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Phenotypic and phytochemical diversity of saffron (*Crocus Sativus* L.)

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Abstract:
Our work focuses on the study of a medicinal plant Crocus sativus. L known as saffron, which is very popular and widely used as a spice and in traditional medicine. The purpose of this work was to compare saffron genotypes derived from three different regions, namely Tlemcen, Khenchela (Algeria) and Taliouine (Morocco). Phenotypic diversity was determined by the Shannon-Weaver diversity index (H ') at different levels. The estimated H showed high phenotypic variability for different traits with an average H of 0.73. The results of the Multiple Correspondence Analysis (PCA) and the Hierarchical Classification (CAH) showed a clear distinction between genotypes. The study of phytochemical parameters it was made from water-acetone extracts of the stigmas. The phytochemical screening revealed the presence of flavonoids, tannins, quinones, terpenoids and reducing compounds in the 3 regions stigma extracts; while Anthraquinones and saponins are absent in prepared extracts. The results showed polyphenols, and total flavonoids. The water-acetone extract of Khenchla's stigmas was higher than Remchi's. The samples from Taliouine is superior in in polyphenolic compounds (0.6 mg EAG / 100g MF), and flavonoids (0.895mg EQ / 100g MF) Crocus sativus. L stigma extract.
Key words: Crocus sativus. L stigma, polyphenols, flavonoids, diversity.

Introduction

The saffron *"Crocus sativus L*. "is a spice used for over 3,000 years. It is not a wild plant because it owes everything to man who has cultivated it, choked it, and imported it around the Mediterranean basin (Palomares, 1988).

Saffron or "red gold" is the world's most expensive food product since it is sold for between 30 and 40 euros per gram. It is obtained from stigmas, and all harvesting operations are carried out by hand (a dry stigma in the saffron plant weighs about 2 mg and each flower contains three, about 150,000 saffron flowers must be carefully cooked harvested for the production of 1 kg of spice) (Winterhalter and Straubinger, 2000; Melnyk et al., 2010). The quantity of saffron petals disposed is +estimated to be over 10,000 tons per year (Kafi et al., 2000).

Saffron (*Crocus Sativus L*.) is a perennial geophyte herbaceous of the Iridaceae family. Crocus L. genus, which comprises about 80 species spread mainly in the Mediterranean and Southeast Asia (Giorgi et al., 2015).

In recent years, the cultivation of saffron has begun to develop throughout the Algerian territory, hence our interest in this plant. The objective of this research is to morpho-metrically characterize and Phytochemical screening of saffron populations *(Crocus Sativus L.)* in three different regions, namely Tlemcen, Khenchela (Algeria) and Taliouine (Morocco).

Materials and Methods

Region of study

The plant material was originated from field surveys in 2018-2019 at the level of the wilaya of: Tlemcen (Remchi and Beni snous) and the wilaya of khanchla and another collection at the level of Morocco (Taliouine) (Figure 01 and 02). A total of 72 plants are studied in Algeria and Morocco

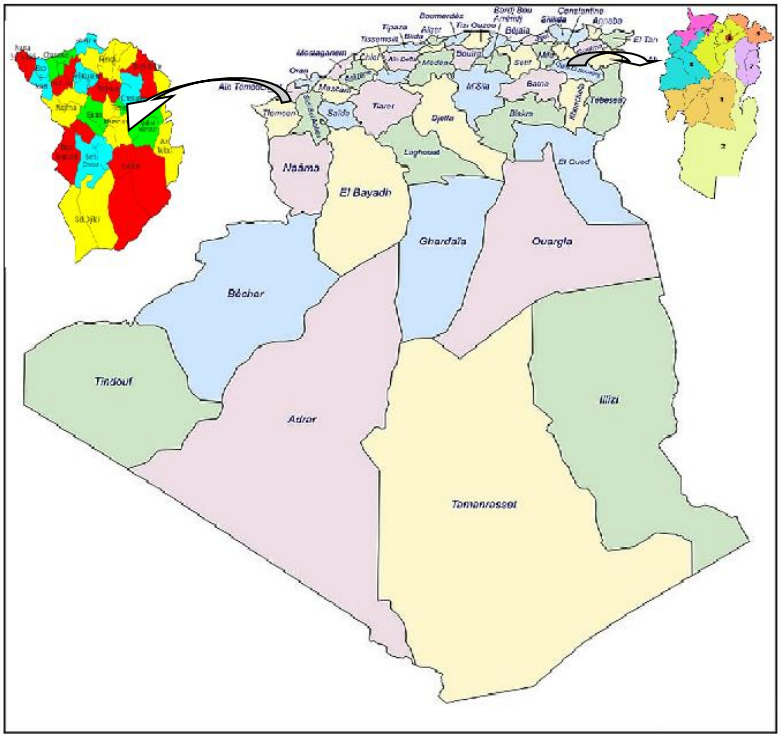


Figure 1: Location of the fields of the saffron samples studied in the wilaya of Tlemcen and khenchela (Algeria).

Figure 2: Geographic location of the Taliouine region (**Morocco**)

Saffron crop cycle

The plant material was planted (saffron bulbs) on September 07, 2018. After 2 months of planting, were harvested the saffron flowers in the morning, then the stigmas were collected and then we dried them (Figure 3).

The land used was chosen according to the following criteria: The availability of water, the deep soil and Soil rich in organic matter
We did a first plowing for the burial of organic matter 1 to 2 months before planting and a second plowing just before planting.

Plantation of saffron:

1. Preparation of soil **2. Start of foliage** **3 .Foliage**

4.flowering **5. Saffron harvest** **6. Drying of the stigma**

Figure 3. Saffron crop cycle

Measured Parameters

The purpose of analyzing these parameters is to identify and characterize the genotypes studied. The following parameters are measured for each plant: flower petals, dry stigma number, length (mm), yellow petals (stamens), total foliage and tufts number, Length (mm), weight(g) and bulbs diameter and the new bulbs numbers.

Figure 04. Measured characters (A, Diameter of bulbs, B: Number of flower petals and C: Length of bulbs).

Statistical analysis

The 08characters were assigned to classes, and analyzed using the Shannon–Weaver diversity index (H; Shannon and Weaver 1949) as defined by Jain et al. (1975) to calculate phenotypical variation of each accession:

$$H = -\sum_{i=1}^{n} Pi\ Ln\ Pi$$

H = Shannon and Weaver diversity index

Pi = Frequency of each phenotypic class *i*of a given character

n = Number of phenotypic classes of each character

The index (H) is converted towards the relative phenotypic diversity index (H ') by dividing it with its maximum value: H max (Ln (n)) to obtain 0 to 1 value.

H: was standardized by converting it to a relative phenotypic diversity index (H') after dividing it by Hmax (Ln (n)) to obtain 0 to 1 values.

$$H' = -\sum_{i=1}^{n} Pi\ Ln\ Pi\ /\ Ln(n)$$

Using FactoMine R software (version R-2.15.3) a multivariate analysis was performed to discriminate accessions with **principal component analysis** (PCA) and **cluster analysis** Hierarchical ascending classification **(**CAH)

Phytochemical analysis

1. *Preparation of the sample :* The stigma after separating from the saffron flower is dried at room temperature and stored in glass corners away from light.
2. *Extraction of phenolic compounds:* Extraction is done by acetone-water with a ratio of 80/20 (v/v). A quantity of 2 g of plant material was mixed with 20 ml of extraction solvent (80% acetone)
3. The mixture was shaken for 30 minutes away from light, followed by centrifugation at 3000 rpm for 10 min.
4. After filtration, the extracts obtained are kept at 4°C (Figure 39) for subsequent use (Figure 05).

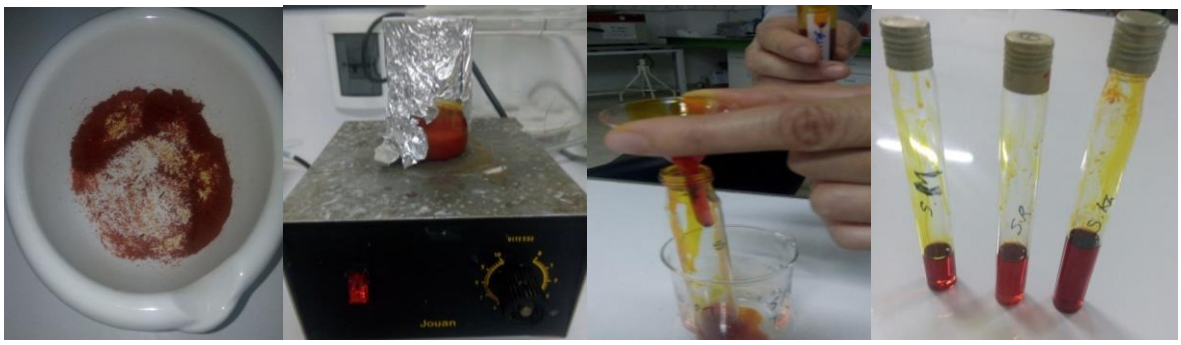


Figure 5. Phytochemical analysis of saffron

Results and discussion

Comparison of means and SD of the different characters of saffron genotypes:

Saffron has great powers, as a curator and as a color for food and drinks. It is not only a spice, but also a popular medicinal plant used in traditional medicine (Abe, 2000). Our work is a contribution to the genetic study, the geographic saffron location culture, biometric measurements and phytochemical studies, the determination of total polyphenols, flavonoids from the crude hydroalcoholic extracts of the stigma of the *Crocus sativus* .

Genetic part

1. The Shannon and Weaver Diversity Index

1-1. **Relative diversity index of the different genotypes studied**:

The relative diversity index (H' mean) of all genotypes studied of saffron of the order 0.73 reflecting the great morphological samples diversity from this collection (Table 01). For the characters studied after consulting several databases, no similar work was found.

Table 1: Relative diversity index of the different genotypes studied:

Genotypes	Petals length	Number of foliage	Number of Tufts	Number of new bulbs	Bulb width	Diameter	Weight of bulb	Dry stigma length	MeanH
G1	0,47	0,98	0,48	0,98	0,95	0,87	0,93	0,16	0,73
G2	0,87	0,97	0,89	0,99	0,81	0,71	0,83	0,16	0,78
G3	0,77	0,85	0,90	0,91	0,78	0,65	0,73	0,00	0,70
mean H	0,70	0,93	0,76	0,96	0,85	0,74	0,83	0,11	0,73

2. Comparison of mean and SD of different characters studied in saffron genotypes:

The mean saffron diameter plant bulbs ranged from 1.83 of the genotypes of the Beni Snous region to 3.76 of the genotypes of the Khenchela region, in a study of saffron cultivated populations in southern Algeria, the overall bulb diameter average ranged from 2.55 to 2.62 (Lahmadi et al., 2013). In addition, southern Algerian populations of Saffron have an average number of new bulbs per plant of 17.17 (Lahmadi et al., 2013), while the average number of new saffron plant bulbs ranged from 7.58 of the Remchi genotype to 8.71 of Khenchla genotype region. For the characters: No difference was seen for the flower petals and dry stigma length, yellow petals number (stamens), total foliage and tufts, and bulbs length and weight between the three regions studied.

3. Major Component Analysis (PCA): It is also noted that the PCA in question represents 100% of the information used for statistical processing, which is very significant. Similar work is being done on saffron varieties at the southeastern level of Algeria, which detect a value of 100/100 of information (Lahmadi *et al.* 2013). Our results are consistent with those of Lahmadi et al. (2013), positive correlations are noted between the new bulbs number and the bulbs diameter. The correlation of these traits can be explained by the influence of genes, i.e. These traits are controlled by a number of common genes or these traits react in the same way to environmental conditions. To exclude thisprobability, we need to have the situation where the same population involves in the same biotope to see if correlations change, otherwise it means that these characters are correlated by a number of common genes.

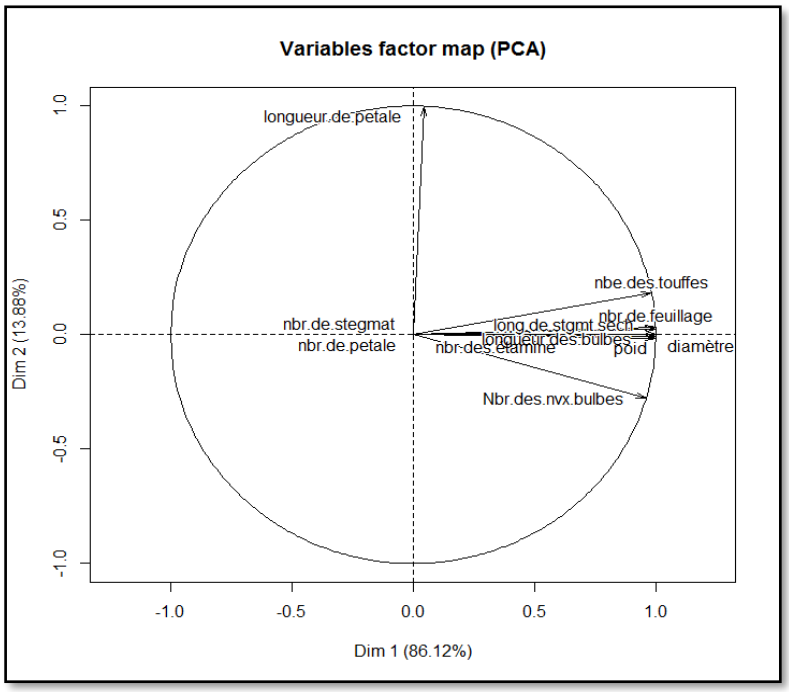
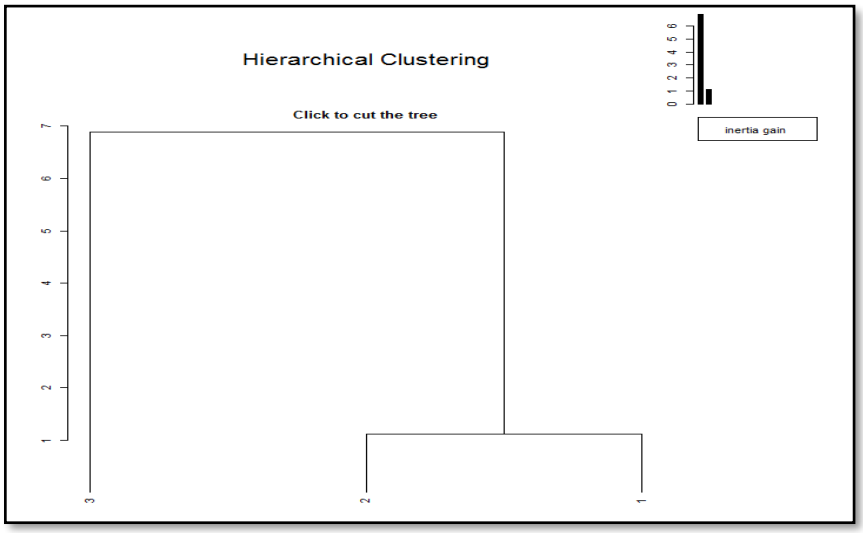


Figure 6: PCA of saffron accessions according to the averages of the different characters for all individuals and regions studied.

4. Hierarchical ascending classification (HAC) of saffron genotypes

in the 3 regions So the phylogenetic tree is explained by a genetic effect because there are two different genotypes in the same region of Tlemcen (Remchi genotype and genotype benisus) Geographical distribution and differentiation of subspecies does not necessarily imply genetic resemblance (Hadjaoui Kamel, 2013)



1- REMCHI **2-MAROC** **3-KHENCHELA**

Figure 07: Hierarchical ascending classification (CAH) of saffron genotypes**.**

2-Phytochemical Part

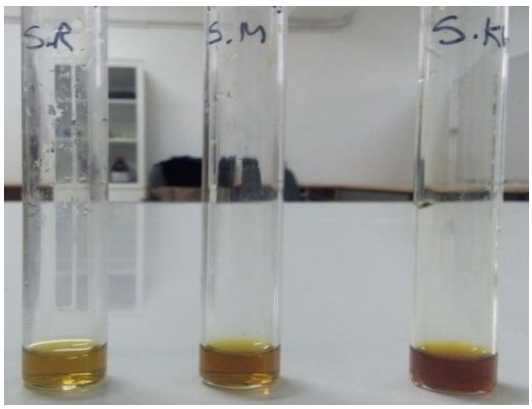


Figure08: phytochemicaltanochemical (Daoud M, 2019)

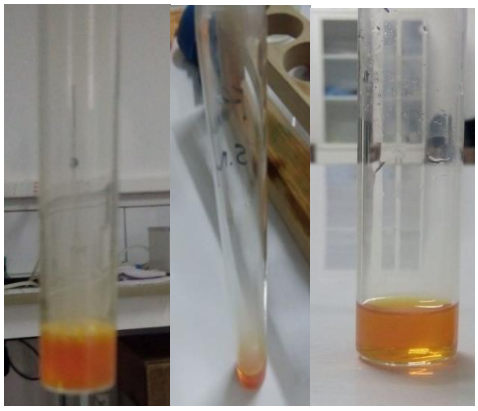


Figure 09: test Flavonoid phytochemical (Daoud M, 2019)

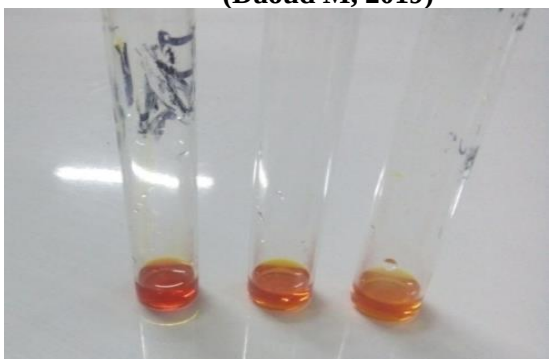


Figure10:test of Quinones phytochemical(Daoud M, 2019)

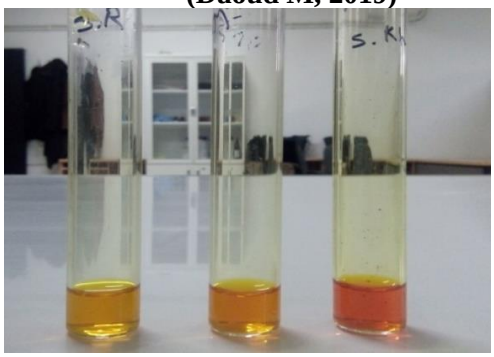


Figure11:test of Anthraquinones phytochemical (Daoud M, 2019)

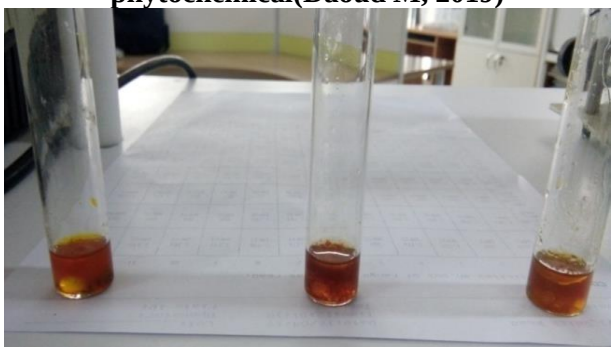


Figure 12: test of Terpenoid (Daoud M, 2019)

Phytochemical testing:

Results obtained with respect to phytochemical tests of the water-acetone destigmatize extracts of Crocus sativus.L (**Table 02).**

Table 2: Results of the phytochemical tests of the Crocus sativus.L stigma of the year 2018 in the regions of Tlemcen, Khenchla (Algeria) and Taliouine (Morocco).

Water-acetone extracts from the stigmas of *Crocus sativus*. 2019			
	Algeria		**Morocoo**
	Khenchela	**Tlemcen**	**Taliouine**
Flavonoids	+++	++	++
Saponins	-	-	-
Free quinines	++	+	+
Anthraquinones	-	-	-
Tannins	+++	++	++
Terpenoids	+++	++	++
Reducing compounds	+++	++	++

(+++): Strongly positive; (++): Positive means; (+): Low positive (-): Negative

Based on the results obtained, we note that our three extracts have tannins. They are very positive in the Khenchla region, followed by Morocco and Remchi, this is confirmed by the greenish coloring. Similarly, flavonoids and quinones are present with significant intensity in the extract from the Khenchela region compared to extracts from the other 2 regions. In addition, our stigma extract shows a strong presence of reducing compounds and terpenoids in the Khenchela region and moderately in the other 2 regions. However, anthraquinones and saponins are absent in all 3 study areas.

2. Determination of phenolic compounds

a. Total polyphenols: The total polyphenols weredetermined by the spectrophotometric method using the Folin-Ciocalteu method.

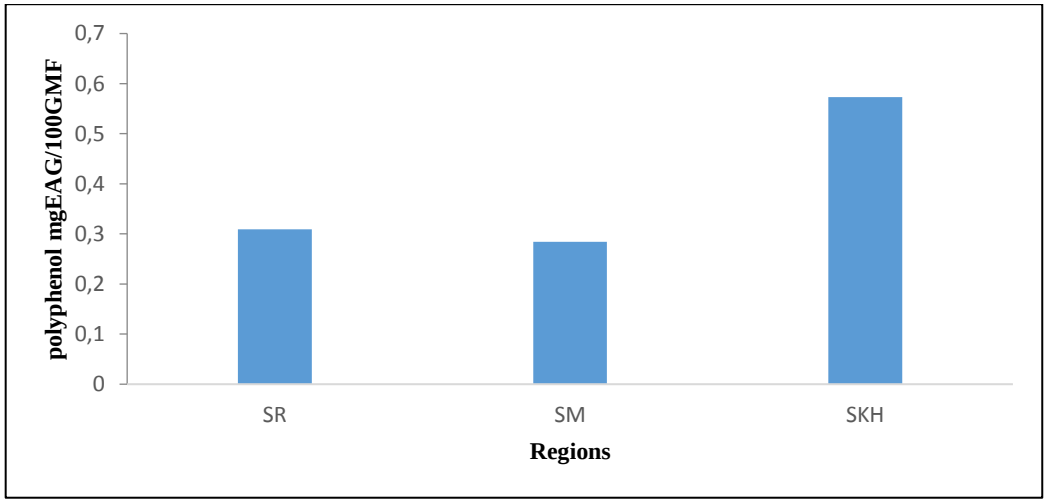


Figure13: The total polyphenol content (mg EAG/100g MF) of Crocus sativus extract.L

Figure13: Showed that the Khenchela region stigma extract contained the highest polyphenol content 0.6 mg EAG/100g MF. AG/100g MF and the Moroccan region 0, 28 mg EAG/100g MF.

b -Total Flavonoids: According to the histogram shown in Figure 15, Khenchela region stigma extract has a higher flavonoid content than the Remchi and Morocco region stigma extract (0.895, 0.302 AND 0.188 mg EQ/100g MF extract) of the stigma of *Crocus sativus* respectively

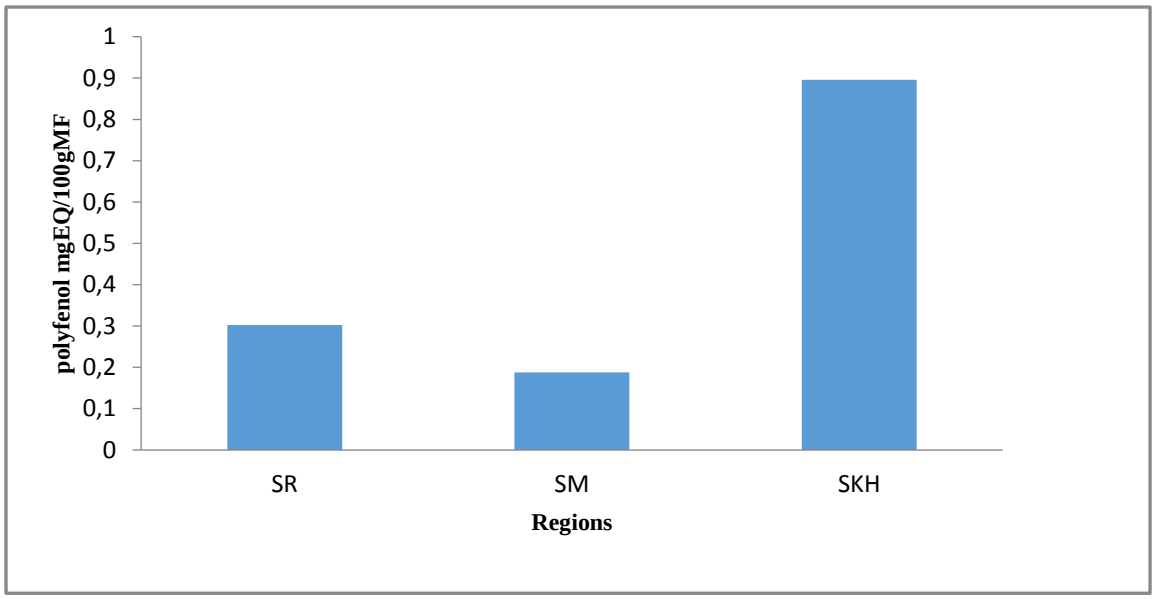


Figure 14: The total flavonoid content (mg EQ/100g MF) of the stigma extract of *Crocus sativus.L*

A phytochemical study was conducted on the stigmas of *Crocus sativus L* to investigate active constituents, determine the total phenolic content of saffron stigmates using reactive Folin-Ciocalteu Saffron (*Crocus sativus L.)* belongs to the iridaceae family. It is used as a condiment, dye and has significant anti-proliferation effects on human cells from colorectal cancer (Aunget al., 2007) and stomach (Al moflehet al., 2006). It is an anti-hyperglycemiant (Kianbakht, 2008). It is used in traditional medicine to treat different diseases, but it can become narcotic at a high dose (Bremness, 2002).

Saffron is a rare spice of great commercial value (Aitoubahouet al., Otmani, 2002). Phytochemical screening showed us the presence of flavonoids, tannins, quinones, terpenoids and reducing compounds in stigma extracts from the 3 regions; anthraquinones and saponins are absent in prepared extracts.

These results are comparable to those published by Mir et al., (2016), which showed the presence of flavonoids, tannins, saponins, terpenoids, Carbohydrates and Phytosterols in the flower of *Crocus sativus.*

Hosseinzadeh et al., (2002) also showed the presence of alkaloids and saponins in the aqueous and ethanolitic extracts of the stigma. Similarly, the work of Karimi (2010) showed the presence of phenolic and flavonoid compounds in the saffron stigma.

The assay of phenolic, flavonoid compounds was performed by chemical reactions using the Folin-Ciocalteu reagent for polyphenol dosing, iron and aluminum for flavonoid dosing The assay of polyphenols, total flavonoids, shows that the water-acetone extract of Khenchla's stigma is richer than the extract of Remchi and Morocco's stigma Polyphenolic compounds (0.6 mg EAG/100g MF), in flavonoids (0.895mg EQ/100g MF of *Crocus sativus* stigma extract.L These results are comparable to those published by (DouzietDjeriri, (2017), The extract prepared from *Crocus sativus* stigma presented the highest levels of total polyphenols (260µg EAG/mgE), while for flavonoids the prepared extract with the highest level of flavonoids (100 µg EC/mgE), It is difficult to compare our results with those of the bibliography, as several factors may influence the concentration of phenolic compounds in our extracts.The distribution of polyphenols is influenced by several factors, including climate, geography, drought, soil quality, depth... (Ebrahimiet al., 2008). In addition, the method of

extraction (extraction solvent and temperature) can also influence the content of total polyphenols and flavonoids (Conde et al., 2009; Lee et al., 2003) prepared extracts.

Conclusion

Depending on the characters studied, the genotype of the region of Khenchelahas the best profile. In addition, the determination of the total phenolic content in the water-acetonenous extracts allowed us to value the stigma of our plant. This study also revealed the importance of selecting the extract. Indeed, the Khenchela stigma extract was the best extract because it possesses the highest phenolic compound content compared to the Remchi stigma extract and that of Morocco. It may conclude that. saffron in the Khenchla region remains the best on the basis of this study. Our prospects are to collect more stigma samples throughout Algeria, in order to value our production for a likely world ranking.

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