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Morphological Variability and Adaptability and Phenolic Content of *Ajuga iva* Collected from Distinct Moroccan Geographical Locations

Laila Lahrizi [1], Faouzi Errachidi [1], Driss Ousaaid [2,*] and Lahsen El Ghadraoui [1]

[1] Laboratory of Functional Ecology and Environmental Engineering, Department of Biology, Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University, Fes 30050, Morocco; lahrizilaila12@gmail.com (L.L.); faouzi.errachidi@usmba.ac.ma (F.E.); lahsen.elghadraoui@usmba.ac.ma (L.E.G.)

[2] Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNAMOPEQ), Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco

* Correspondence: driss.ousaaid@usmba.ac.ma

Abstract: Adaptation plasticity constitutes a key factor in the development of such plants under different ecoclimatic conditions. The current study was designed to determine the morphological and phenotypic variability of *Ajuga iva* collected from distinct geographical locations in Morocco and their phenolic content. Four samples of *Ajuga iva* were collected to evaluate the morphological variability and adaptability to ensure the sustainable growth of this medicinal plant known for its unique biological properties. Eleven morphological parameters were selected, including length, width, number, distance, and the thickness of different parts, as well as total phenolic content. Statistical tools, such as principal component analysis, and correlation were used to assess the change in the parameters under study based on the geographical origin. Treatment of the obtained results revealed a high variability of morphological parameters of different samples according to the site and altitude, and the interaction between the studied factors. The sample collected from Jbel Zerhoun registered the highest values of the following morphological parameters: APL (12.47 ± 2.09 cm), UPL (6.56 ± 0.40 cm), APW (3.28 ± 1.59 g), UPW (1.24 ± 0.19 g), LW (0.40 ± 0.10 cm), LN (44 ± 4), and NN (21.33 ± 2.51). The samples collected from an altitude above 1000 m showed the highest values of different morphological parameters (aerial part length and weight, underground part weight, leaf number and weight, and node number) and registered the maximum of TPC (124.12 mg GAE/g, 128.86 mg GAE/g, and 164.75 mg GAE/g for samples collected from Immouzzer Kander, Jbel Zerhoun, and Azrou, respectively). Therefore, the samples from high elevations can resist environmental critical conditions by the emergence of different biochemical processes to synthesize bioactive compounds with multifaceted effects.

Keywords: *Ajuga iva*; morphological attributes; environmental conditions; total phenolic content

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1. Introduction

Environmental conditions are limiting factors in developing numerous cultures [1–3]. Several plants grow naturally in distinct pedoclimatic conditions tolerating unfavorable conditions such as drought and cold [4–6]. To resist these critical conditions, medicinal plants produce a broad spectrum of phytochemicals to minimize the negative effects of abiotic and biotic stresses [7]. One of the most potent plants, *Ajuga iva* is utilized as a natural remedy in numerous traditional medicines, including Ayurveda, Chinese, Arabic, Iranian, and European ones [8,9]. It is a member of the Lamiaceae family and reaches 20 cm in height with dense and tight leaves. The inflorescence of the plant is made up of lone flowers that grow in the leaf axils from May through June. Its fruits are nutlets that are crosslinked [10–12].

Ajuga iva develops in different regions of North Africa and South Europe [10,11]. Morphological transitions are one of the strategies employed by plants to adapt to constant variations in climatic conditions [13]. Critical situations like biotic and abiotic stressors can be resisted by plants thanks to various metabolic changes [1,5,14]. *A. iva* occupies various geographical areas that can impose several environmental constraints on its normal growth and survival [10]. In response, different mechanisms are involved to determine and respond appropriately to multiple environmental stresses [15,16]. Morphological changes are one of the most common strategies employed to cope with different stressor agents [17,18]. Different factors control the nutritive value of plants, including intrinsic (phytochemistry and variety) and extrinsic factors (pedoclimatic conditions, maturity, and storage) [19,20]. The escalating demand for this herb, coupled with climatic changes and unsustainable harvesting practices, has led to the depletion of this medicinal plant. Habitat loss and fragmentation constitute a real challenge that disrupts gen flow, population size, and increases the probability of extinction [21]. *A. iva* provides a real source of bioactive compounds and serves as the basis for the development of traditional and conventional medicines. In fact, mounting evidence confirms the utility of this plant against numerous human diseases such as diabetes, obesity, inflammation, infection, and cardiovascular diseases [10,22]. The delve into the phytochemistry of *Ajuga iva* showed a broad spectrum of biologically active compounds, including steroids, terpenoids, flavonoids, phenolic acids, and fatty acids [12]. LC/UV/MS revealed 32 bioactive compounds detected in different amounts in the *A. iva* aqueous extract, including ferulic acid (19.06%), quercetin (10.19%), coumaric acid (9.63%), apigenin 7-(2-O-apiosylglucoside (6.8%), cholesterol (6.17%), luteolin (4.53%), ajugasterone D (4.29%), kaempferide (4.2%), epigallocatechin gallate (3.94%), and vanillin (3.17%) [23].

It has been shown that light conditions have a direct impact on the morphological and photosynthetic responses of *Brassica oleracea* var. sabellica by increasing plant height, and leaf number, length, and width [24]. Plant height plays an important role in ameliorating the light access and resisting lodging and crowding [25]. Pedoclimatic factors firstly alter the plant physiology, phenology, and its geographical distribution [26]. Consequently, a significant reduction in plant productivity was observed [27].

Within this framework, the current study was undertaken to determine the morphological variability and adaptability of different samples of *Ajuga iva* collected from distinct geographical locations.

2. Materials and Methods

2.1. Sampling Site Characteristics

Four samples of *Ajuga iva* were collected from distinct Moroccan geographical locations, including Jbel Zerhoun, Immouzzer Kandar, Azrou, and Fez. The period of sampling extends from June to August 2022. Table 1 summarizes the geographical characteristics of different sites.

Table 1. Sample symbols and climatic characterization of different geographical locations.

Geographical Zone	Jbel Zerhoun (JZ)	Immouzzer Kandar (IM)	Fez	Azrou (Az)
Altitude (ALT) (m)	1005	1317	580	1285
Latitude	34°02′02″ N	33°44′0.24 N	34°01′26″ N	33°27′14″ N
Longitude	5°30′42″ W	5°0′37.8 W	5°00′06″ W	5°12′34″ W
Bioclimatic stage	Sub-humid	Sub-humid	Semi-arid	Sub-humid
Rainfall (mm)	511	468.2	690	664
Temperature average (°C)	17.1	17.1	17.75	12.1

2.2. Morphological Parameters

The morphological parameters of the plants under study were determined on the basis of a list of descriptors according to previous published studies [18,24]. The principal stems, roots, and leaves were prepared for different measurements, including length, width, thickness, and so on. The weight of both aerial and underground parts of *A. iva* were determined using an analytical balance (Sartorius Entris 64-1S). For morphological attributes, we used a digital caliper (Carrera Precision Instrument, reading at 0.01 mm) to measure the length and width of leaves, roots, and stems, as presented in Figure 1. In the same way, the thickness was determined and the number of leaves were counted (Figure 1). Ten plants were randomly chosen from each sample for morphological analysis.

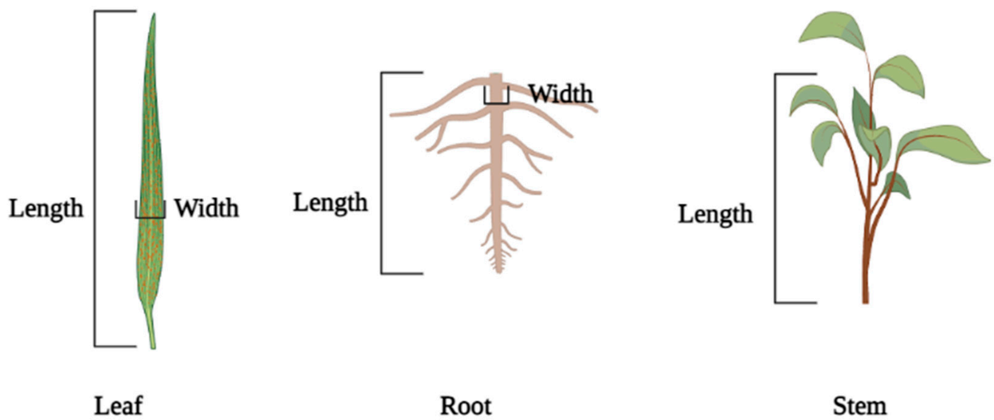

Figure 1. Different measured parts of *Ajuga iva* (image generated by Biorender.com).

2.3. Extraction Method

All samples collected from different geographical locations were cleaned and air dried to prepare different extracts. Three extractor solvents were selected to assess the extraction procedure, including water, ethanol, and methanol. The solid-to-liquid ratio was 1/10 (w/v). The obtained extracts were filtrated (Whatman, n°1) and kept in the refrigerator at 4 °C until experimental measurements were taken.

2.4. Determination of Total Phenolic Content

The quantification of TPC of different extracts (water, methanol, and ethanol) was determined by the Folin–Ciocalteu reagent using the method described by [28] with slight modifications. Briefly, 50µL of each extract was blended with 450 µL of freshly prepared Folin–Ciocalteu reagent and 450 µL of Na_2CO_3. The mixture was incubated at room temperature in darkness. After 2 h, the optic density was read at 760 nm. Then, the concentration of total phenolic compounds was determined by reference to the calibration curve using gallic acid as a standard.

2.5. Statistical Analysis

The obtained results are displayed as mean ± SD. Graph Pad Prism 6 software was used to do the Tuckey test for the comparison of various values; then, the principal component analysis two-way ANOVA was performed. A probability value of $p < 0.05$ was used to determine the statistical significance. Then, using PAST 3 software, the Pearson correlation coefficient was used to determine the correlation between all of the analyzed parameters.

3. Results

3.1. Morphological Parameters

Table 2 displays the obtained results of different determined parameters of *Ajuga iva*. The treatment of results indicates the highest variability of different parameters of samples under study. The sample from Jbel Zerhoun registered the highest length of aerial part (12.47 ± 2.09 cm), aerial part weight (3.28 ± 1.59 cm), leaf width (0.4 ± 0.10 cm), leaf number (44 ± 4), and node number (21.33 ± 2.51). On the other hand, the sample from Azrou showed the lowest values for the following parameters: aerial part length (8.73 ± 2.04 cm), aerial part weight (1.04 ± 0.02 g), leaf length (0.8 ± 0.10 cm), and leaf width (0.25 ± 0.05 cm). The samples collected from high altitude, such as JZ, IM, and AZ, recorded the highest values of different parameters under study.

Table 2. Morphological parameters of different samples under study.

	JZ	IM	FEZ	AZ
Aerial part length (APL) (cm)	12.47 ± 2.09 [a]	9.36 ± 2.28 [a]	10.47 ± 0.95 [a]	8.73 ± 2.04 [a]
Underground part length (UPL) (cm)	6.56 ± 0.40 [a]	4.70 ± 0.26 [a]	4.23 ± 0.25 [a]	7.76 ± 2.02 [a]
Aerial part weight (APW) (g)	3.28 ± 1.59 [a]	1.64 ± 0.22 [a]	1.70 ± 0.21 [a]	1.04 ± 0.02 [a]
Underground part weight (UPW) (g)	1.24 ± 0.19 [a]	0.50 ± 0.08 [a]	0.78 ± 0.03 [a]	0.61 ± 0.27 [a]
Leaf length (LL) (cm)	2.56 ± 0.30 [a]	3 ± 0.26 [a]	3.13 ± 0.15 [a]	0.80 ± 0.10 [a]
Leaf width (LW) (cm)	0.40 ± 0.10 [a]	0.33 ± 0.05 [a]	0.38 ± 0.02 [a]	0.25 ± 0.05 [a]
Leaf number (LN)	44 ± 4	34.66 ± 10.06 [a]	36 ± 2 [a]	35.33 ± 2.30 [a]
Node number (NN)	21.33 ± 2.51 [a]	18.33 ± 3.51 [a]	18 ± 1 [a]	18.66 ± 3.05 [a]
Stem thickness (ST) (cm)	0.26 ± 0.05 [a]	0.30 ± 0.10 [a]	0.23 ± 0.05 [a]	0.23 ± 0.15 [a]

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$).

3.2. Total Phenolic Content

The obtained results of total phenolic content of different samples under study are displayed in Table 3. Different solvents with different polarities were used to assess the extraction of phenolic content and showed high variabilities of total phenolic content. The water extract showed the highest values of TPC: 128.86 mg GAE/g for JZ, 124.12 mg GAE/g for IM, and 164.75 mg GAE/g for AZ. The lowest value of TPC was registered in the sample collected from the Fez region, followed by Immouzzer, Jbel Zerhoun, and Azrou, respectively, for ethanol extract.

Table 3. Total phenolic content of different samples under study.

	JZ	IM	FEZ	AZ
Water	128.86 [a]	124.12 [a]	25.27 [b]	164.75 [a]
Ethanol	74.03 [b]	61.65 [b]	53.84 [a]	74.29 [b]
Methanol	29.82 [c]	36.64 [c]	25.87 [b]	62.60 [c]

Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test.

Statistical analysis of the obtained results showed a high variability of phenolic content between all studied samples and extracts of the same sample (Table 3). A statistically significant difference between the aqueous extract and other extracts under study was observed ($p < 0.05$). Water thus proved to be the most effective solvent for extracting phenolic content from the studied vegetal matrix. From all the samples that were examined, methanol resulted in the least phenolic content (Table 3).

Concerning the correlation analysis, a positive correlation was observed between TPC and leaf length, leaf number, and aerial part length ($r = 0.71394$, $r = 0.88403$, $r = 0.75402$, respectively) (Table 4).

Table 4. Pearson correlation coefficients between morphological parameters, total phenolic content, and altitude of different samples under study.

	APL	UPL	APW	UPW	LL	LW	LN	NN	ST	TPC
APL		0.91899	0.033646	0.047797	0.5412	0.13776	0.073632	0.19273	0.91682	0.75402
UPL	−0.0811007		0.99435	0.79674	0.4257	0.4257	0.71318	0.54421	0.68336	0.1892
APW	0.96635	−0.0056527		0.088698	0.57961	0.20851	0.053638	0.11428	0.70873	0.95743
UPW	0.9522	0.20326	0.9113		0.82837	0.31901	0.027697	0.11679	0.87904	0.92274
LL	0.4588	−0.90742	0.42039	0.17163		0.16574	0.87547	0.95891	0.53435	0.28606
LW	0.86224	−0.5743	0.79149	0.68099	0.83426		0.38697	0.57398	0.78956	0.37535
LN	0.92637	0.28682	0.94636	0.9723	0.12453	0.61303		0.034921	0.941	0.88403
NN	0.80727	0.45579	0.88572	0.88321	−0.0411091	0.42602	0.96508		0.83633	0.63131
ST	0.083178	−0.31664	0.29127	−0.12096	0.46565	0.21044	0.059001	0.16367		0.74013
TPC	−0.24698	0.8108	−0.042568	−0.077261	0.71394	−0.62465	0.11597	0.36869	0.25987	

3.3. Multivariate Analysis

To discriminate between all samples under study, the principal component analysis was carried out as an excellent statistical tool to discover the relationship between all investigated parameters. Figure 2 summarized the two principal components extracted in the PCA model of samples under study. The sum of the first two PCs presented an accumulative variance of 86.713%. The PC1 explained 54.264% and showed in its negative part the samples collected from Azrou and Immouzer Kandar, while the positive part contained samples collected from Jbel Zerhoun and FEZ. The second PC presented an accumulative variance of 32.449%. The negative part of this PC contained samples collected from Immouzzer Kandar and FEZ, while the positive part presented the other samples under study. Analyzing the score plot (Figure 2), it is worth noting that the sample collected from Jbel Zerhoun correlated with the highest values of the following morphological parameters: NN, LN, UPW, APW, and APL, whereas the sample collected from FEZ correlated with LW, LL, and to a minor extent, ST. Samples collected from Immouzzer Kandar (IM) and Fez (FEZ) were very close, while other samples were clearly estranged. All parameters under study, except UPL and TPC, were highly positively related to PC1, whereas ST, LW, and LL were negatively associated with PC2.

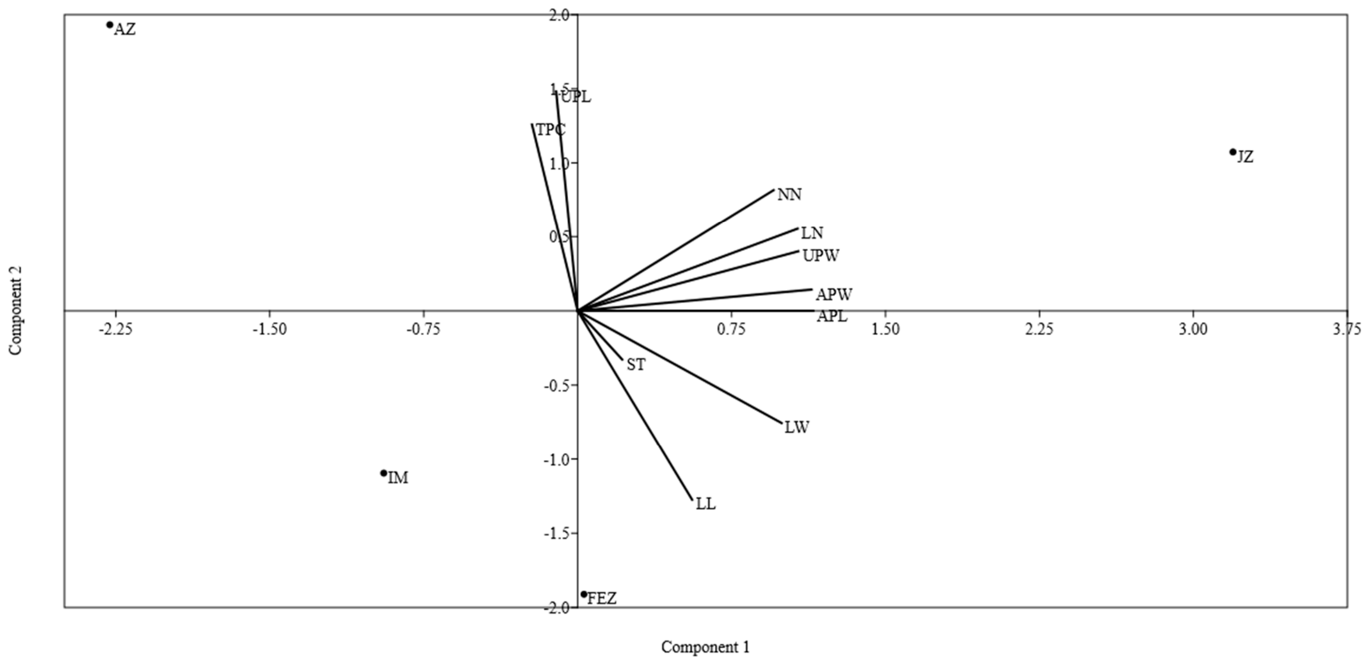

Figure 2. PCA score and loading plots of different parameters and samples; JZ, IM, FEZ, and AZ represent samples as mentioned in Table 1. APL: Aerial part length, UPL: underground part length, APW: aerial part weight, UPW: underground part weight, LL: leaf length, LW: leaf width, LN: leaf number, NN: node number, ST: stem thickness, and TPC: total phenolic content.

The obtained findings of the correlation study performed using all morphological parameters and total phenolic content are shown in Table 4. The treatment of findings showed a positive correlation between underground length (UPL) and leaf length (LL) and total phenolic content (r = 0.8108 and r = 0.71394, respectively), while negative correlation has been found between the following parameters: aerial part length (APL), aerial part weight (APW), underground part weight (APW), and leaf weight (LW) with total phenolic content (TPC) (r = −0.24698, r = −0.042568, r = −0.077261, r = −0.62465, respectively).

4. Discussion

Morphological attributes of plants constitute a form of adaptation to diverse pedoclimatic conditions. Multiple physiological changes are dependent on several factors, including drought, cold, light, altitude, and biostimulants [29,30]. The current study was undertaken to investigate for the first time the impact of pedoclimatic conditions on the morphological attributes and phenolic compound accumulation of different samples of *Ajuga iva* collected from Moroccan distinct geographical origins. Treatment of the obtained results showed a significant variability of morphological parameters of the samples under study. The sample collected from Jbel Zerhoun (JZ) registered the highest values of morphological parameters, including aerial part height and weight, underground part weight, leaf weight, leaf number, and node number. Concerning the phenolic content, the samples from Jbel Zerhoun and Azrou were the richest in phenolic content (Table 3). Pioneer plants need to cope with a series of environmental challenges by morphological adaptability and phenolic accumulation in the plant.

The different morphological features of *Ajuga iva* are impacted by critical environmental conditions. Poor leaf growth parameters, such as form, size, leaf area, intensity, cuticle waxiness and pubescence composition, dry weight, density, and root length, are the result of unfavorable conditions [31]. Different markers of a plant's adaptability to adverse conditions can be manifested by early maturity, leaf rolling, erect leaf habit, deep root system, and reduced leaf area [32–34].

A plant typically uses three methods, namely escape, tolerance, and avoidance, to counteract the negative effects of drought [35]. The first strategy enables the plant to complete its life cycle fast before stress manifests, which implies that fewer seeds will be produced [34]. Low tissue water potential and osmotic changes that support plant turgor make up the drought tolerance mechanism. In addition, the plant may preserve its water potential, develop deeper roots in the ground, increase transpiration, and lessen water loss from tissues due to the drought avoidance mechanism [34,36]. According to this theory, the varying pedoclimatic conditions at each study station could account for the great variety of morphological indicators of *Ajuga iva* sampled from various geographic areas. Furthermore, altitude, light, temperature, relative humidity, and wind speed constitute important elements in plant life [30]. They affect the morphological characteristics of plants and their ecophysiological reactions [30,37]. The elevation of altitude induces a decrease in the height of plants and increases the number of branches, thickening of branches, chlorophyll content, photosynthetic potential, and reduces stomatal breathing [38]. Additionally, the expression levels of genes involved in the formation of chlorophyll (*HEMA*, *CLH*, *CHLI*, *CHLH*, and *CHLG*) and photosystem II (*PsbB*, *PsbD*, and *PsbO*) are directly influenced by light intensity [39]. The two processes of acclimation and adaptation, which represent environmental and genetic factors, respectively, confuse the link between altitude and leaf morphology [40]. During plant life, photosynthesis is a crucial biological process that affects the bioaccumulation of energy and dry matter in plants [39].

The sample collected from Jbel Zerhoun showed the highest values of the following morphological parameters: aerial part length, underground part weight, leaf width, leaf number, and node number. It is worth noting that all samples were collected from geographical sites with an altitude of more than 1000 m except the Fez region, which registered the lowest values of UPL, APW, NN, and ST. The findings evoked by Hovenden et al. demonstrated that although leaf length, width, and area are partially influenced by hereditary factors, they are malleable and can change response to environmental conditions at a given [41]. Abello et al. found that Kale (*Brassica oleracea* var. sabellica) cultivated under different light conditions physiologically responded differently. The authors found that green and blue light were the most appropriate for plant height development and photosynthetic rate, while ambient light boosts the number, length, and width of leaves [24]. In addition, soil plays a key role in plant development by providing all nutritious essential elements [42,43]. All these factors can have a direct impact on enhancing or diminishing the quantity and quality of herb performance [44]. Importantly, *Ajuga iva* plays an important role in the mountain ecosystem in many aspects, such as water retention, soil amelioration, forest biodiversity, ecological markers, and nutrient recycling [45].

Environmental stresses modify a plant's membrane fluidity, water, and ionic composition, which results in reactive oxygen species that reduce the plant capacity to photosynthesis and destabilize its DNA, RNA, and proteins [46]. Numerous factors, including microhabitat and microclimate, have a strong correlation with the chemical composition and distribution of medicinal plants [47].

Concerning the total phenolic content, various reports have investigated the phenolic content of *Ajuga iva* sampled from various regions of Morocco. Senhaji et al. found that the macerated methanol extract was the richest in TPC with a value of 25.26 ± 0.95 μg GAE/g, followed by ethyl acetate extract with a value of 24.19 ± 1.29 μg GAE/g [48]. Bouyayhya et al. investigated the TPC of *Ajuga iva* (L.) Schreb sampled from the Ouezzane area (North Morocco) [49]. The authors found that the ethanol extract registered an amount of 49.75 ± 2.08 mg GAE/g extract. The aerial part of *A. iva* contained a quantity of TPC of 44.41 ± 0.22 mg GAE/g extract [50].

The type and quantity of extractor solvents have also had a significant impact on the extractability of phenolic compounds [51]. Additionally, the type and polarity of the targeted phytocompounds may be strongly correlated with this heterogeneity of TPC levels found in different extracts. The less polar solvents, such as acetone 80%, were found to have the largest concentrations of phenolic content [51]. In the same context, Taneva et al. evoked that the ethanol 50% was effective to extract the maximum phenolic content [52]. The obtained results are in line with our previous study, which documented that water was the most appropriate extractor solvent to recover the highest amounts of TPC [53].

The variability of the phenolic content of different samples under study is highly dependent on the ecoclimatic characteristics of different geographical sites under study. The obtained results agree with several studies that demonstrated the impact of climatic conditions, soil characteristics, and elevation above sea level [54–58]. The study by Zargoosh et al. found a direct impact of ecological conditions on phenolic accumulation in *Scrophularia striata*, which enhanced the emergence of different chemical processes in the plant [30]. Authors found that the plants harvested at high elevations registered the highest amounts of TPC (47.62 μg GAE/g), which significantly correlated with altitude and all soil properties [30]. The phenolic composition of plants is highly controlled by genetic factors and the environment [59]. In fact, rainfall, temperature, soil characteristics, and environmental factors can directly alter plant chemical composition and consequently their biological properties [59–61].

Therefore, the morphological attributes and phenolic content constitute a direct criterion to predict the development conditions of medicinal plants. The critical ecoclimatic conditions boost phenolic accumulation. The obtained results could be of crucial importance to select the right area to harvest *A. iva* with high TPC, which could be a candidate for further experimental investigation in vitro and in vivo.

5. Conclusions

According to the results of the current study, ecological factors including height, rainfall, temperature, and bioclimatic stage significantly affect the morphological characteristics and phenolic accumulation of *A. iva*. The samples that came from the highest altitudes had the highest concentrations of the examined morphological characteristics and total phenolic contents. The best location to grow *A. iva* producing a considerable amount of phenolic compounds is at Jbel Zerhoun.

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