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Original Research Article

Biochemical and Climatological Analysis of Three Spontaneous Populations of the *Chamaerops humilis*L. (Palm Doum) Collected from Semi-Arid and Sub-Humid Regions, Morocco

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ABSTRACT

Chamaerops humilis L. is one of the medicinal herbs consumed as food or used to treat different ailments in folk medicine. It contains numerous phytochemical compounds such as polyphenols, flavonoids, tannins, and so on. The present study was designed to investigate the biochemical variability of three ecotypes of *Chamaerops humilis* L. and climatological parameters and their influence on the distribution and development of *Chamaerops humilis* L. leaves of both heritage and emblematic value in the Moroccan herbarium. Three samples of *Chamaerops humilis* L. were collected from different regions viz El Hajeb, Fez, and Sefrou. Biochemical examination (Total soluble sugars (Dinitrosalicylic acid (DNS), proteins (Lowry's method), and vitamin C (Colorimetric assay), as well as the content of polyphenols (Folin-Ciocalteu method) and flavonoids (aluminum trichloride method) of the leaves of *Chamaerops humilis* L. were determined. The results of the biochemical quantification revealed that the sample of Fez was the richest in phenolic and flavonoid contents with values of 75.390 mg AGE/g DM and 3.257 mg QE/g DM, respectively, while the sample collected from El Hajeb contained high levels of primary metabolites with the following; total soluble proteins content 631.145 mg/g FM and vitamin C content 0.613 mg/g FM. The present work showed significant diversity in the different studied parameters of *C. humilis*, due to the characteristics of each station, such as pedoclimatic conditions.

Keywords: *Chamaerops humilis* L., biochemistry variability, climatology variability, Morocco.

Introduction

Chamaerops humilis L. (*C. humilis*) is a wild species that grows naturally in warm and temperate climate.[1] It is a member of the Arecaceae family.[2] According to Merlo and his colleagues, this species prefers calcareous soils but can grow in any soil.[3] This plant is a major biological indicator of the thermo-mediterranean vegetation stage, according to the ecological plan. *C. humilis* is a remarkable species due to its ecological, socioeconomic, dietary, and medicinal value.[4,5] *C. humilis* provides several pharmacologicla effects, for instance, hypoglycemic effect, anti-inflammatory effect, anabolic and antiseptic properties.[4,6] It also aids in the fight against erosion and desertification.[4,6] The fruits of *C. humilis* are well known for their nutritional enrichment.[7] Because of these characteristics, *C. humilis*. is a plant of universal value that colonizes arid and semi-arid ecological surfaces. However, this species is constantly subjected to increasing and uncontrolled human pressures and significant ecological effects (type of climate, altitude, temperature, precipitation, and soil type). These factors directly jeopardize the preservation and renewal of this biological resource, as well as the region's ecological balance. The aim of our work is to assess the biochemical variability of three ecotypes of *C. humilis* located in different areas based on climatic conditions, to better understand the influence of ecolologic factors on the development of this plant cover. In addition, to ensure long-term production and a higher value for this species.

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Materials and Methods

Sampling area

Samples of *C. humilis* were collected from three different bioclimatic areas: Sefrou, Fez, and El Hajeb (Figure 1). Table 1 summarizes the climate data from each station. Samples were collected in May 2018. Professor Amina Bari a botanist identified the plant sample under voucher number CH185181S1 (FSDM, USMBA, FEZ, MOROCCO).

Biochemical study

The biochemical study sought to identify and quantify the macronutrients and micronutrients of *C. humilis* extracted from leaves of studied samples. Total soluble sugars, proteins, and vitamin C content, as well as polyphenols and flavonoids content, were determined in *C. humilis*. The total soluble sugars content was determined using dinitrosalicylic acid (DNS), which formed a chromophore between the reagent and ending-reducing sugars.[8]Lowry's method was used to determine protein content based on the color test with the Folin-Ciocalteu method.[9]Colorimetric assay was used to determine the vitamin C content.[9]

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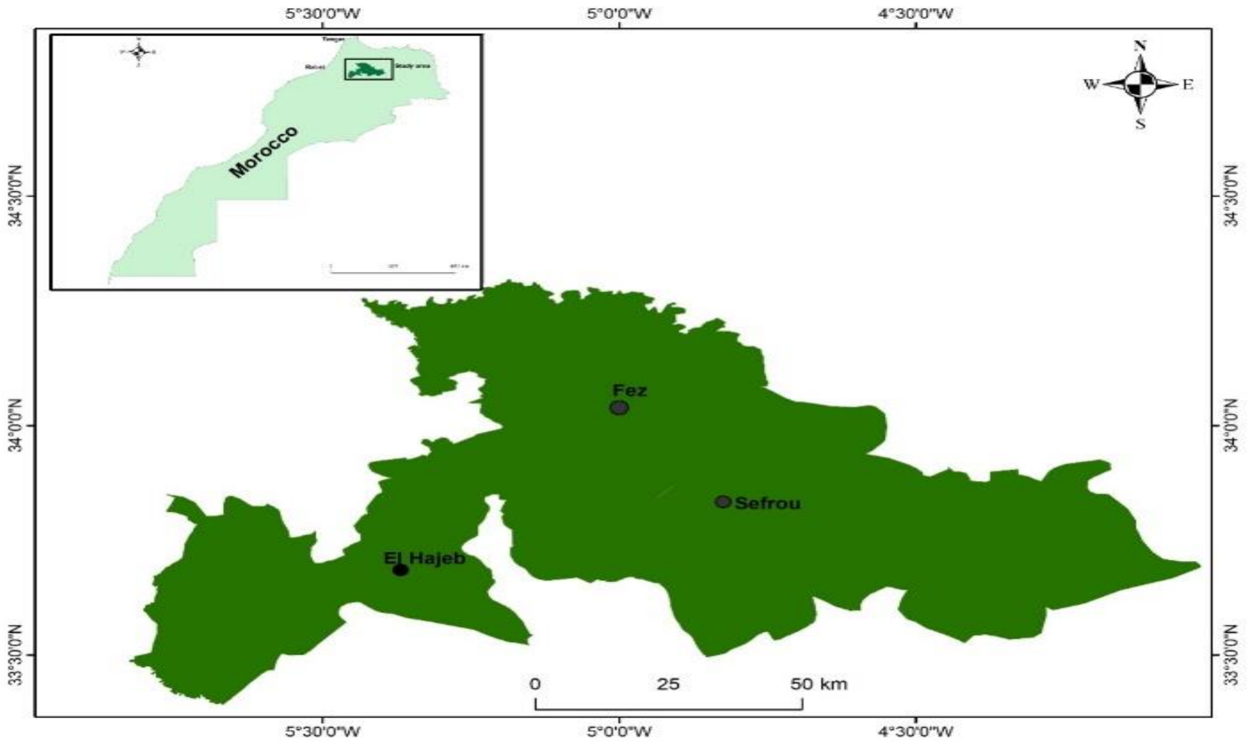

Figure 1: Location of sampling stations.

The phenolic compounds were quantified by modifying the Folin-Ciocalteu method described by Singleton *et al*.[10] Flavonoids were quantified using the aluminum trichloride method ($AlCl_3$) described by Bahorun *et al*.[11] All biochemical analyses were performed on both dry and fresh materials.

Climate study (Emberger rainfall quotient)

The goal was to determine the type of climate that governed each studied area. The current study focused on Emberger's rain quotient to accomplish this. This allows for the placementof weather stations in the appropriate bioclimatic stage, allowing for a better understanding of the aridity of the Mediterranean regions based on precise climatic data (temperature and precipitation). The following formulas were used to calculate Emberger's quotient (Table 2).

Statistical analysis

SPSS 18 was used for statistical analysis (SPSS IBM, 2009). The data were subjected to analysis of variance (ANOVA) to determine whether there were any significant differences between the treatments. $P < 0.05$ was used to determine whether the results were significant. Following that, the Pearson correlation test was used to determine quantitative variables, Finally, the Emberger diagram was mapped using Arc GIS version 10.5 software to determine the climate type of each the studied stations.

Table 1: Ecological Characteristics of Sampling Station

Station	Sefrou	Fez	El Hajeb
Coordinates	33°49'54'' N 4°49'40''O	34°02′13''N 4°59′59'' O	33°41'45'' N 5°22'00'' O
Rainfall (mm)	468	438	612
Altitude (m)	850	403	1050
m C°	2.6	4	2
M C°	34	35	27.5
Soil type	Clay-Silty	Clay –SiltySandy	Bedrock
Climate	Semi-arid to temperate winter	Semi-arid to temperate winter	Sub-humid to cool winter

m: an average of the minimums of the coldest month; M, an average of the best months of the warmest month, N: North; O: West

Table 2: Emberger's bioclimatic formula

Formula	Climatological parameters	Reference
Q2=1000∗P/ ((M'+ m'/2)*(M'-m'))	P : Annual precipitation mm /an	22
Q2' =3.43P∗/ (M-m)	M: Maximum temperature of the warmest monthly Annual precipitations	
	TK°= t°+ 273	
	m: Minimum temperature of the coldest month TK°= t°+ 273	
	P: Annualprecipitation mm /an	25
Q2''=2000P/ (M+m+546.4) ∗ (M-m)	M: Maximum temperature of warmest month ∘C	
	m: Minimum temperature of coldest month ∘C	

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Results and Discussion

Biochemical analysis

Total soluble sugars content (TSS)

Figure 2A depicts the obtained results, which highlight the variation in the total soluble sugar content at the leaf part level in the three studied populations (El Hajeb, Sefrou, and Fez). The Fez population's leaves have the highest TSS content, with a value of 27,426 mg/g FM (fresh material), compared to other populations, which have TSS content ranging from 20,573 mg/g FM for the Sefrou population to 16,543 mg/g FM for the El Hajeb population. The variance analysis of TSS content revealed a significant difference (F=18.15; ddl=2; P $\leq$0.05) between the three of *C. humilis* populations studied. The variation in total soluble sugars composition in *C. humilis* from different regions studied is generally due to climatic stress. The Fez region has the highest concentration of TSS (27.426 mg/g FM); this increase is usually due to the weather conditions that characterize this region, therefore when plants face these diverse conditions, they react by increasing the concentration of soluble sugar, This accumulation in the leaves of the plants can be explained as a mechanism of tolerance to the growing conditions, which was also reported by,[12] they found that the accumulation of TSS is widely recorded in *Ziziphus lotus* in different stress conditions (Hydric and thermal). This increase in total soluble sugarsconcentration was very clear in the Guercif station, which is characterized by a stressful climate (average rainfall: 185 mm/year) Temperature: 18.5C; Humidity: 56%; Elevation: 367 m) compared to other stations (Fez and AinChifa) which results in a significant accumulation of TSS in plants in this region that has suffered from very high water and thermal stress. Many studies have shown that soluble sugars are one of the mechanisms plants adaptations to stress.[13] They allow plants to withstand the lack of water by reducing the osmotic potential. This phenomenon is known as osmotic adjustment.[14] Also, soluble sugars were reported to be an indicator of stress levels, and their accumulation is a sign of stress resistance.[13] Metabolic sugars such as glucose, galactose, sucrose, and fructose have also been reported to resist various types of stress.[13] Stressed plants can respond under complex conditions by increasing the number of soluble sugars in their cells.[13,14]

Total soluble protein content (TSP)

Figure 2B depicted a variation in total soluble protein content at leaf level in three different Middle Atlas populations (El hajeb, Sefrou, and Fez). Analysis of these results revealed that the population of El Hajeb accumulates high levels of TSP than the populations of Fez and Sefrou.

When compared to other populations, it has a value of 631.145 mg/g FM, with 348,259 mg/g FM, 256,219 mg/g FM of protein levels inSefrou and Fez, respectively. At the level of the different study populations, the variance analysis of total soluble protein content revealed a significant effect (F=82.703; ddl=2; P< 0.005).

Vitamin C content

The analysis of figure 2C revealed that the El Hajeb population had the highest vitamin Ccontent, with a value of 0.613 mg/g FM, while the other two populations had the lowest values, varying between 0.347 mg/g FM and 0.264 mg/g FM for Fez and Sefrou respectively.

Statistical analysis of the results reveled significant differences between vitamin C levels among the three studied populations (ANOVA: F =297, 71; ddl =2; P <0,0001).

Based on the results obtained, the population of El Hajeb seems to be the wealthiest station of vitamin C compared to the population of other stations. This variation could be related to different factors, such asclimatic and environmental factors, namely light, precipitation, season and type of soil,[15] and also genetic heritage.[12]

To obtain a clear idea of the vitamin C status in *C.humilis* leaves, the results were compared to those reported in other studies that were conducted on different plant species such as the Phoenix fruit of actylifera that have a vitamin C content of 0.66 mg/g,[16] this value is roughly equal to the one obtained in the population of El Hajeb (0.613 mg/g FM), and higher than those obtained in the other two populations Sefrou and Fez 0.264 mg/g FM and 0.347 mg/g FM respectively.

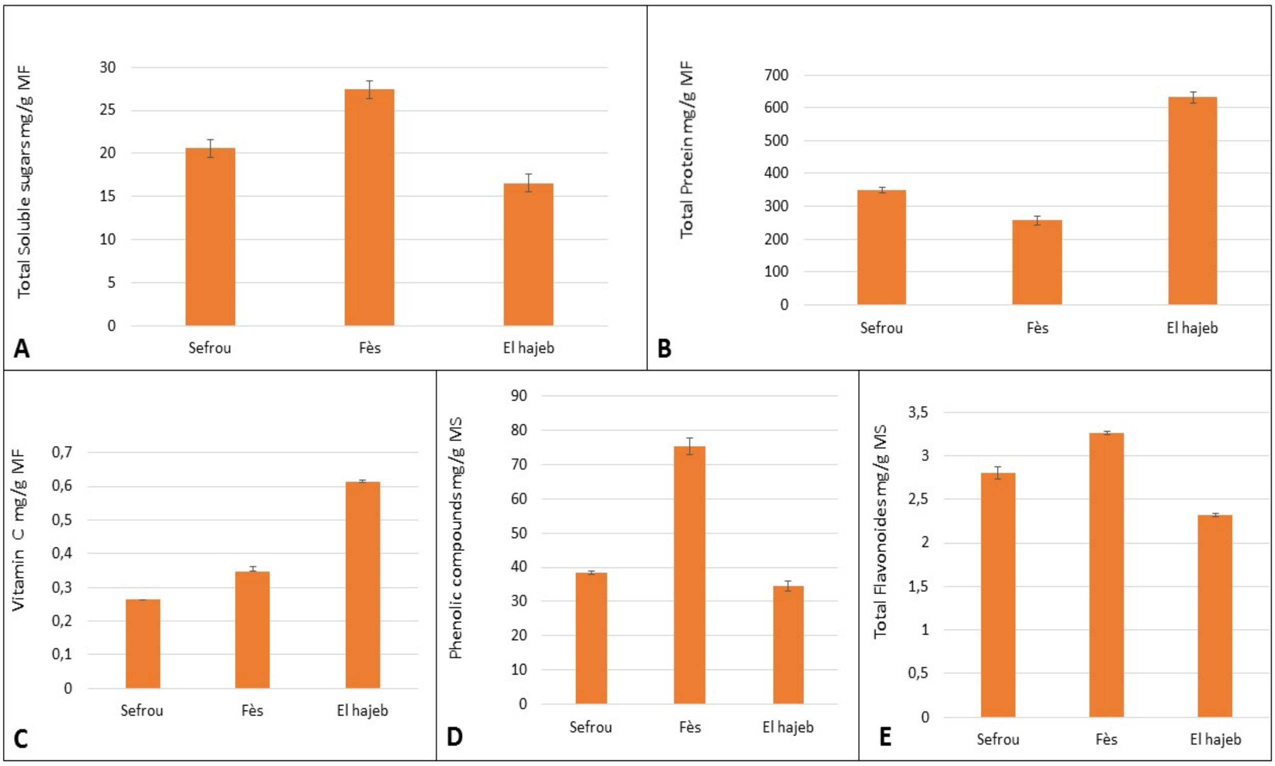

Figure 2: Change in the biochemical compounds of three populations of *C. humilis*:(A) Total soluble sugar content, (B) Soluble protein, (C) Vitamin C content, (D) Polyphenol content, and (E) Flavonoid content. Value presented as mean ± SEM

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Therefore, it could be concluded that the leaves of this species have significant nutritional importance and a potential source of natural anti-oxidant.

Total polyphenol content

Figure 2D depicts the total phenolic compound content in the three *C. humilis*populations' leaves (El Hajeb, Sefrou, and Fez). The findings a significant difference in phenolic compounds content between the three populations.The Fez population had the highest content, with a value of 75,390 mg GAE/g DM (dry material).Other samples yielded calues of 34,571 mgGAE/g DM, and 38,449 MG GAE/g DM for the populations of El Hajeb and Sefrou respectively.A highly significant difference was found between the stations ofSefrou, Fez, and El Hajeb (ANOVA: F =171.95; ddl =2; P = 0.001).

Secondary metabolite analysis showed that the total polyphenol content tested on *C. humilis* leaves generally leaves low compared to the results obtained by,[17] who reported polyphenol content of leaves of 99.8 mg/g DM. Other studies highlighted low polyphenol levels compared to our results, including the value of 25,209 mg/g DM.[18]

Total flavonoid content

Figure 2E depicts the flavonoid content of plant leaves extracts from three stations (El Hajeb, Sefrou, and Fez). The quantitative dosage of flavonoids revealed that the population of Fez had the highest flavonoid content, with 3.257 mg QE/g DM, followed by the Sefrou population with 2,808 mg QE/g DM, and Fez with 2,313 mg QE /g DM.

Statistical analysis of the results reveals a significant difference in flavonoid levels between the three studied regions (Sefrou, Fez, and El Hajeb) (ANOVA: F =111.19; ddl= 2; P ≤ 0.002).

Concerning flavonoid content, the results obtained are different from those reported by,[19] who reported that the flavonoid content of the *C. humilis* leaves has 17,269 mg/g MS. However, according to the study conducted by,[17] the flavonoid concentration of the plant leaf studied is 3.70 mg/g MS which is found in our study. Moreover, the total protein content obtained in this study were slightly higher than those reported by,[19] which has reported a value of 22.04 mg/g DM, the high total protein content (631.145 mg/g FM), is an indication that C. *humilis* leaves were good sources of protein for humans and could also be used as food stocks for animals.

Correlations between characteristics

The correlations for the studied parameters are presented in Table 3. The majority of these correlations are highly significant (R >0.700). The highest correlations are those associating biochemical traits with climatic traits. The biochemical data are clearly positively and negatively correlated with each other.

Environmental factors play a significant role in the discrimination of plant groups.[20]

The present study attests that the ecological factors tested have an essential effect on the distribution of *C. humilis.* However, the variation in biochemical parameters is intimately related to the surrounding conditions of the species.

Climate analysis

Ombrothermic diagram of study stations

According to the analysis of average temperatures and precipitation (Figure 3a, 3b, 3c), the three regions have distinct thermal and hydrological profiles.

The seasons in the Sefrou and Fez regions are distinct: a rainy season that lasts from November to May and is characterized by rainy months, and a dry season that lasts from May to October. This period is distinguished by an increase in temperature and a significant decrease in precipitation, signaling the end of the dry season and the beginning of the wet season (Figure 3a, 3b).

The diagram analysis (Figure 3c) confirms that the climate of the El Hajeb region is characterized by a long wet period that runs from October to April, during which the rainy episodes are almost all-weather, accounting 80 percent of the annual rainfall, and a dry season that runs from May to September. The altitude of the Middle Atlas largely influences this difference recorded in the region. These climatological parameters show a noticeable difference between the three stations.

Rainfall Quotient

The Emberger bioclimatic index (Table 4) is one of the most widely used bioclimatic indices in North Africa and the Mediterranean region.[21]It includes annual precipitation (P), the warmest month's maximum temperature (Min C or K) and the coldest month's average minimum temperature (min C or K),[22] the corrected quotients Q'2 and Q"2 proposed by,[22,23]which are particularly suitable for Mediterranean regions, reflect the ratio of rainfall to average thermal extremes (hottest and coldest months), all corrected by extreme thermal amplitude (M - m).

According to the bioclimatic diagram, the following types of climate can be distinguished based on the values of Q and m (limiting factor): Wet floor, Sub-wet floor, Semi-arid floor, Arid floor, and Saharan floor.

The rainfall quotient calculated using three methods,[22–25] is shown in the table below (Table 4).Emberger's patient analysis demonstrates that the formulas proposed by,[24,25]produce results similar to those described by.[22,23] We also discovered that the value of Q2 obtained at the El Hajeb region level is significant (82.3) when compared to the other two areas (Sefrou and Fez), which have values close to the rainfall quotient (51.1 and 48.2, respectively). We can deduce that the three study stations belong to the different bioclimatic stages based on the values of Q2.

Table 3: Correlations between studied characteristics

	T-Poly	T-Flav	TSP	TSS	VC	Altitude	Rainfall
T-Poly	1						
T-Flav	0,877***	1					
TSP	-0,753***	-0,936***	1				
TSS	0,932***	0,940***	-0,875***	1			
VC	-0,295	-0,387	0,412	-0,335	1		
Altitude	-0,971***	-0,950***	0,877***	-0,972***	0,355*	1	
Rainfall	-0,682**	-0,916***	0,973***	-0,840***	0,409*	0,829***	1

*** Correlation is significant at the 0.001 level ** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

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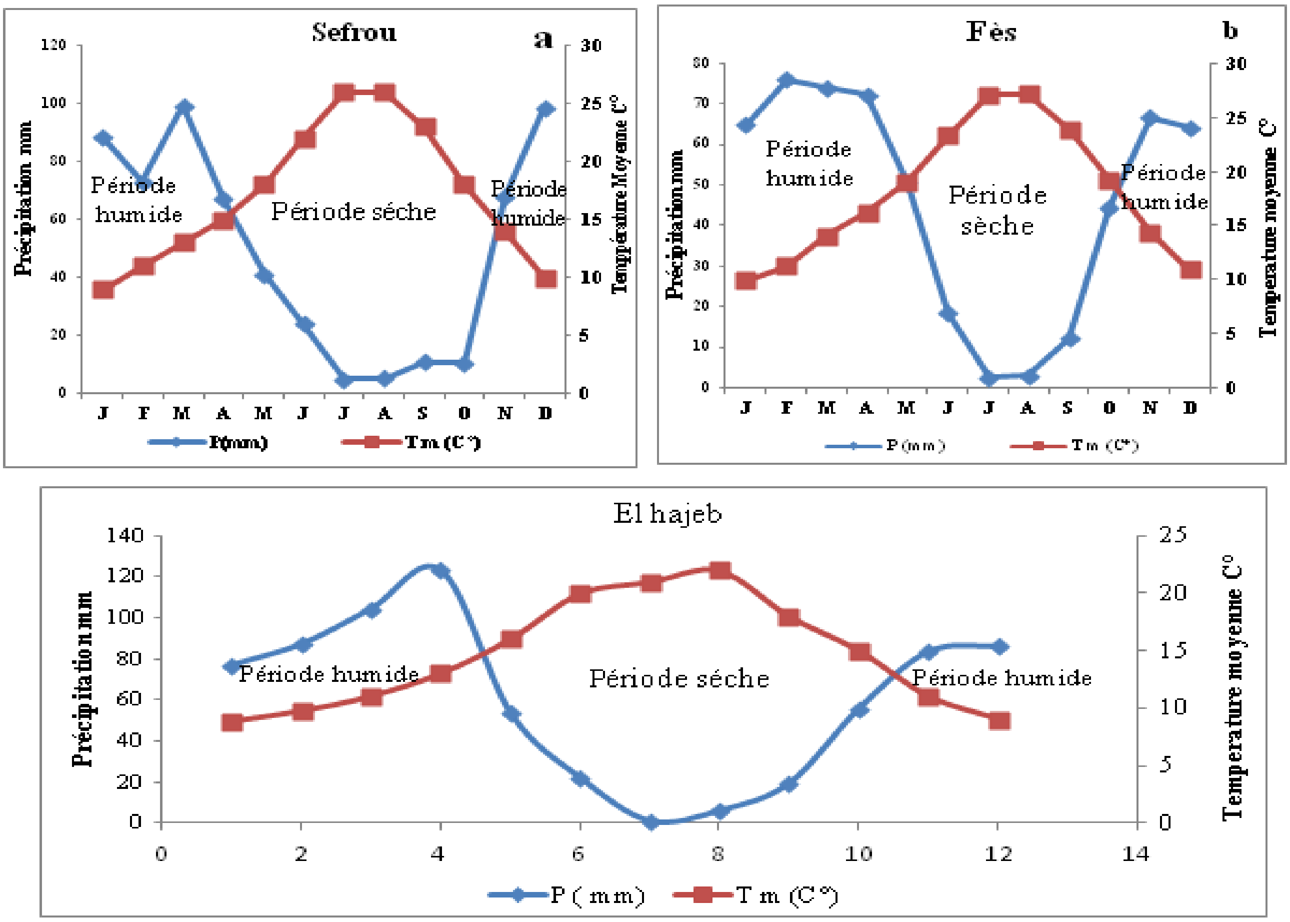

Figure 3: Ombrothermic diagram of the three stations in semi-arid and sub-humid area: Sefrou, FEZ, and El Hajeb.

Table 4: Climate Analysis of Middle Atlas Regions

	Stations	Sefrou	Fez	El hajeb
Climate parameters	Minimum temperature of the coldest month °C	2.6	4.3	2
	Maximum temperature for the warmest month °C	34	35.5	27.5
	Annual precipitation mm /an	468	438	612
Emberger Quotient	Q2 [22,23]	51.1	48.2	82.3
	Q2' [24]	51.12	48.15	82.32
	Q2'' [25]	49.41	47.89	83.34

Emberger Climagram

Emberger subdivided the climatic zone into characteristic zones by growing the arid from top to bottom by combining the rain-thermal quotients obtained (Q2) and the minimum temperatures of the coldest month (m) (limiting factor). These are the ''Bioclimatic Vegetation Stages'' (Figure 4).

We were able to trace the Embergerclimagram to identify the bioclimatic floors that characterize each study station using the average value of the coldest month's minimums and the Q (Emberger rain-efficient quotient). The projection of the stations studied in the EMBERGER climagram is shown in Figure 4. The analysis of this climagram reveals that the El Hajeb station is subject to the sub-wet bioclimatic stage in cool winter with Q2 is around 82.3, whereas the other two Sefrou and Fez stations are classified in semi-arid climate in temperate winter, with Q2 neighbors of 51.1 and 48.2 respectively.

Conclusion

Biochemical parameters of *C. humilis* collected from different locations were determined in the present work. The results obtained showed that *C. humilis* contains considerable amounts of primary and secondary metabolites. Furthermore, the geographical location had a remarkable effect on the content of primary and secondary metabolites.

Conflict of Interests

The authors declare no conflict of interests.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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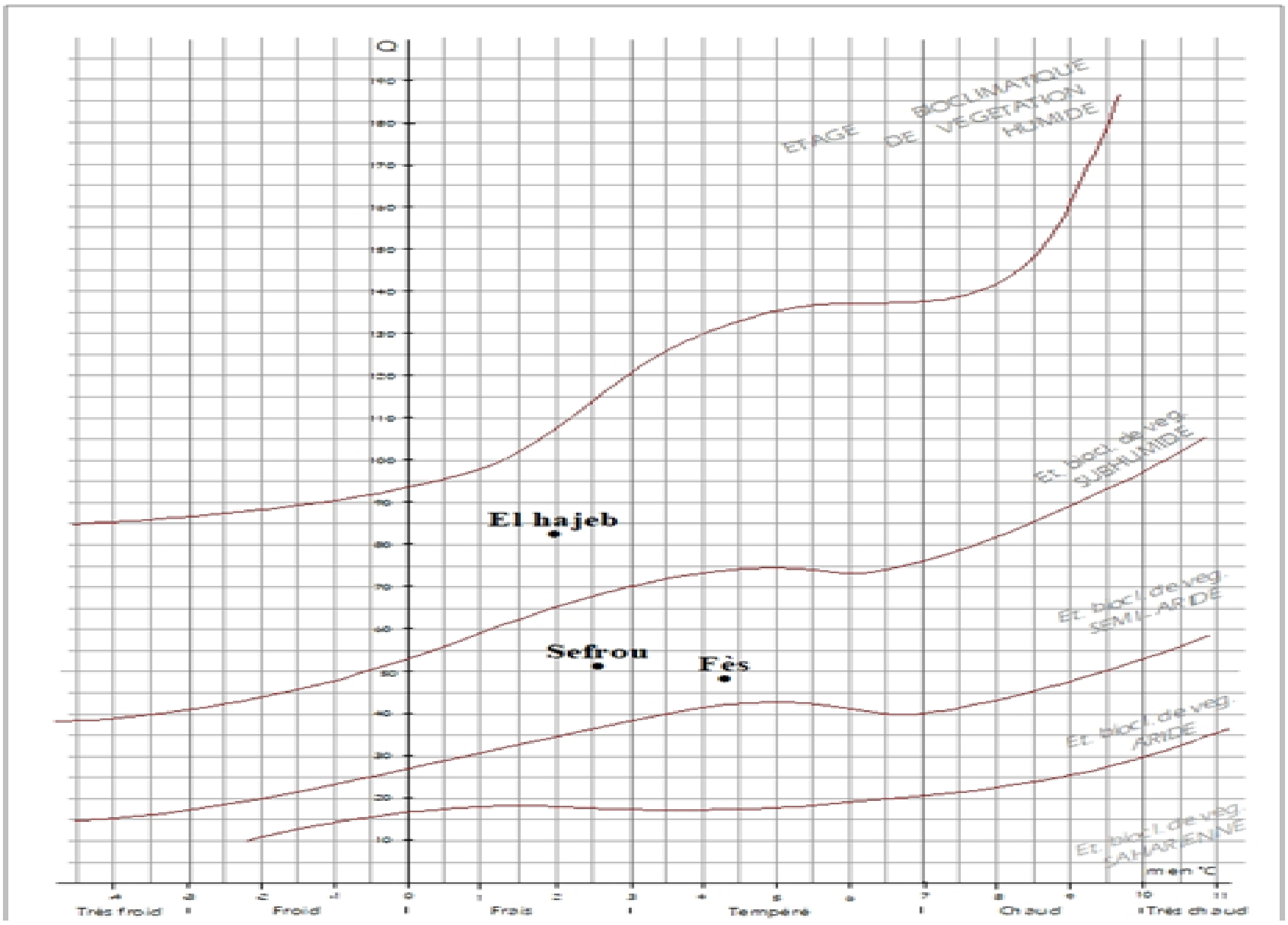

Figure 4: Projection of stations studied in the EMBERGER climagram

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