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Solvent-Dependent Variation in Extraction Yield and Antioxidant Activity of *Artemisia herba-alba* from Arris and Ain Yagout, Algeria

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ABSTRACT: *Artemisia herba-alba* is an important medicinal and aromatic plant whose bioactive properties may vary according to extraction conditions and geographical origin. This study investigated the effects of extraction solvent and sampling site on the extraction yield and antioxidant activity of *A. herba-alba* extracts collected from Arris and Ain Yagout. Aqueous, ethanolic, and methanolic extraction systems were evaluated. Because the assumptions required for parametric analyses were not satisfied, non-parametric statistical approaches were used to assess the effects of extraction solvent, sampling site, and their interaction. Significant effects of both extraction solvent and sampling site were observed for extraction yield and antioxidant activity, whereas their interaction was not significant. Methanolic extracts exhibited the highest extraction yield and antioxidant activity at both sampling sites, followed by ethanolic extracts, while aqueous extracts showed the lowest values. Heatmap visualization provided an integrated representation of the mean responses across solvent and sampling-site combinations. Furthermore, linear regression analyses revealed strong positive relationships between extraction yield and antioxidant activity within each extraction solvent and sampling site. Overall, the findings demonstrate that extraction solvent and geographical origin are important factors influencing the extraction performance and antioxidant potential of *A. herba-alba*. The consistent solvent-related patterns observed across both sampling locations indicate that the effect of extraction solvent was broadly maintained between sites. These results emphasize the importance of optimizing extraction conditions when evaluating the antioxidant properties of *A. herba-alba*.

INTRODUCTION

Medicinal plants constitute an important reservoir of natural molecules with diverse biological activities and have been used for centuries in traditional healthcare systems (Cragg and Newman, 2013; Wink, 2015). Their biological effects are largely attributed to secondary metabolites such as phenolic acids, flavonoids, terpenoids, tannins, and other phytochemicals (Wink, 2015; Yang et al., 2018). Among the biological properties associated with these compounds, antioxidant activity has received particular attention because oxidative stress is involved in numerous physiological and pathological processes (Pizzino et al.,

2017; Xu et al., 2017). Natural plant extracts capable of scavenging reactive species or reducing oxidative reactions are therefore increasingly investigated as potential sources of antioxidant compounds for pharmaceutical, food, nutraceutical, and cosmetic applications (Dai and Mumper, 2010; Xu et al., 2017). Within this context, *Artemisia herba-alba* Asso is of particular interest. This perennial aromatic species belongs to the Asteraceae family and is widely distributed throughout arid and semi-arid areas of the Mediterranean region and North Africa (Mohamed et al., 2010; Belhattab et al., 2014). It has a long history of traditional use and has been investigated for several biological properties (Mohamed et al., 2010). The chemical profile of

A. herba-alba includes different groups of secondary metabolites, among which phenolic

compounds, flavonoids, terpenoids, and volatile constituents have been reported (Mohamed et al., 2010; Belhattab et al., 2014; Bakchiche et al., 2022). The presence of these compounds is thought to contribute to the biological activities attributed to the species, including its antioxidant potential (Bakchiche et al., 2022; Ayad et al., 2022). However, the concentration and relative abundance of these constituents may vary according to environmental conditions, plant development, and the procedures used for their extraction (Figueiredo et al., 2008; Belhattab et al., 2014; Sultana et al., 2009). The extraction process is consequently a critical stage in the evaluation of the biological potential of plant materials. The type of solvent can strongly affect both the amount of material recovered and the chemical profile of the resulting extract (Sultana et al., 2009; Do et al., 2014). This is mainly because plant metabolites differ in polarity, solubility, and affinity for particular extraction media (Dai and Mumper, 2010; Azmir et al., 2013). Polar solvents such as water are particularly suitable for highly polar constituents, whereas solvents such as ethanol and methanol can facilitate the recovery of a wider range of moderately to highly polar secondary metabolites (Dai and Mumper, 2010; Xu et al., 2017). Previous research on medicinal plants has demonstrated that changing the extraction solvent can substantially modify extraction yield as well as antioxidant activity (Sultana et al., 2009; Do et al., 2014; Ayad et al., 2022). However, no single solvent can be considered universally optimal because extraction efficiency depends on the plant matrix, the targeted compounds, and the experimental conditions employed (Azmir et al., 2013; Do et al., 2014). The antioxidant response of plant extracts is therefore not necessarily determined by the total quantity of material extracted. Rather, it depends on the nature and concentration of the compounds recovered during the extraction process (Sultana et al., 2009; Dai and Mumper, 2010). Phenolic compounds and flavonoids, for example, are frequently associated with antioxidant activity because of their ability to donate hydrogen atoms or electrons and to interact with reactive species (Rice-Evans et al., 1996; Dai and Mumper, 2010). Consequently, an increase in extraction efficiency may be accompanied by enhanced antioxidant activity when antioxidant-active constituents are efficiently co-extracted. Nevertheless, the relationship between extraction yield and antioxidant capacity may vary according to the composition of the extract, making it important to

investigate this relationship experimentally rather than assuming that a higher yield always corresponds to greater antioxidant activity (Sultana et al., 2009; Do et al., 2014). Another factor that may contribute to variation in the biological properties of medicinal plants is geographical origin. Plants belonging to the same species may exhibit substantial differences in their chemical composition when growing under different environmental conditions (Figueiredo et al., 2008; Belhattab et al., 2014). Climatic parameters, soil properties, water availability, altitude, light exposure, and environmental stress can affect plant metabolism and consequently influence the synthesis and accumulation of secondary metabolites (Ramakrishna and Ravishankar, 2011; Yang et al., 2018; Figueiredo et al., 2008). Geographical variation has therefore been considered an important source of differences in the antioxidant capacity and phytochemical characteristics of medicinal and aromatic plants (Figueiredo et al., 2008; Yang et al., 2018). For *A. herba-alba*, which is naturally distributed across heterogeneous arid and semi-arid environments, the investigation of geographical variability is particularly relevant (Belhattab et al., 2014; Mohamed et al., 2010). Although the antioxidant properties and phytochemical characteristics of *A. herba-alba* have been addressed in previous investigations, the combined assessment of extraction solvent, geographical origin, and the relationship between extraction yield and antioxidant activity remains comparatively limited (Mohamed et al., 2010; Ayad et al., 2022; Bakchiche et al., 2022). Previous studies have often concentrated on individual aspects of the extraction process or on the biological properties of the resulting extracts (Ayad et al., 2022; Bakchiche et al., 2022; Belhattab et al., 2014). A simultaneous evaluation of these factors may provide additional information on whether differences between plant populations are maintained across extraction systems and whether the association between extraction efficiency and antioxidant activity is consistent among different geographical origins. Such an approach is particularly useful for distinguishing the effect of the extraction procedure from the intrinsic variability associated with the plant material itself. Accordingly, the present study investigated *A. herba-alba* collected from two distinct sampling locations, Arris and Ain Yagout, using three extraction media with different extraction characteristics: water, ethanol, and methanol. The study aimed to determine how solvent selection and sampling site affect

extraction yield and antioxidant activity and to assess whether these two factors interact in determining the observed responses. A further objective was to characterize statistically the relationship between extraction yield and antioxidant activity according to both extraction solvent and geographical origin. We hypothesized that the extraction solvent would significantly modify both responses, that plant material originating from different locations would display different extraction and antioxidant profiles, and that the effect of solvent could potentially vary according to sampling site. We also hypothesized that extraction yield would be positively related to antioxidant activity if increased recovery of extractable material was accompanied by greater recovery of antioxidant-active constituents. By combining these comparisons with statistical analyses of main effects, interactions, and relationships between variables, this study provides an integrated assessment of the factors contributing to the variability of *A. herba-alba* extracts. The results are expected to contribute to a better understanding of solvent-dependent extraction behavior and geographical variation in the antioxidant potential of this species.

2. MATERIALS AND METHODS

2.1 Plant Material and Study Sites

Leaves of *Artemisia herba-alba* Asso were collected in March 2026 from two locations in Batna Province, northeastern Algeria: Ain Yagout and Arris. Ain Yagout is situated approximately 35 km northeast of Batna city, within a transitional zone between the mountainous areas and the High Plateaus. Arris is located in the southeastern part of Batna Province, within the Aurès Mountains and the Oued Abiod Valley. The two sites were selected to represent geographically distinct locations within the Batna region. Species identification was carried out using the floristic references of Quézel and Santa (1962–1963). The geographical location of Arris within Batna Province is shown in Figure 1.

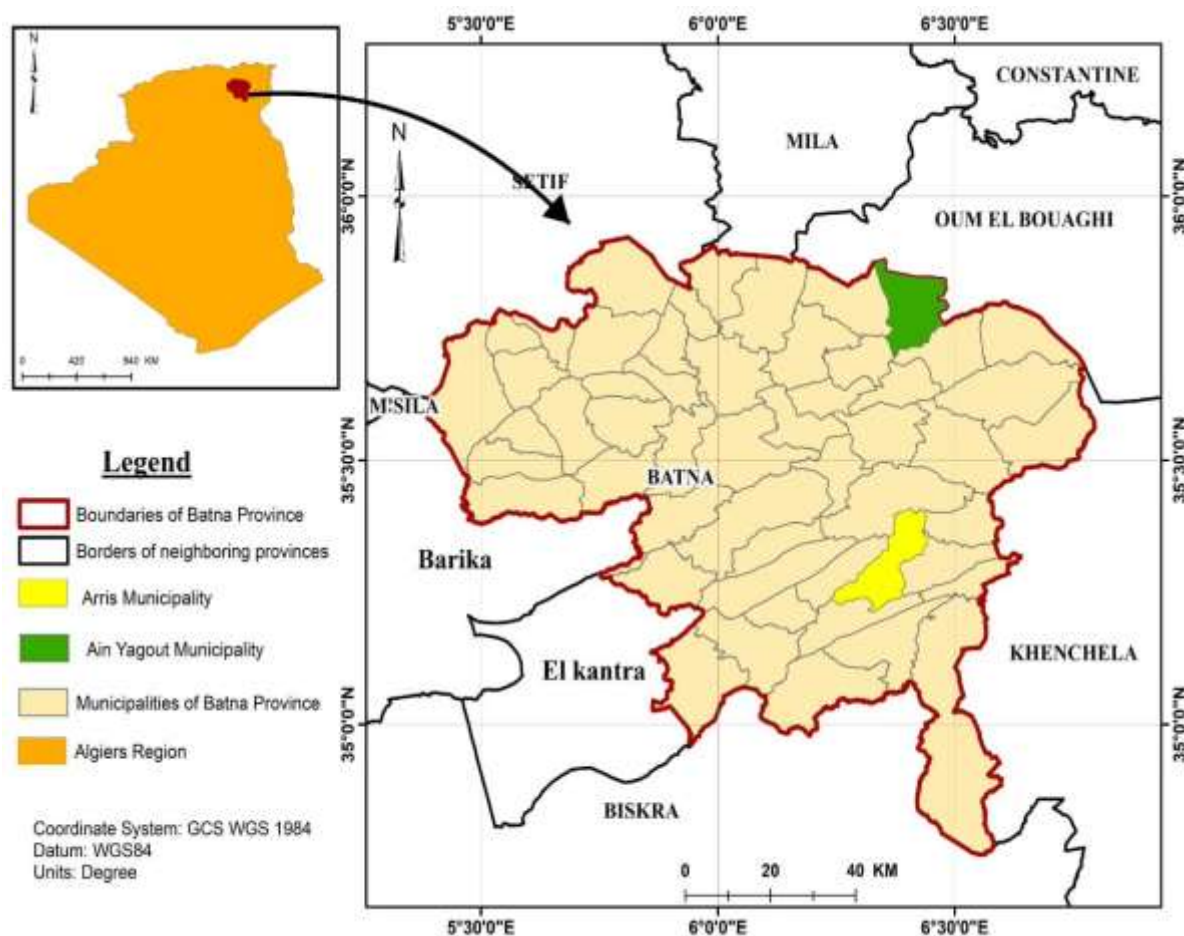


Figure 1. Geographic location of Arris municipality and Ain Yagout within Batna Province, Algeria (AMAMRA et al, 2026)

2.2 Preparation of Plant Material

Following collection, the leaves were separated from the plant material and dried for 15 days in a location protected from direct light. The dried leaves were subsequently ground and sieved to obtain a homogeneous fine powder. The powdered material was stored until extraction.

2.3 Preparation of Plant Extracts

Three extraction systems were evaluated: aqueous, ethanolic, and methanolic. All extractions were performed by maceration under mechanical agitation at room temperature.

2.3.1 Aqueous Extract

Ten grams of powdered plant material were suspended in 100 mL of distilled water and subjected to mechanical agitation for 24 h at room temperature. The mixture was then filtered, and the aqueous filtrate was dried in an oven at 40 °C.

2.3.2 Ethanolic Extract

Fifty grams of plant powder were mixed with 250 mL of 80% ethanol and macerated under mechanical agitation for 3 days at room temperature. After extraction, the mixture was filtered and the solvent was concentrated.

2.3.3 Methanolic Extract

Ten grams of plant powder were mixed with 100 mL of methanol/water (70:30, v/v) and subjected to mechanical agitation for 3 days at room temperature. The mixture was subsequently filtered, and the solvent was concentrated using a rotary evaporator.

Table 1. Operational conditions used for preparation of the aqueous, ethanolic, and methanolic extracts.

Extract	Plant material / solvent	Extraction conditions	Final treatment
Aqueous	10 g / 100 mL distilled water	24 h, mechanical agitation, room temperature	Filtration and oven drying at 40 °C
Ethanolic	50 g / 250 mL ethanol (80%)	3 days, mechanical agitation, room temperature	Filtration and solvent concentration
Methanolic	10 g / 100 mL methanol/water (70:30, v/v)	3 days, mechanical agitation, room temperature	Filtration and rotary evaporation

Note. All extraction procedures were conducted at room temperature under mechanical agitation. The methanol/water ratio is expressed as volume/volume (v/v).

2.4 Determination of Extraction Yield

Following solvent removal and drying, the crude extract obtained from each extraction procedure was weighed. Extraction yield was calculated as the percentage of dry extract recovered relative to the initial mass of plant material:

$$\text{Extraction yield (\%)} = \frac{\text{mass of dry extract}}{\text{initial mass of plant material}} \times 100$$

This measure was used to compare extraction efficiency among the three solvent systems and between the two sampling sites.

Statistical Analysis

All statistical analyses and graphical representations were performed using R software (version 4.5.1; R Core Team, 2025). Prior to inferential analyses, the distributional characteristics of the data were assessed to determine the most appropriate statistical procedures. Normality was evaluated using the Shapiro–Wilk test implemented in the stats package, while homogeneity of variances was assessed using Levene’s test from the car package (Fox and Weisberg, 2019). These preliminary analyses were conducted to verify the assumptions required for parametric statistical tests. Significant deviations from normality and/or homogeneity of variances justified the use of non-parametric statistical approaches. To evaluate the effects

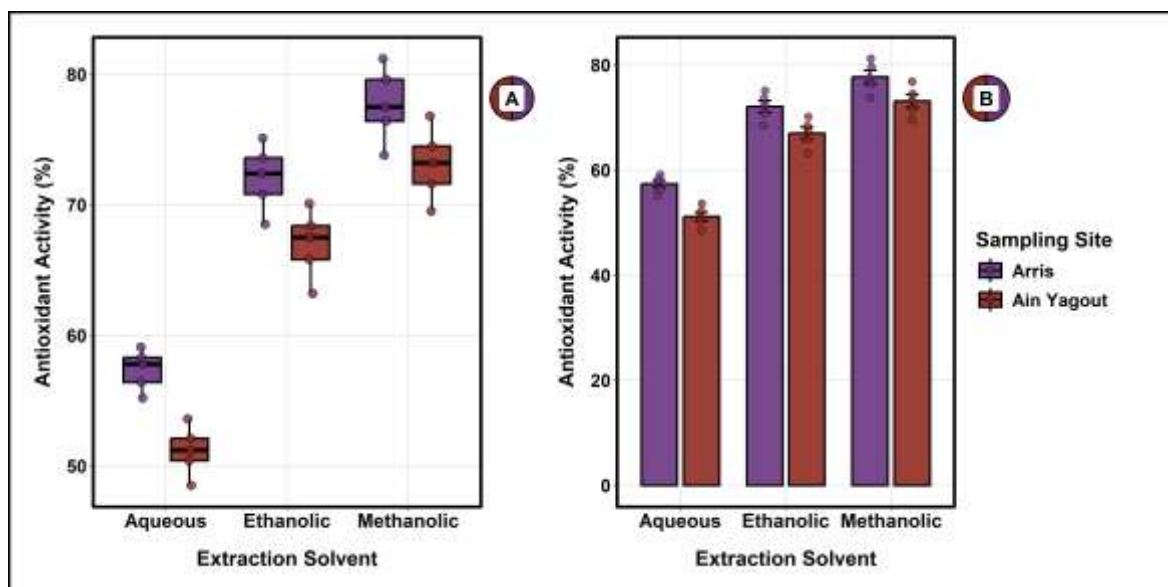
of extraction type and sampling site on extraction yield and antioxidant activity of *Artemisia herba-alba*, a non-parametric two-way analysis of variance was performed using the Scheirer–Ray–Hare (SRH) test. This test was selected because it permits the simultaneous evaluation of two categorical factors and their interaction when the assumptions of conventional two-way ANOVA are not adequately satisfied. Extraction Type and Sampling Site were considered as the main explanatory factors, while their interaction was included to determine whether the effect of one factor depended on the level of the other. The SRH analysis was performed using the ‘rcompanion’ package (Mangiafico, 2026). When significant effects were identified by the SRH analysis, post-hoc pairwise comparisons were performed to determine which experimental groups differed significantly. Dunn’s multiple comparison test (Dunn, 1964), implemented using the ‘FSA’ package (Ogle et al., 2026), was used for pairwise comparisons. P-values obtained from multiple comparisons were adjusted using the Benjamini–Hochberg (BH) procedure to control the false discovery rate. Statistical significance was considered at $p < 0.05$. Because the normality assumptions were not satisfied, the association between extraction yield and antioxidant activity was additionally evaluated using Spearman’s rank correlation. This non-parametric correlation analysis was selected to assess the strength and direction of the monotonic relationship between the two quantitative variables without requiring normally distributed data. Spearman’s correlation coefficient (ρ) was calculated using the ‘stats’ package in R. This analysis was used to complement the regression analysis and provide a non-parametric assessment of the association between extraction yield and antioxidant activity. A correlation heatmap was subsequently generated to provide a graphical representation of the strength and direction of the association between the quantitative variables. Spearman correlation coefficients were calculated using the `cor()` function from the stats package and visualized using `ggplot2` (Wickham, 2016). In the heatmap, the color and intensity of each cell represented the direction and magnitude of the correlation coefficient, respectively. The correlation heatmap was used as an exploratory graphical tool to facilitate the interpretation of relationships among the measured quantitative traits and to complement the results of the inferential analyses. The purpose of this analysis was to quantify the linear relationship between extraction yield and antioxidant activity and to estimate the coefficient of determination (R^2), regression coefficient, and statistical significance of the fitted relationship. Linear regression was performed using the stats package. Regression relationships were also examined separately according to sampling site and extraction type when appropriate, allowing potential differences in the relationship between the two traits among experimental groups to be visualized. Comprehensive graphical visualization was performed using the ‘ggplot2’ package (Wickham, 2016). Boxplots combined with jittered individual observations were used to visualize the distribution and variability of extraction yield and antioxidant activity across extraction types and sampling sites. Bar charts presenting group means with standard error (SE) bars and individual observations were used to illustrate group-level variation and the precision of the estimated means. Linear regression scatter plots with fitted regression lines and 95% confidence intervals were used to visualize the relationship between extraction yield and antioxidant activity. Heatmaps based on mean values were used as complementary exploratory visualizations to summarize and compare the magnitude of each quantitative parameter across extraction types and sampling sites, providing an integrated overview of the variation observed among experimental groups. Data preparation, filtering, grouping, and summarization were conducted using the ‘dplyr’ and ‘tidyr’ packages (Wickham et al., 2023). Composite figures and multi-panel graphical layouts were assembled using the ‘patchwork’ package. All analyses and graphical representations were conducted in R version 4.5.1, and statistical significance was evaluated at $p < 0.05$.

RESULTS

Variation in Antioxidant Activity and Extraction Yield of *Artemisia herba-alba* Extracts as a Function of Solvent and Sampling Site

The antioxidant activity and extraction yield of *Artemisia herba-alba* extracts varied according to both extraction solvent and sampling site (Figure 2). The distribution of antioxidant activity across the three extraction solvents and the two sampling sites is presented in the boxplots (Figure 2A), while the corresponding mean values $\pm$ standard error (SE) are shown in Figure 2B. For extraction yield, the distribution of individual observations is presented in Figure 2C, and the corresponding mean values $\pm$ SE are shown in Figure 2D. The statistical analysis demonstrated significant effects of extraction solvent and sampling site on both response variables. Extraction solvent had a highly significant effect on antioxidant activity ($F_{2,24} = 105.64$, $p < 0.001$) and extraction yield ($F_{2,24} = 79.71$, $p < 0.001$). Consistent with these statistical results, the graphical distribution showed clear differences among the aqueous, ethanolic, and methanolic extracts for both antioxidant activity (Figure 2A,B) and extraction yield (Figure 2C,D). Sampling site also significantly affected antioxidant activity ($F_{1,24} = 29.80$, $p < 0.001$) and extraction yield ($F_{1,24} = 36.16$, $p < 0.001$). As illustrated in Figure 1A–D, extracts obtained from Arris generally

exhibited higher antioxidant activity and extraction yield than those obtained from Ain Yagout. This site-related variation indicates that the geographical origin of the plant material contributed significantly to differences in extraction performance and antioxidant response. For antioxidant activity, the methanolic extracts showed the highest values at both sampling sites, followed by ethanolic extracts, whereas aqueous extracts generally exhibited the lowest values (Figure 2A,B). A similar pattern was observed for extraction yield, with methanolic and ethanolic extraction generally producing higher yields than aqueous extraction (Figure 2C,D). These trends suggest that solvent selection strongly influenced both the quantity of extract recovered and its antioxidant activity. In contrast, the interaction between extraction solvent and sampling site was not significant for antioxidant activity ($F_{2,24} = 0.19$, $p = 0.829$) or extraction yield ($F_{2,24} = 0.74$, $p = 0.488$). Thus, although the absolute values differed between Arris and Ain Yagout, there was no statistical evidence that the effect of extraction solvent depended on sampling site. The similar solvent-related patterns observed at both locations in Figure 2A–D are consistent with this non-significant interaction. The Dunn post-hoc test with Benjamini–Hochberg adjustment further identified the specific pairwise differences among the solvent $\times$ site combinations. For antioxidant activity, significant differences were mainly observed in comparisons involving aqueous and methanolic extracts. The strongest difference was detected between methanolic extracts from Ain Yagout and aqueous extracts from Arris (adjusted $p = 0.000208$). Significant differences were also observed between aqueous and methanolic extracts within and between sampling sites (adjusted $p \leq 0.00475$), supporting the clear separation of these groups observed in the distribution plots (Figure 2A). For extraction yield, significant pairwise differences were likewise observed mainly between aqueous extracts and methanolic or ethanolic extracts. The strongest difference occurred between methanolic extracts from Ain Yagout and aqueous extracts from Arris (adjusted $p = 0.000264$). Additional significant differences were observed between aqueous and methanolic extracts and between selected aqueous and ethanolic comparisons (adjusted $p < 0.05$), consistent with the differences in mean extraction yield shown in Figure 1C,D. Overall, the results demonstrate that extraction solvent was a major determinant of both antioxidant activity and extraction yield, while sampling site also contributed significantly to the observed variation. However, the absence of a significant solvent $\times$ site interaction indicates that the effect of solvent was broadly consistent across the two sampling locations. The graphical patterns in Figure 2A–D therefore provide visual support for the statistical findings, showing distinct solvent-related responses while maintaining a similar overall pattern between Arris and Ain Yagout.



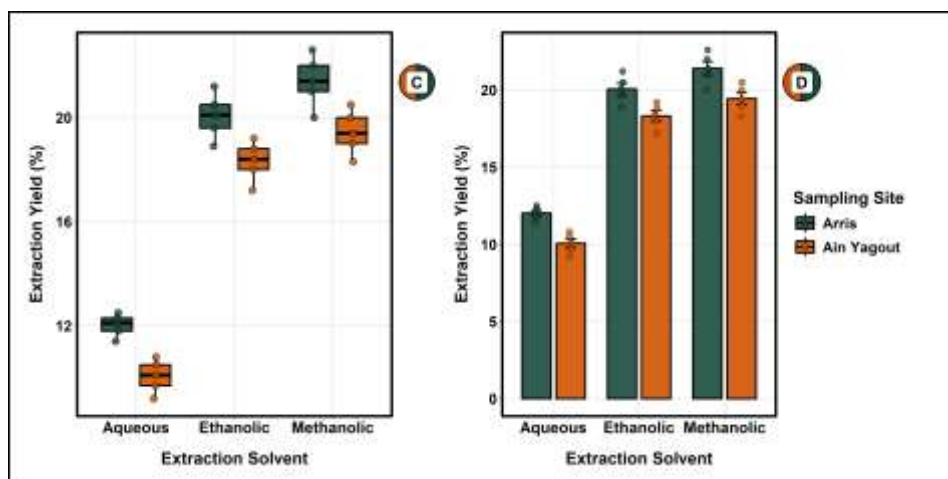


Figure 2. Antioxidant activity and extraction yield of *Artemisia herba-alba* extracts obtained using three extraction solvents from two sampling sites (Arris and Ain Yagout). (A) Boxplots showing the distribution and individual observations of antioxidant activity. (B) Mean antioxidant activity $\pm$ standard error (SE). (C) Boxplots showing the distribution and individual observations of extraction yield. (D) Mean extraction yield $\pm$ standard error (SE).

Effect of Extraction Solvent and Sampling Site on Antioxidant Activity and Extraction Yield of *Artemisia herba-alba*

The heatmaps show that both extraction solvent and sampling site influence the antioxidant activity and extraction yield of *Artemisia herba-alba* extracts. For antioxidant activity (Figure 3.A), the aqueous extracts showed the lowest values at both sites (51.2% for Ain Yagout and 57.4% for Arris), whereas ethanolic and methanolic extracts exhibited substantially higher activities. The highest antioxidant activity was observed for the methanolic extract from Arris (77.7%), followed by the methanolic extract from Ain Yagout (73.1%). For extraction yield (Figure 3.B), the aqueous extracts again showed the lowest yields (10.1–12.0%), while ethanol and methanol produced considerably higher yields. The highest extraction yield was obtained with methanol at Arris (21.4%), followed by methanol at Ain Yagout (19.4%). Overall, the results indicate that organic solvents, particularly methanol and ethanol, were more efficient than water for extracting antioxidant compounds from *A. herba-alba*, with the Arris samples generally exhibiting higher values than those from Ain Yagout.

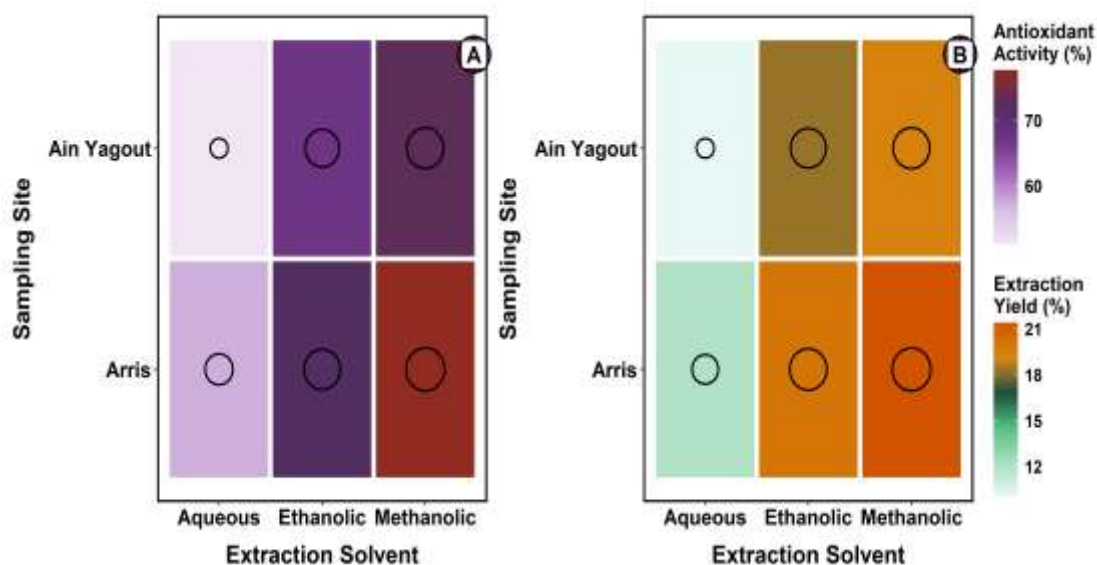


Figure 3. Heatmap visualization of antioxidant activity and extraction yield of *Artemisia herba-alba* extracts as affected by extraction solvent and sampling site. (A) Antioxidant activity (%) and (B) extraction yield (%). Color intensity represents the mean value for each solvent $\times$ sampling-site combination, while filled circles indicate the corresponding sampling sites.

Solvent- and Site-Dependent Relationships Between Extraction Yield and Antioxidant Activity

Figure 4. A illustrates the relationship between extraction yield (%) and antioxidant activity (%) according to the extraction solvent. A clear and highly consistent positive linear relationship was observed for all three extraction systems. The aqueous extracts showed the strongest linear association, with antioxidant activity increasing from approximately 49% to 59% as extraction yield increased from about 9.3% to 12.5% ($y = 3.163x + 19.337$; $R^2 = 1.00$; $p < 0.001$). This exceptionally high coefficient of determination indicates that, within the experimental range investigated, virtually all of the variability in antioxidant activity was linearly associated with extraction yield. The ethanolic extracts also exhibited a very strong positive relationship ($y = 3.006x + 11.856$; $R^2 = 0.99$; $p < 0.001$). Antioxidant activity increased progressively from approximately 63% to 78% over the corresponding increase in extraction yield. Similarly, methanolic extraction showed a strong and statistically significant relationship ($y = 2.627x + 27.769$; $R^2 = 0.98$; $p < 0.001$), with antioxidant activity reaching approximately 81% at the highest extraction yield. The differences in regression slopes provide additional information about the relationship between extraction yield and antioxidant activity. The slope represents the approximate increase in antioxidant activity associated with a one-unit increase in extraction yield within each solvent system. The aqueous regression displayed the steepest slope, followed by ethanol and methanol. However, the intercepts differed substantially among the three models, and the solvent groups occupied distinct ranges of extraction yield and antioxidant activity. Therefore, the slopes should not be interpreted independently of the experimental range. Overall, the results indicate that solvent type is associated with distinct extraction-performance profiles, and that increasing extraction yield was systematically accompanied by increased antioxidant activity. Importantly, the high R^2 values obtained for all three solvents ($R^2 \geq 0.98$) demonstrate that the observed relationships were not random and that extraction yield was strongly associated with antioxidant activity under the experimental conditions. This pattern may reflect the co-extraction of antioxidant-active constituents as the efficiency of compound recovery increased. Nevertheless, because correlation/regression does not by itself establish causality, the results should be interpreted as evidence of a strong association rather than proof that increasing extraction yield directly causes the increase in antioxidant activity. **Figure 4. B** presents the same relationship according to sampling site, namely Arris and Ain Yagout. Both sites displayed strong, positive, and statistically significant linear relationships between extraction yield and antioxidant activity. For Arris, the regression equation was $y = 2.077x + 32.017$, with $R^2 = 0.98$ and $p < 0.001$. For Ain Yagout, the corresponding equation was $y = 2.209x + 28.542$, with $R^2 = 0.97$ and $p < 0.001$. Thus, in both sampling locations, higher extraction yields were consistently associated with higher antioxidant activity. The regression lines are relatively close to one another, and their slopes are comparable (2.077 for Arris versus 2.209 for Ain Yagout). This suggests that the strength and direction of the extraction yield–antioxidant activity relationship were broadly similar between the two sampling sites. Nevertheless, the slightly different intercepts indicate that the predicted antioxidant activity at a given extraction yield was not identical between sites. Such differences may be related to variations in the chemical composition of the plant material associated with geographical origin, environmental conditions, soil characteristics, climatic factors, maturity stage, or other site-specific variables. These factors are potential explanations and would require independent chemical or environmental measurements to be demonstrated. A notable feature of **Figure 4. B** is the high degree of linearity despite the samples originating from two different locations. The R^2 values of 0.98 and 0.97 indicate that approximately 98% and 97% of the variation in antioxidant activity, respectively, is explained by the linear relationship with extraction yield within Arris and Ain Yagout. This consistency supports the reproducibility of the association across the two sampling sites. Overall, the results suggest that extraction efficiency and antioxidant potential are closely linked, while the magnitude of the response varies according to the extraction solvent and, to a lesser extent, the sampling site. The exceptionally high R^2 values further indicate that extraction yield can serve as a useful quantitative indicator of antioxidant activity within the experimental conditions examined. However, extrapolation beyond the observed extraction-yield ranges should be avoided, and formal statistical comparison of regression slopes/intercepts would be required to determine whether the differences between solvents or sites are statistically significant rather than simply numerically different.

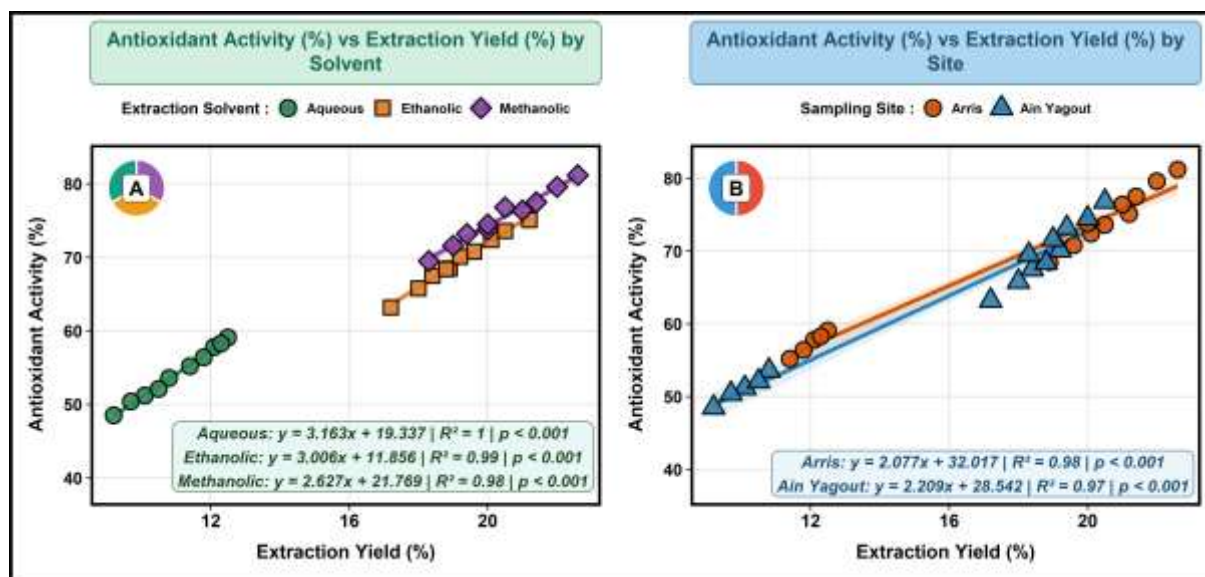


Figure 4. Linear relationships between extraction yield (%) and antioxidant activity (%) across different extraction solvents (A) and sampling sites (B).

DISCUSSION

The present study highlights the importance of both extraction conditions and geographical origin in determining the antioxidant potential and extraction efficiency of *Artemisia herba-alba*. A major

finding of this work is that the choice of solvent markedly influenced both the quantity of extract recovered and its antioxidant activity. In addition, samples collected from the two investigated sites displayed distinct levels of extraction performance, indicating that geographical origin may contribute to the variability observed in the bioactive properties of this species. The combined assessment of solvent effects, sampling-site variation, and the relationship between extraction yield and antioxidant activity provides a broader perspective on the factors influencing the extraction of bioactive compounds from *A. herba-alba* (Ayad et al., 2022; Belhatab et al., 2014). Among the solvents investigated, methanol generally produced the extracts with the highest antioxidant activity, followed by ethanol, whereas aqueous extraction resulted in the lowest antioxidant response. A similar tendency was observed for extraction yield, with methanol and ethanol recovering a greater amount of extractable material than water. This pattern suggests that the solvent used during extraction has a strong influence on the recovery of compounds contributing to antioxidant activity. Similar observations have been reported for medicinal and aromatic plants, where organic solvents or hydroalcoholic systems frequently provide greater recovery of phenolic and flavonoid compounds than water alone (Sultana et al., 2009; Do et al., 2014; Dai and Mumper, 2010). The higher efficiency of methanol may be related to its ability to solubilize a broad range of secondary metabolites, particularly compounds with antioxidant properties (Dai and Mumper, 2010; Xu et al., 2017). The relatively high performance of ethanol is also noteworthy. Although methanol gave the strongest response under the conditions investigated, ethanol showed a comparable overall extraction pattern and clearly outperformed aqueous extraction. This finding is consistent with previous studies showing that ethanol can efficiently recover antioxidant compounds from medicinal plants (Do et al., 2014; Ayad et al., 2022). From a practical perspective, the effectiveness of ethanol may also be relevant for future applications involving food, nutraceutical, or cosmetic products, where the use of solvents with greater suitability for these applications is generally preferred (Chemat et al., 2012). The geographical origin of the plant material also contributed significantly to the observed variability. Overall, the samples collected from Arris exhibited higher extraction yields and stronger antioxidant responses than those collected from Ain Yagout. Such differences may reflect variations in the environmental conditions prevailing at the two locations. Factors such as soil characteristics, temperature, water availability, altitude, solar exposure, and climatic stress can influence plant metabolism and consequently modify the accumulation of secondary metabolites (Ramakrishna and Ravishankar, 2011; Yang et al., 2018). In *A. herba-alba* and other medicinal plants, geographical and ecological conditions have been associated with differences in phytochemical composition and biological activity (Belhatab et al., 2014; Figueiredo et al., 2008). An important aspect of the present study is that the effect of the extraction solvent was broadly similar at both sampling sites. Although the absolute responses differed between Arris and Ain Yagout, the same general pattern was

maintained, with methanol and ethanol performing better than water. The absence of a significant interaction between solvent and sampling site suggests that the influence of solvent was relatively stable across the two geographical origins. This observation may be of practical importance because it indicates that the choice of extraction solvent remains a major determinant of extraction performance even when the plant material originates from different environments. Another relevant finding was the strong positive association observed between extraction yield and antioxidant activity. Across the different extraction systems, samples producing greater amounts of extract generally exhibited higher antioxidant activity. A similar relationship was observed when the data were considered according to sampling site, suggesting that this pattern was

not restricted to a single geographical origin. Such an association may be explained by the simultaneous recovery of antioxidant-active metabolites as the extraction efficiency increases. Phenolic compounds, flavonoids, and other secondary metabolites are known to contribute substantially to the antioxidant properties of many medicinal plants (Rice-Evans et al., 1996; Dai and Mumper, 2010; Xu et al., 2017). Therefore, conditions that favor the extraction of a larger fraction of these constituents may result in both higher extraction yields and stronger antioxidant responses (Sultana et al., 2009; Do et al., 2014).

Nevertheless, extraction yield should not be considered a direct measure of antioxidant quality. The total amount of extract reflects the overall quantity of compounds recovered from the plant matrix, but it does not provide information about their chemical identity or relative contribution to antioxidant activity. An extract with a high yield may contain large amounts of compounds with limited antioxidant capacity, whereas a lower-yield extract may be particularly rich in highly active constituents. Consequently, the strong relationship observed in the present study probably reflects, at least in part, the co-extraction of antioxidant compounds, but this hypothesis requires confirmation through detailed phytochemical characterization (Bakchiche et al., 2022; Altemimi et al., 2017). The differences observed between the two sampling sites may also indicate that the chemical composition of *A. herba-alba* is influenced by local environmental conditions. The slightly different relationships between extraction yield and antioxidant activity at the two sites suggest that plant material from each location may possess a distinct chemical profile. Differences in phenolic composition, flavonoid abundance, or other secondary metabolites could potentially explain these variations (Yang et al., 2018; Belhatab et al., 2014). However, such an interpretation remains hypothetical in the absence of direct chemical measurements. Future studies combining antioxidant assays with chromatographic and spectrometric analyses would therefore be valuable for identifying the compounds responsible for the observed geographical differences. The high consistency of the relationship between extraction yield and antioxidant activity across both solvents and sampling sites represents an additional contribution of this study. Rather than being restricted to a particular extraction condition or geographical origin, the positive association was maintained across the experimental groups. This suggests that extraction efficiency may provide useful preliminary information about antioxidant performance under the conditions investigated. However, this relationship should be considered specific to the experimental range examined and should not be extrapolated beyond it without further validation. Taken together, the findings demonstrate that the antioxidant potential of *A. herba-alba* extracts is influenced by both the extraction solvent and the geographical origin of the plant material. The superiority of methanolic and ethanolic extraction compared with aqueous extraction highlights the importance of solvent selection for recovering antioxidant-active constituents. At the same time, the differences between Arris and Ain Yagout emphasize the contribution of geographical variability to the bioactive properties of the species. The strong association between extraction yield and antioxidant activity further suggests that extraction efficiency and antioxidant potential are closely related under the conditions investigated.

From a broader perspective, these findings contribute to a better understanding of the factors controlling the recovery of bioactive compounds from *A. herba-alba*. They also underline the importance of considering both environmental origin and extraction strategy when comparing the antioxidant properties of plant extracts. Further investigation of the phenolic and flavonoid profiles of the extracts, together with targeted chromatographic analyses, would help clarify the chemical

basis of the differences observed between solvents and sampling sites and would provide stronger evidence linking extract composition to antioxidant activity (Bakchiche et al., 2022; Altemimi et al., 2017).

CONCLUSION

The present study demonstrates that both extraction solvent and sampling site significantly influenced the extraction yield and antioxidant activity of *Artemisia herba-alba*. Among the three extraction systems investigated, methanol produced the highest extraction yield and antioxidant activity at both sampling sites, followed by ethanol, whereas aqueous extraction consistently

resulted in the lowest values. The higher values observed for samples from Arris compared with Ain Yagout further indicate that geographical origin contributed to the variation in extraction performance and antioxidant response. Importantly, the solvent $\times$ sampling site interaction was not significant for either antioxidant activity or extraction yield. This indicates that, despite differences in absolute values between Arris and Ain Yagout, the relative pattern associated with the three extraction solvents remained broadly consistent across the two locations. The heatmaps provided an integrated visualization of these mean responses and clearly illustrated the differences among solvent $\times$ site combinations. The regression analyses further revealed strong positive linear relationships between extraction yield and antioxidant activity within each solvent and sampling site, with very high coefficients of determination. These findings indicate that higher extraction yields were consistently associated with higher antioxidant activity under the experimental conditions investigated. However, these regression relationships should be interpreted as associations rather than evidence of causality, and differences among regression slopes or intercepts would require formal statistical comparison before concluding that the relationships differ significantly between solvents or sites. Taken together, the results highlight the importance of solvent selection and geographical origin in determining the extraction efficiency and antioxidant characteristics of *A. herba-alba*. The consistently higher responses obtained with methanolic extraction suggest that solvent polarity and extraction efficiency may play an important role in the recovery of antioxidant-active constituents. Nevertheless, chemical profiling of the extracts would be necessary to identify the specific compounds responsible for the observed differences and to clarify the mechanisms underlying the relationship between extraction yield and antioxidant activity.

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