under mechanical agitation for 24 h. The mixture was filtered through Whatman filter paper, and the filtrate was evaporated at 40 °C.

Ethanolic extract. Ten grams of plant powder were macerated in 100 mL of 80% ethanol with agitation for 3 days, then filtered.

Methanolic extract. Ten grams of plant powder were macerated in a methanol–water mixture (70:30, v/v) with mechanical agitation for 3 days, then filtered through Whatman filter paper.

The resulting extracts were stored in amber glass containers until analysis.

Qualitative Phytochemical Analysis

Qualitative phytochemical screening was performed using specific colorimetric or precipitation reactions to detect the principal groups of primary and secondary metabolites.

Primary metabolites. Proteins were assessed using the $NaOH/CuSO_4$ test, for which a violet color indicated a positive reaction. Lipids were assessed using petroleum ether followed by H_2SO_4, with a violet color indicating a positive reaction.

Secondary metabolites in the ethanolic extract. Sterols were assessed using acetic anhydride, chloroform, and H_2SO_4; the formation of a reddish-brown ring indicated a positive reaction. Coumarins were assessed using KOH followed by HCl; a reddish-brown precipitate indicated a positive reaction. Glycosides were assessed using Fehling's reagent; a brick-red precipitate indicated a positive reaction. Tannins were assessed using ferric chloride; green, blue-green, or greenish-brown coloration indicated a positive reaction, depending on the tannin type.

Secondary metabolites in the aqueous extract. Saponins were assessed using the stable, persistent foam test. Steroids were assessed using acetic anhydride and H_2SO_4; a violet-to-blue-to-green color change indicated a positive reaction.

Crude Extract Yield

The crude extracts were weighed after evaporation and drying. Extraction yield (R, %) was calculated as the mass of the dry extract obtained (M_e) divided by the mass of the plant material processed (M_v), multiplied by 100:

$$R\ (\%) = (M_e / M_v) \times 100$$

Antioxidant Activity: DPPH Assay

Free-radical-scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. In the presence of an antioxidant, the stable violet DPPH radical is reduced, producing a yellow color; this reduction was monitored by ultraviolet–visible spectrophotometry at 517 nm.

Preparation of the DPPH solution. DPPH (0.004 g) was dissolved in 100 mL of methanol, and the solution was agitated mechanically for 3 h.

Assay procedure. A series of dilutions of each extract was prepared in the milligram-per-milliliter range, with ascorbic acid used as the positive control. The assay mixture contained 3 mL of methanolic DPPH solution and increasing volumes of extract (0.5, 1, 2, and 3 mL); the final volume was adjusted to 5 mL with methanol. Three dilution series were incubated at room temperature, protected from light, for 15, 30, and 60 min, respectively. Absorbance was then measured at 517 nm using an ultraviolet–visible spectrophotometer against the corresponding DPPH–methanol control.

Statistical Analysis

Qualitative Phytochemical Screening

All statistical analyses and graphical visualizations were performed in R (Version 4.5.1; R Core Team, 2025). The qualitative data were arranged in a binary matrix (1 = presence, 0 = absence) across three independent biological replicates per site (N = 3). The design included eight phytochemical compounds—two primary metabolites (proteins and lipids) and six secondary metabolites (sterols, coumarins, glycosides, tannins, saponins, and steroids)—which were evaluated at two collection sites, yielding 48 binary observations.

Compound-specific differences in detection frequency between sites were evaluated using Fisher's exact test because the expected cell counts did not meet the assumptions of the chi-square test. Detection probabilities were also modeled using a generalized linear model with a binomial distribution and logit link to assess the effects of collection site and metabolite class (primary vs. secondary). Model output was organized using the broom package (Robinson et al., 2024), and main effects were evaluated using Type II Wald chi-square tests implemented in the car package (Fox & Weisberg, 2019).

Multivariate differences in qualitative composition were evaluated using Jaccard dissimilarities, which are appropriate for asymmetric binary data because joint absences do not contribute to similarity. Global compositional differences between sites were tested using permutational multivariate analysis of variance (PERMANOVA), and sample relationships were visualized using principal coordinates analysis (PCoA). Both analyses were conducted using the vegan package (Oksanen et al., 2024). Complete-linkage hierarchical

clustering based on Jaccard dissimilarity was displayed as a heatmap using the pheatmap package (Kolde, 2019). Figures were produced using ggplot2 (Wickham, 2016).

Extraction Yield and Antioxidant Activity

Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively. Because the normality assumption was violated, nonparametric analyses were used. For each response variable, the effects of extract type (aqueous, ethanolic, or methanolic), collection site (Ain Touta or Forage Park), and their interaction were evaluated using the Scheirer–Ray–Hare test implemented in the rcompanion package (Mangiafico, 2026). The antioxidant and extraction-yield data included five observations for each site-by-extract combination ($N = 30$).

When a main effect was statistically significant, Dunn's test (Dunn, 1964), implemented in the FSA package (Ogle et al., 2026), was used for post hoc pairwise comparisons. The Benjamini–Hochberg procedure was applied to control the false discovery rate. Simple linear regressions were used to examine the association between extraction yield and antioxidant activity, stratified by extract type and collection site. The coefficient of determination (R^2) and exact p values are reported. The significance threshold was set at $\alpha = .05$.

Results

Qualitative Phytochemical Screening

A global Fisher's exact test based on pooled presence–absence records showed identical distributions at Ain Touta and Forage Park: 15 presence records and 9 absence records across 24 observations at each site (62.5% presence). Accordingly, the global detection frequencies did not differ between sites ($p = 1.000$).

Compound-specific Fisher's exact tests likewise showed no statistically significant between-site differences (all ps = 1.000; Figure 1 and Table 1). Proteins were not detected in any replicate from either site. Lipids were detected in all Ain Touta replicates and in two of the three Forage Park replicates. Sterols, coumarins, and saponins were detected in every replicate at both sites. Glycosides and steroids were not detected at Ain Touta but were detected in one replicate of Forage Park. Tannins were detected in all Ain Touta replicates and in two Forage Park replicates.

Table 1. Qualitative Phytochemical Screening at Ain Touta and Forage Park

Metabolite class	Compound	Ain Touta, *n*/*N* (%)	Forage Park, *n*/*N* (%)	*p*	Result
Overall	All compounds	15/24 (62.5)	15/24 (62.5)	1.000	Not significant
Primary	Proteins	0/3 (0.0)	0/3 (0.0)	1.000	Not significant
Primary	Lipids	3/3 (100.0)	2/3 (66.7)	1.000	Not significant
Secondary	Sterols	3/3 (100.0)	3/3 (100.0)	1.000	Not significant
Secondary	Coumarins	3/3 (100.0)	3/3 (100.0)	1.000	Not significant
Secondary	Glycosides	0/3 (0.0)	1/3 (33.3)	1.000	Not significant
Secondary	Tannins	3/3 (100.0)	2/3 (66.7)	1.000	Not significant
Secondary	Saponins	3/3 (100.0)	3/3 (100.0)	1.000	Not significant
Secondary	Steroids	0/3 (0.0)	1/3 (33.3)	1.000	Not significant

Note. Fisher's exact tests were used for all comparisons. For the overall analysis, 24 observations were pooled per site.

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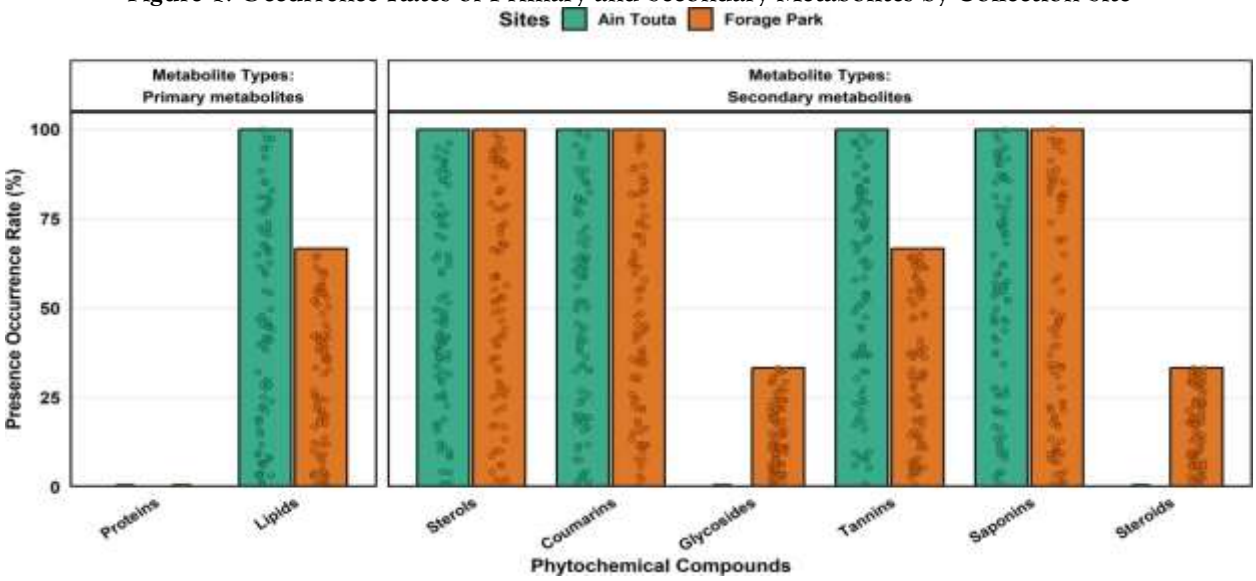
Figure 1. Occurrence Rates of Primary and Secondary Metabolites by Collection Site



Note. Jittered points are graphical representations of the binary occurrence frequencies and should not be interpreted as additional biological replicates. All compound-specific Fisher's exact tests yielded $p = 1.000$.

Multivariate Profiles and Generalized Linear Modeling

PERMANOVA based on Jaccard dissimilarities did not identify a statistically significant difference in the global qualitative phytochemical profile between Ain Touta and Forage Park, $F(1, 4) = 1.00$, $R^2 = .20$, $p = 1.000$ (Table 2). The collection site accounted for 20% of the observed variation, with the remaining 80% attributable to residual variation. The PCoA showed substantial overlap between sites. Five of the six biological replicates—three from Ain Touta and two from Forage Park—shared identical ordination coordinates. In contrast, one Forage Park replicate was separated along the first axis because of differences in lipid, glycoside, tannin, and steroid detection (Figure 4).

In the binomial generalized linear model, collection site was not associated with detection probability (Wald $\chi^2(1) = 0.00$, $p = 1.000$). Metabolite class was also not statistically significant (Wald $\chi^2(1) = 2.89$, $p = .089$), although secondary metabolites had a higher descriptive occurrence rate (26/36, 72.2%) than primary metabolites (5/12, 41.7%; Figure 2).

Table 2. PERMANOVA of the Effect of Collection Site on the Qualitative Phytochemical Profile

Source	*df*	Sum of squares	R^2	Pseudo-*F*	*p*
Collection site	1	0.05442	.200	1.000	1.000
Residual	4	0.21769	.800	—	—
Total	5	0.27211	1.000	—	—

Note. The analysis used Jaccard dissimilarities and 719 unrestricted permutations under the reduced model.

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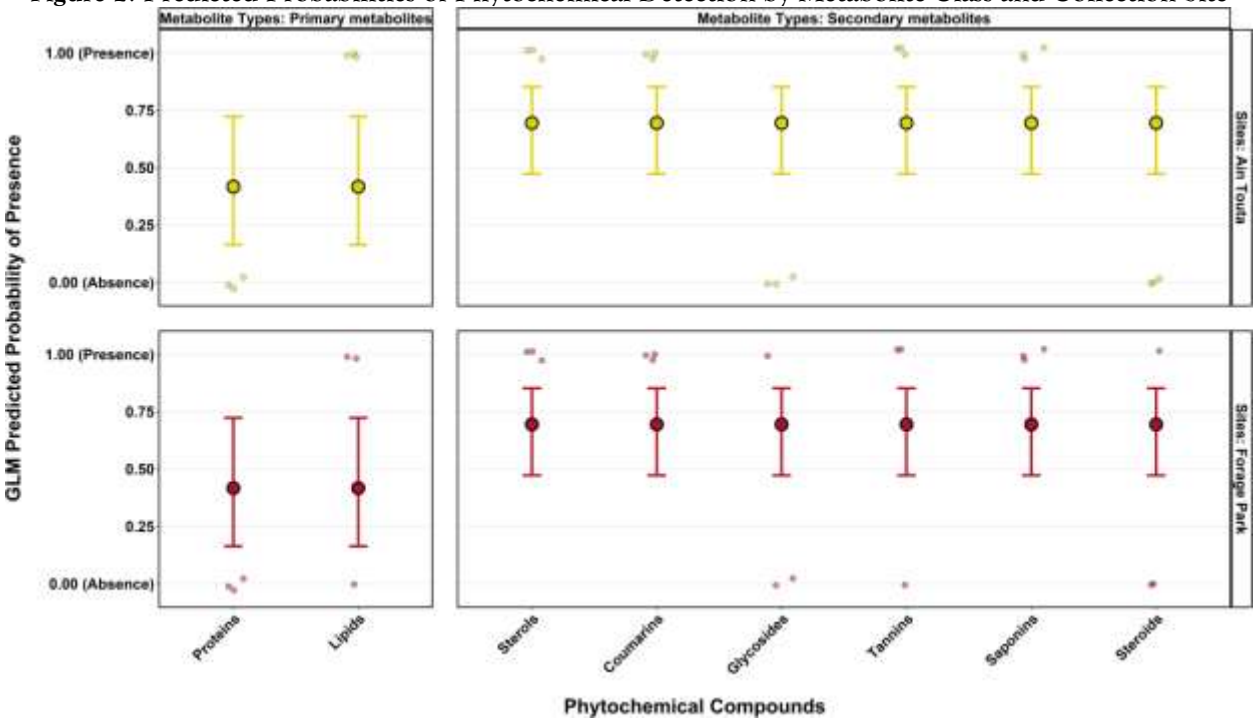
Figure 2. Predicted Probabilities of Phytochemical Detection by Metabolite Class and Collection Site

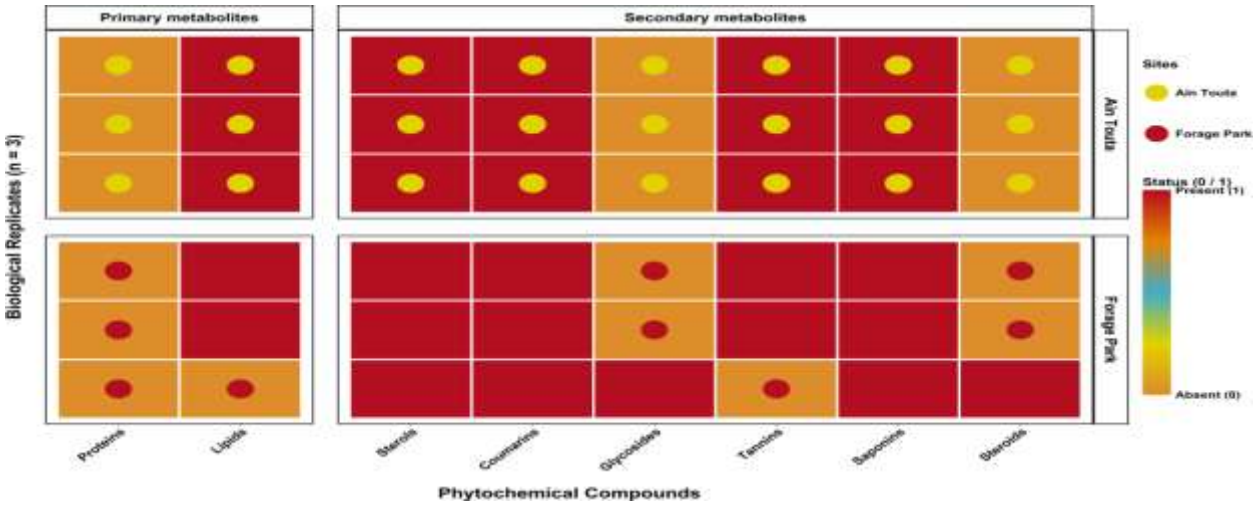


Note. Points show raw binary observations (1 = presence, 0 = absence). Error bars represent 95% confidence intervals. Type II Wald tests: collection site, p = 1.000; metabolite class, p = .089.

Heatmap and Hierarchical Clustering

The heatmap showed a high degree of qualitative compositional similarity between Ain Touta and Forage Park (Figure 3). Proteins were absent from all replicates, whereas lipids were detected in most replicates. Sterols, coumarins, and saponins were consistently detected across both sites. Variation was limited to sporadic detection of glycosides and steroids and the absence of tannins in one Forage Park replicate. The clustering dendrogram did not separate samples into site-specific groups; instead, replicates from both sites were intermingled within the same clusters.

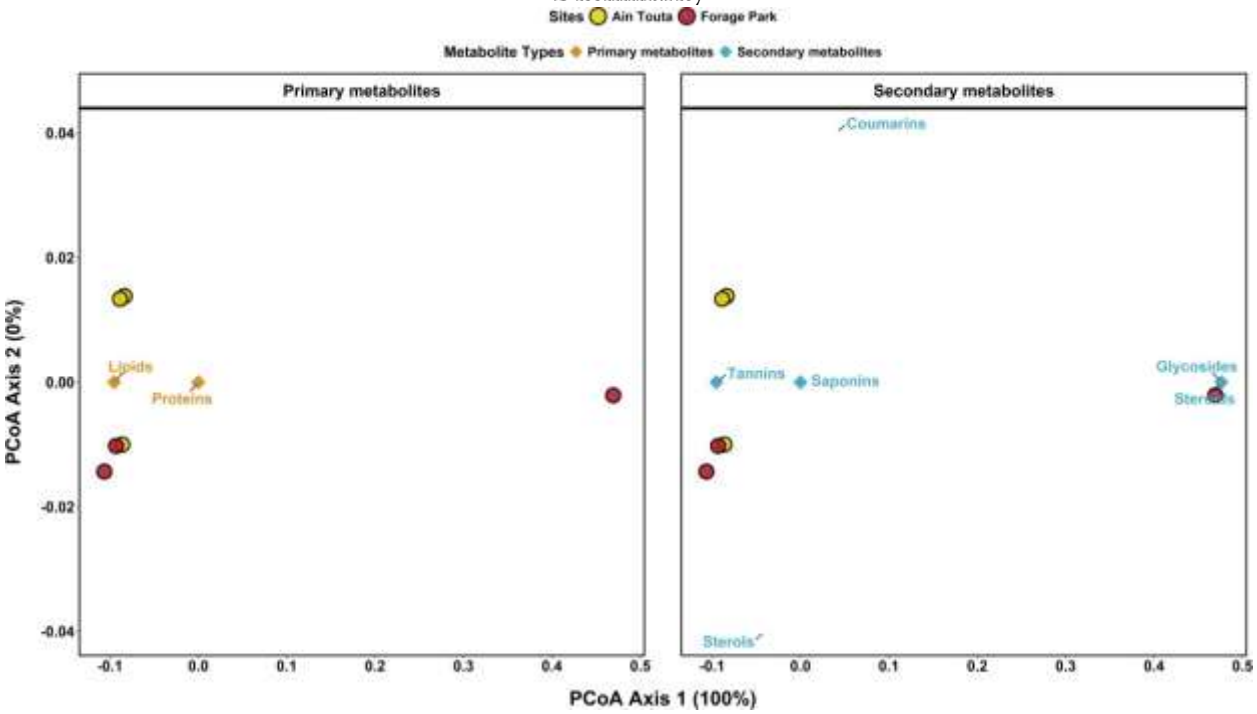
Figure 3. Phytochemical Presence–Absence Heatmap and Hierarchical Clustering Across Collection Sites



Principal Coordinates Analysis

The PCoA based on Jaccard dissimilarities further illustrated the strong overlap between the two sites (Figure 4). The first axis accounted for 100% of the represented variation, whereas the second axis contributed approximately 0%. Five of the six replicates occupied the same position in ordination space, and only one Forage Park replicate was separated along the first axis. This pattern was consistent with the nonsignificant PERMANOVA result.

Figure 4. Principal Coordinates Analysis of Qualitative Phytochemical Profiles Based on Jaccard Dissimilarity



Antioxidant Activity and Extraction Yield

A Scheirer–Ray–Hare test was used to evaluate antioxidant activity across extract types and collection sites (N = 30; Figure 5). The extract type × collection site interaction was not statistically significant, $H(2) = 0.008$, p = .996. The main effect of collection site was also not statistically significant, $H(1) = 2.82$, p = .093. In contrast, extract type had a statistically significant effect on antioxidant activity ($H(2) = 24.59$, $p < .001$).

Benjamini–Hochberg-adjusted Dunn comparisons showed that the methanolic extract had greater antioxidant activity than both the aqueous extract ($z = -4.95$, adjusted $p < .001$) and the ethanolic extract ($z = -2.29$, adjusted $p = .022$). The ethanolic extract also had greater antioxidant activity than the aqueous extract, $z = -2.67$, adjusted $p = .011$. Thus, antioxidant activity followed the order methanolic > ethanolic > aqueous.

Extraction yields were lowest for the aqueous extracts at both sites, whereas the ethanolic and methanolic extracts produced higher yields (Figure 6). Ain Touta samples showed descriptively higher yields than Forage Park samples across all three solvent systems.

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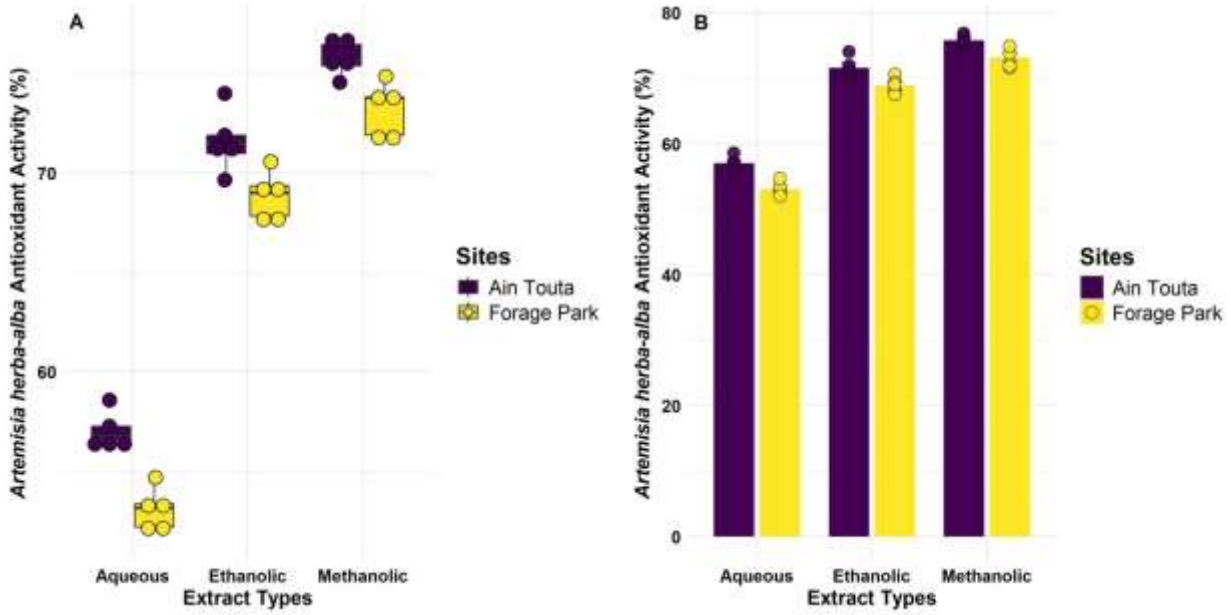
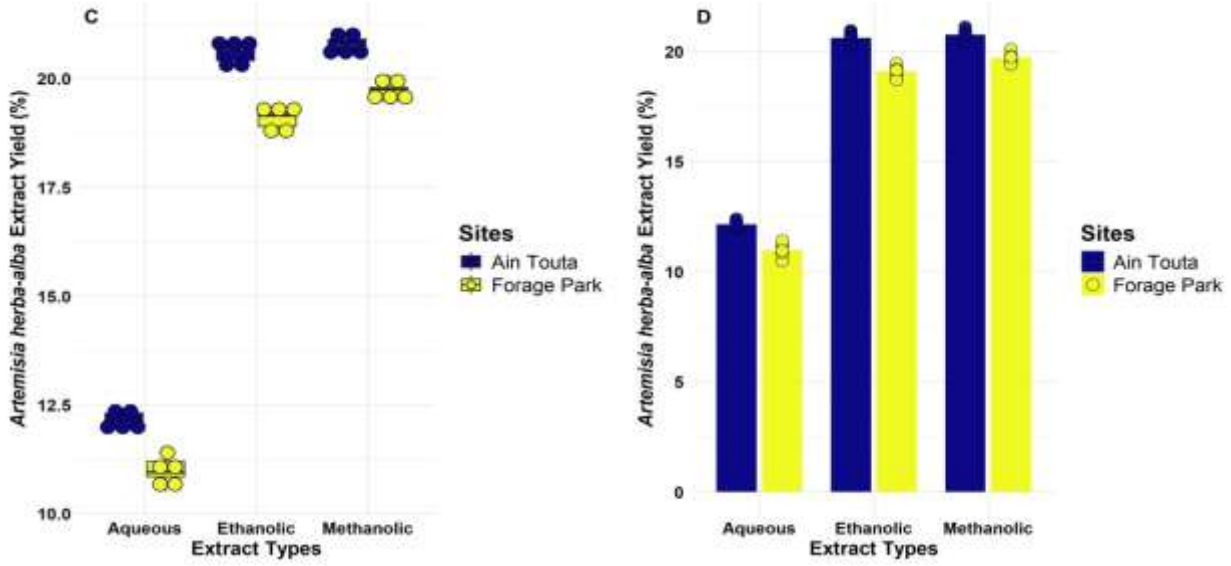
Figure 5. Antioxidant Activity of Artemisia herba-alba by Extract Type and Collection Site



Note. Panel A presents boxplots with individual observations; Panel B presents means with standard error bars and individual observations.

Figure 6. Extraction Yield of Artemisia herba-alba by Extract Type and Collection Site



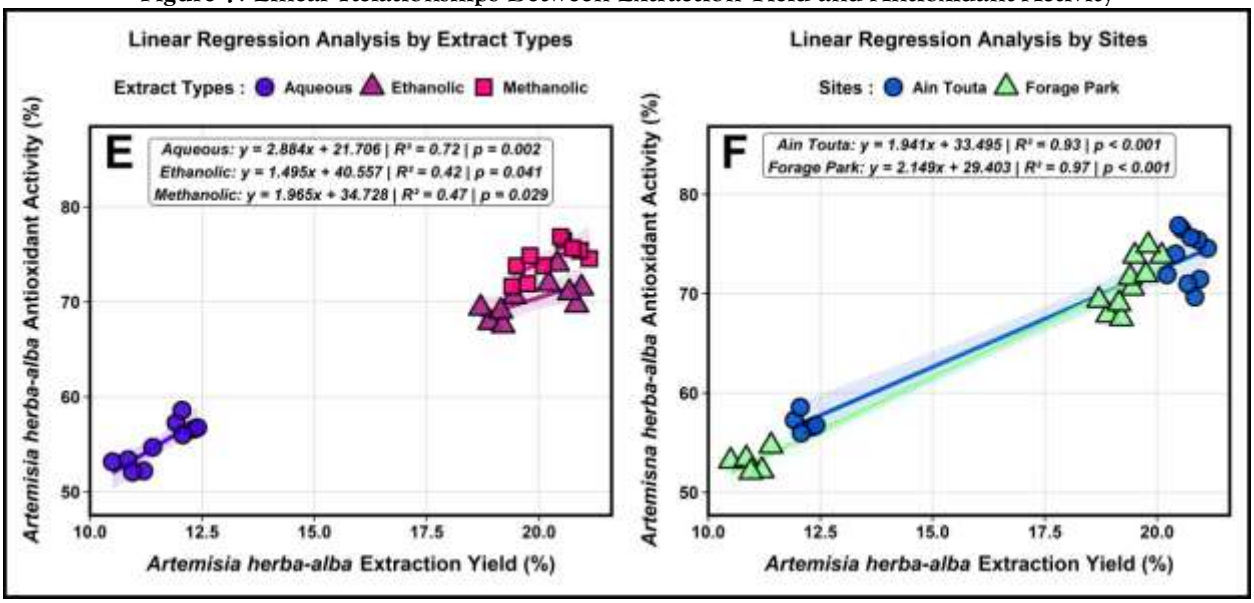
Note. Panel C presents the distribution of yield values with individual observations; Panel D presents means with standard error bars and individual observations.

Association Between Extraction Yield and Antioxidant Activity

The relationship between extraction yield and antioxidant activity differed by extract type (Figure 7E). The aqueous extract showed the strongest linear association, $y = 2.8838x + 21.7058$, $R^2 = .7158$, $p = .002$, followed by the methanolic extract, $R^2 = .4667$, $p = .029$, and the ethanolic extract, $R^2 = .4243$, $p = .041$. Site-specific regression lines were similar in direction and slope (Figure 7F). The Forage Park regression was $y = 2.1486x + 29.4032$, $R^2 = .9659$, $p < .001$, and the Ain Touta regression was $y = 1.9415x + 33.4954$, $R^2 = .9316$, $p < .001$.

These descriptive patterns indicate that extract type strongly structured the yield–activity association, whereas the two collection sites produced visually similar regression trajectories.

Figure 7. Linear Relationships Between Extraction Yield and Antioxidant Activity



Note. Panel E shows regressions stratified by extract type; Panel F shows regressions stratified by collection site.

DISCUSSION

The principal findings were the marked qualitative phytochemical similarity between Ain Touta and Forage Park and the strong influence of extraction solvent on antioxidant activity. Neither the univariate nor multivariate analyses identified statistically significant between-site differences in qualitative metabolite detection. In contrast, antioxidant activity differed substantially among solvent systems, with methanolic extracts consistently showing the highest activity, followed by ethanolic and aqueous extracts. These findings should be interpreted in light of the limited sensitivity of binary phytochemical tests, which cannot detect subtle differences in metabolite concentration.

Phytochemical Uniformity Across Collection Sites

The absence of statistically significant differences between Ain Touta and Forage Park in Fisher's exact tests, PERMANOVA, and PCoA suggests that the qualitative phytochemical profile of *A. herba-alba* was relatively stable at the local geographic scale examined. Secondary-metabolite diversity is often influenced by pronounced environmental gradients, including altitude, aridity, and soil composition; such gradients may need to reach a sufficient level of heterogeneity before producing detectable chemical differences (Alami et al., 2024; Ogwu et al., 2025). The two sites were separated by approximately 30 km and shared broadly comparable semi-arid conditions, which may explain the absence of clear qualitative divergence.

The present findings are broadly consistent with studies of *A. herba-alba* from Algeria, Jordan, and Morocco that reported recurring detection of sterols, coumarins, saponins, and tannins despite geographic differences (Alghonmeen et al., 2024; El Hajli et al., 2024; Mahboub et al., 2024; Medjeldi et al., 2024). Taken together, these studies suggest a relatively conserved qualitative chemical profile, although geographic effects may be more evident in compound concentrations than in simple presence–absence patterns.

Proteins were not detected by the qualitative assay, whereas sterols, coumarins, and saponins were detected consistently. One possible explanation is that aromatic plants exposed to chronic water stress allocate resources toward protective secondary metabolism (Alami et al., 2024). However, the present tests establish only whether a colorimetric reaction was observed; they do not demonstrate the complete absence of proteins

or quantify metabolic allocation. Quantitative assays are therefore required before drawing physiological conclusions from this pattern.

Effect of Extraction Solvent on Antioxidant Activity

Extract type had a statistically significant effect on antioxidant activity, with methanolic extracts outperforming ethanolic and aqueous extracts. This pattern is consistent with the role of solvent polarity in determining the range and quantity of phenolic compounds recovered from plant material. Solvents of intermediate polarity, including methanol and hydroalcoholic mixtures, may solubilize a broader range of polyphenols and flavonoids than water alone (Arya et al., 2025; Zreen et al., 2022). Similar solvent-dependent differences in free-radical-scavenging activity have been reported in other Mediterranean and subtropical plant species (Arya et al., 2025; El Mannoubi, 2023).

The aqueous extract showed the strongest within-solvent association between extraction yield and antioxidant activity (R^2 = .72), despite having the lowest absolute antioxidant activity. This pattern may indicate that, within the aqueous fraction, antioxidant activity is closely related to the total amount of polar material extracted, potentially including glycosylated phenolics. By contrast, the weaker yield–activity relationships observed for methanolic and ethanolic extracts may reflect greater chemical heterogeneity, such that antioxidant performance depends more on compound identity than on total extract mass (Zreen et al., 2022). Similar dissociations between extraction yield and biological activity have been reported for organic-solvent extracts (Arya et al., 2025; El Mannoubi, 2023).

Collection site did not have a statistically significant effect on antioxidant activity, and the site-specific regression lines relating yield to activity were visually similar. At this limited geographic scale, local edaphoclimatic variation may therefore have had less influence than solvent selection. This reproducibility is potentially useful for the standardized development of *A. herba-alba* extracts because it suggests that extraction conditions are a key determinant of antioxidant performance.

Limitations and Future Directions

Several limitations should be considered. First, the qualitative screening included only three biological replicates per site. Second, binary phytochemical tests cannot detect differences in concentration or identify individual compounds. Third, the geographic comparison was limited to two relatively close sites with broadly similar climates. Finally, the regression analyses were stratified descriptively, and formal comparisons of regression slopes were not reported. Future studies should include a larger number of environmentally contrasting sites, quantitative measurement of total phenolic and flavonoid contents, and compound-level profiling using high-performance liquid chromatography or gas chromatography–mass spectrometry. These approaches would provide a more precise estimate of geographic and solvent-dependent variation in the phytochemical composition and antioxidant capacity of *A. herba-alba*.

CONCLUSION

The collection site did not have a detectable effect on the qualitative phytochemical profile or antioxidant activity of *Artemisia herba-alba* within the limited geographic area examined. Ain Touta and Forage Park showed closely similar presence–absence profiles, particularly for sterols, coumarins, and saponins. In contrast, extraction solvent was the principal factor associated with antioxidant performance, with methanolic extracts consistently exhibiting greater activity than ethanolic and aqueous extracts.

The solvent-dependent relationship between extraction yield and antioxidant activity further indicates that optimization of the extraction protocol is more important than selection between these two collection sites for maximizing the bioactive potential of the species. The apparent local stability of the qualitative profile may support future standardization efforts. Studies involving more geographically and environmentally contrasting sites, larger sample sizes, and quantitative characterization of bioactive compounds are needed to confirm these findings and clarify the mechanisms underlying the antioxidant activity of this medicinal plant.

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