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RESEARCH ARTICLE

THE MICRONUTRIENT COMPOSITION OF CROCUS SATIVUS L. AND ITS CORRELATIONS BASED ON STATISTICAL ANALYSIS

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Abstract

This work focused on determining the micronutrient status of *Crocus sativus L.*stigmas with studying of the different correlations between physical-chemical compounds (moisture, minerals, ash, total phenolic content, total carotenoids, zeaxanthin, vitamins and antioxidant activity). The mineral composition (Na, K, Ca, Mg, P, Fe, Cu, Mn, Zn, Mo and B) was analyzed by ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy). The total phenolic content, total carotenoids and DPPH activity were evaluated using UV-Vis spectrophotometer. The retention times of zeaxanthin, vitamin E and vitamin B9 molecules as well as their contents in the saffron stigmas were determined by UHPLC and were respectively (11.473 min, 15.561 ppm), (4.588 min, 1.048 ppm) and (3.977 min, 103.382 ppm). The obtained total phenolics value was 9.51 ± 1.10mg GAE/g dry weight (DW) and for total carotenoids, 28.53 ± 2.27 mg β-carotene /g DW. Positive correlations ($p < 0.05$) were noted between moisture-calcium, molybdenum-magnesium, manganese-potassium, manganese-phosphorus, boron-calcium, copper-iron,total phenolic-antioxidant activity and total carotenoids-zeaxanthin. However, negative correlations were found between moisture-sodium, ash-carbon, calcium-sodium and boron-sodium. No significant correlationswere observed between ash-total phenolic, ash-carotenoids,ash-vitamin E and ash-vitamin B9.

Introduction:-

Aromatic and medicinal plants have been used since ancient times as culinary spices and as medicines for curing wide variety of human diseases. Several drugs are used to be made from these plants and continue to be exploited in this way until today. As a result of numerous studies demonstrating that spices with phenolic and flavonoid components have antioxidant properties, they are often used as food supplements and constitute a reservoir for the pharmacopoeias of the world [1].

Saffron (*Crocus sativus L.*) is considered as one of the plants that are used as herbal medicine and food coloring, with less frequent uses as a component of textile dyes or perfumes. In Morocco, saffron is cultivated in a small southern region called Taliouine, located in the Anti-Atlas Mountains [2].

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Biomedical research has shown that the *Crocus sativus L.* is rich in bioactive compounds consisting of carotenes as crocin, crocetin, α-carotene, β-carotene and lycopene which not only contribute to its sensory profile, but also in the treatment of a variety of diseases in vivo and in vitro [3]. It is recognized by its anticancer effects, its cardiovascular effect [4], its antioxidant activity [5], anti-tumor [6,7], antidepressant [8,9], antispasmodic and anti-inflammatory [10,11].

Except these carotenes, the *Crocus sativus L.* contains xanthophylls, another group of carotenoids, integrating lutein and zeaxanthine (isomer of lutein) [12]. Zeaxanthin has a nutraceutical function in eye health, due to its presence as a yellow pigment in the macula which is a central area of the retina at the back of the eye where light rays converge in diurnal vision. This problem is a global concern in eye health. It has been noted that cataract and macular degeneration are age-related diseases in people over the age of 40 and 65, respectively. These consequences are not related to age alone, but primarily to poor nutrition, in particular carotenoid deficiency and an absence or high cost of effective treatments based on lutein and zeaxanthin in industrialized countries [13,14].

Saffron does not only contains carotenoids, but it is also rich in vitamins including vitamin B9 also called folic acid, vitamin E and mineral salts. The *Crocus sativus L.* has a complex chemical composition, made up of several substances, some of them present in very low concentrations. This particular complex composition results obviously from the interaction of the plant with its environment (Water, Soil and Air). The plant extracts its nutrients from the soil using its roots, namely water, main nutrients (N, P and K), trace elements (Fe, Cu, Zn, Mn, Mo, B, ...) and other minerals called major (Ca, Mg, Na and K) necessary for the growth of the saffron flower. This plant performs photosynthesis in its leaves, it develops complex molecules called organic compounds. The content of these constituents in the plant varies considerably depending on several factors such as the place and time of harvest, method of harvest, drying method and storage [15].

The study aims to identify the various significant correlations between physical-chemical compoundsof *Crocus sativus L.* while relying on different analytical methods: spectrometry UV-Vis, ICP-OES and chromatographic by UHPLC, as well as statistical methods such as the principal component analysis(PCA) for a better understanding of herein presented results.

Materials And Methods:-

Sample collection and preparation

The samples of the *Crocus sativus L.* (Saffron stigmas) used in this study were taken in November 2020 in the Taliouine region more particularly in the village of Tisslit which, is located at 30000 m from the center of Taliouine in the direction of Taznaght, south center of Morocco. Hygrothermal measurements and geographical coordinates of the sampling area are given in the table 1.

The flowers of *Crocus sativus L.* were collected early in the morning manually, which limits the length of their storage. These samples stigmaswere immediately removedwhich is commonly known as pruning operation. Shortly afterward, the stigmas were subjected to drying using convective solar dryer at 50 °Cas described in [16] to preserve the physical-chemical quality of saffron while extending its shelf life.

Table 1:- Climatic and geographical conditions of the sampling.

Settings	**Measures**
Temperature	8 °C
Humidity	43 %
Wind air velocity	2.2 m s^{-1}
GPS altimeter	1692 m
Altitude on the ellipsoid	1734 m
Latitude	30°33'56" N
Longitude	7°36'06" W

Analysis of physical and chemical composition of *Crocus sativus L.*

Determination of moisture and ash contents

The samples moisture content and ash content were determined according to the standard methods [17,18].

Determination of nitrogen content
The nitrogen content is determined by theKjeldahl method in two steps: the mineralization and titration. A test portion of 0.1 or 0.15 g of the previously dried product were placed in a tube of suitable Kjeldahl and mineralized with 10 mL of sulfuric acid and 2.5 g of the catalyst mixture (10: 1: 1): potassium sulphate, copper sulphate and titanium dioxide. The mixture was heated to a temperature not exceeding 400 °C for 2 h 15 min. After the mineralization step, the mixture was subjected to a distillation step followed by titration with 0.05 M of sulfuric acid (EN 16169: 2012).

The nitrogen content is expressed in % by the following equation (Eq. 1):

$$W_N = \frac{(V_1 - V_0) \times c \times M_N}{m_p} \times 10^{-4} \tag{1}$$

V_1: Volume of sulfuric acid used during the titration of the sample (mL).
V_0: Volume of sulfuric acid used during the titration of blank test (mL).
c: Concentration of H_2SO_4 used for the titration (0.05 mol L^{-1}).
M_N: Molar mass of nitrogen (g mol^{-1}).
m_p: Mass of the test sample (kg).

Determination of zeaxanthine, vitamin E and B9 in Crocus sativus L. using UHPLC method
A mass of 0.5-1.0 g of the stigmas of *Crocus sativus L.* were added to 10-20 mL of 80 % methanol and then extracted using an Anton Paar MAS 24 type microwave extractor (60 °C, 10 min, 600 rpm). The mixture was filtered and the filtrate was stored in brown glass vials. A volume of hexane was added to the extract prepared to separate the phases, then the solution recovered is filtered through a 0.45 μm nylon syringe in vials reserved for reading by UHPLC [19].

The determination of vitamin E (alpha-tocopherol) and vitamin B9 (folic acid), as well as zeaxanthin was carried out by using Ultra HPLC type Thermo Scientific (DionexUltiMate 3000 ISQ EM, Germering, GERMANY). The column used is Thermo Scientific C18 (250 × 4.6 mm, size 5 µm). The temperature of the column was maintained at 25 °C. Pump flow rate (1 mL min^{-1}), volume injected (20 µL). The mobile phase is acetonitrile: methanol (30:20, v/v) for the zeaxanthine and methanol: water (48:2, v/v) for vitamin E and B9. The peak height represents the intensity of absorbance in units of mAU (milli-Absorbance Units) for each compound. It was measured on the ultraviolet detection wavelengths: 452, 292 and 280 nm respectively for zeaxanthin, vitamin E and vitamin B9.

The calibration curves were obtained by concentrations prepared from the solutions standards of each molecule.

Determination of mineral content by ICP-OES
A number of 11 elements was determined in triplicate using an ICP-OES of the PerkinElmer (Optima 8000, Shelton, U.S.A) characterized by the following optimization and adjustment parameters: air (500 bar), nitrogen (7 bar) and pressure gauge argon (8 bar); flow rate of plasma gas (12 L min^{-1}), auxiliary (0.2 L min^{-1}), nebulizer (0.7 L min^{-1}); power (1400 Watts); plasma temperature (7000 - 15000 K); pump flow rate (1 mL min^{-1}).

Minerals are salts necessary for all living organisms [20]. Analysis of the mineral content of the dried stigmas of the Crocus sativus L. was carried out by dissolving the ash with 5 mL of 2N HCL, the mixture is transferred to a 50 mL flask and made up with distilled water. Then the solution is filtered using an ashless filter paper followed by detection by ICP-OES [21]. The minerals to be analyzed are: Na, Mg, K, Ca, P, Cu, Mn, Zn, Fe, B, and Mo.

Determination of total phenolic compounds by UV-Vis
The content of total polyphenols (TPP) was determined according to themodified protocol described by Ghedadba et al. (2015) [22]. This methodwas based on the use of the Folin-Ciocalteu reagent. A 0.5 ml volume of the methanolic extract was mixed with 1 ml of Folin-Ciocalteu reagent (diluted 10 times). After 5 minutes of incubation at room temperature, 0.8 ml of 7.5% (w/v) sodium bicarbonate was added. The mixture was then incubated in the dark for 1 hour at room temperature. The absorbance of the resulting blue color was measured at 765 nm against a blank. TPP content wasestimated using a calibration curve prepared from gallic acid and results were expressed as milligrams of gallic acid equivalents (GAE) per g of dried extract.

Determination of antioxidant activity: DPPH test

DPPH (2,2-diphenyl 1-picrylhydrazyl) (Figure 1) is a free radical and is frequently used to evaluate the antioxidant activity of a product [23].

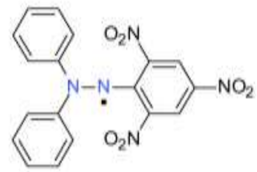


Figure 1:- Chemical structure of DPPH.

This method aims to evaluate the antioxidant potential of different methanolic extracts of saffron stigmas. A series of concentrations were prepared for each sample. A volume of 200 µl was taken from each concentration and mixed with 1.8 ml of DPPH (0.5mM). Then, the mixture was incubated in the dark for 20-30 minutes at room temperature [24]. The absorbance was measured at 517 nm by a UV-vis spectrophotometer against a blank.

All measurements were performed in triplicate. The percentage of inhibition was calculated according to the equation 2:

$$\% \text{ inhibition of DPPH activity} = \frac{\left(Abs_{control} - Abs_{sample}\right)}{Abs_{control}} \times 100 \quad (2)$$

Determination of total carotenoids

Total carotenoids were extracted using a modified version of the method described by Meléndez-Martínez et al. (2007) [25]. Samples were homogenized using a blender, and 0.5 g of the stigmas were weighed into a centrifuge tube and extracted with HPLC-grade solvents from 2.5 ml of extraction solution (hexane/acetone/methanol, 50/25/25, with 0.1% butylated hydroxytoluene). The mixture was homogenized and then centrifuged for 10 min at 4000 rpm and 4 °C. The supernatant phase was used for absorbance measured at450 nm by a UV-vis spectrophotometer. Total carotenoids (T) were calculated using the extinction coefficient of β-carotene (Eq.3) and expressed as mg β-carotene/g DW:

$$T = A / (F \times C) \quad (3)$$

A: absorbance of the sample;
F: 2505, extinction coefficient (E1%) of β-carotene;
C: concentration of the sample (g/ml).

Statistical Analysis

The statistical analysis was carried out by the PCA (Principal Component Analysis) in order to valorize the values obtained, as well as to look for the correlations between the different elements. It is both a geometric and a statistical mathematical tool. The results of the PCA include: a statistical description (mean, variance and standard deviation), a Pearson’s correlation and correlation graphs. Correlations between parameters are considered significant if they are different from 0 at a significance level α = 0.05. XLSTAT 2020 is the software used for statistical analysis.

Results And Discussion:-

Mineral Analysis

Table 2 puts together the experimental results of the chemical composition of *Crocus sativus L.* with a statistical description. The water content is on average 11.47 % (3 tests). The ash content is estimated at an average value of 5.80 % (3 tests). Potassium and phosphorus exist in a large quantity in saffron compared to other minerals with values up to 1343.35 mg/100 g and 340.89 mg/100 g respectively. The results of the ratios between carbon, nitrogen, phosphorus and potassium in the *Crocus sativus L.* are also represented in table 2. There are six stoichiometric ratios: C/N, C/P, C/K, N/P, N/K and P/K. The C/N, C/P and C/K ratios have larger averages than the N/P, N/K, and P/K ratios, which range from 25.00 to 138.59 and 35.16 correspondingly.

The value of the ash content (5.80 %) in the *Crocus sativus L.* agrees with the literature concerning the mineral content of a plant which varies between 1-12 % [17]. This value alongside to with that of the water content (11.47 %) corroborates with the results reported by USDA (United States Department of Agriculture) [26]. The dispersion of the micro-nutritional composition results of *Crocus sativus L.* (Table 2) may be due to the growth rates of the plant, as well as its absorption system. This allowed us to establish relationships in constituent elements of the plant studied and compared them as indicative in Table 5. The values displayed in this table explain the availability of nutrients C, N, K and P in large quantities in the *Crocus sativus L.* The C/N and C/P of a plant represents its production which can be supported by a nutritional provisioning. The availability of nutrients and their stoichiometry in the soil can thus be the cause of variations in the nutrient ratios in the plant [27].

Table 2:- Statistical description of the experimental data of the *Crocus sativus L.*

	Average	**Variance**	**Standard deviation**
Moisture (%)	11.47	0.40	0.63
Ash (%)	5.80	0.54	0.74
C (%)	47.10	0.14	0.37
N (%)	1.89	0.01	0.10
Na (mg/100 g)	32.48	0.10	0.31
K (mg/100 g)	1343.35	6765.29	82.25
Ca (mg/100 g)	165.59	388.36	19.71
Mg (mg/100 g)	130.32	21.74	4.66
P (mg/100 g)	340.89	462.90	21.52
Zn (mg/100 g)	5.58	0.39	0.63
Mn mg/100 g)	1.78	0.21	0.45
Fe (mg/100 g)	24.76	25.34	5.03
Cu (mg/100 g)	1.14	0.01	0.08
Mo (µg/100 g)	74.47	294.71	17.17
B (µg/100 g)	30.93	27.37	5.23
C / N	25.00	1.31	1.14
C / P	138.59	103.60	10.18
C / K	35.16	6.25	2.50
N / P	5.56	0.45	0.67
N / K	1.41	0.03	0.16
P / K	0.25	0.00	0.00

Correlation between minerals, moisture, carbon and nitrogen

The statistical analysis of Table 3 showed significant correlations between the different parameters analyzed (moisture, ash, nitrogen, carbon and minerals) for the *Crocus sativus L.*to better understand these different links, an analysis by PCA was carried out.

Pearson'scorrelation analysis revealed a very high correlation between moisture-calcium and molybdenum-magnesium, where the coefficient tends to 1. In addition, positive correlations were observed between manganese-potassium ($r = 0.998$), manganese-phosphorus ($r = 0.998$), boron-calcium ($r = 0.997$) and copper-iron ($r = 0.998$). However, negative correlations have been observed between moisture-sodium ($r = -0.998$), ash-carbon ($r = -1$), calcium-sodium ($r = -0.999$) and boron-sodium ($r = -1$).

Moreover, given that the correlation coefficient between iron and copper was $r = 0.998$ (Table 3), the increase in the copper content has a positive influence on iron absorption. Calcium absorption is conditioned by the boron content ($r = 0.997$). This result corroborates with the literature which states that boron plays a role in the absorption of cations, especially calcium [28]. Boron is used with calcium in the synthesis of cell walls and is essential for cell division (creation of new cells), thus it contributes to the metabolism of calcium, magnesium and vitamin D. However, its deficiency may causes damage to the roots and reduced absorption ofP and K [29]. All of these minerals are essential for reaction mechanisms in the human body [30]. Iron is the most abundant trace element in our body. It is essential for the synthesis of hemoglobin in red blood cells which carry oxygen to all cells. It is also a fundamental constituent of the myoglobin, a substance similar to hemoglobin, which helps muscles store oxygen. In addition, iron has an important role in many biological processes including DNA synthesis, lipid metabolism, and detoxification [31]. Copper is also very important; it is involved in cellular respiration and it is a cofactor of enzymes. It also behaves as an antioxidant by stimulating superoxide dismutase, thus protecting the cell against the toxic effect of free radicals [32]. In addition, excess copper can induce iron deficiency. Like copper and iron, zinc is the second most important trace element due to its abundance in the human body and its participation in the metabolism of carbohydrates, fats and proteins. It is involved in controlling appetite by acting on the central nervous system [33]. Manganese is an activator of enzymes and not an enzyme component. It is responsible for blood coagulation and hemostasis in conjunction with vitamin K. Manganese is also involved in the synthesis, secretion and action of insulin in combination with zinc and copper. It participates in the synthesis of vitamins E and B1 [34]. Molybdenum is also essential for the functioning of several enzymes. It is implicated in the metabolism of some amino acids as well as purines, which are constituents of DNA. It is complicated in the fixation of gaseous nitrogen by the Rhizobium radicicola and in the reduction of nitrates (nitrate reductase). In fact, molybdenum deficiency causes a decrease in nitrate reductase activity. Note also that copper, iron and zinc have positive effects on molybdenum metabolism [35].

Table 4 includes the inertia also called the percentage of information carried by each of the axes of the PCA of the *Crocus sativus L.* and that the experimental data can be represented in two axes. The variability represents the percentage of total inertia explained by each axis. The percentage carried by the first axis (F1) is 66.16 %, and by the 2^{th} axis (F2) is 33.84 %.

Two tools help in the interpretation of PCA results: contribution and quality of representation.

At the level of the contributions which represent the participation of each parameter in the construction of an axis, it is possible to observe the elements which contribute more to the construction of the axes. Table 5 represents the contributions (%) of the elements in the construction of axes F1 and F2. It shows that Mo and Mg contribute more to the construction of axis 1, while Na, Zn, B, Ca and humidity contribute more to the construction of axis 2.

The length of a vector of a parameter reflects the quality of its representation in the plane (F1/F2) or in the considered axis. The quality of the representations is measured by the cosine squared of the angle between the parameter and it's projected on an axis. The closer the squared cosine of the angle is to 1, the better the variable is projected. The circle of correlations (Figure 2) shows that most of the constituent elements of *Crocus sativus L.* are well projected on axis 1, except zinc, nitrogen and carbon which are well projected on axis 2. Axis F1 is related to Mn, Mg, B, Ca, moisture, ash, Cu, Fe, Mo, K and P which explains why all these elements mentioned are linked together in the micronutrient composition of saffron, and therefore it will be interesting to take this result into consideration when making food supplements.

Table 3:- Linearity between different parameters analyzed from the plant *Crocus sativus L.*

	Moisture	**Ash**	**C**	**N**	**Na**	**K**	**Ca**	**Mg**	**P**	**Zn**	**Fe**	**Mn**	**Cu**	**Mo**	**B**
Moisture	**1**														
Ash	0.043	**1**													
C	-0.043	**-1.000***	**1**												
N	0.067	-0.994	0.994	**1**											
Na	**-0.998***	0.014	-0.014	-0.123	**1**										
K	0.353	0.950	-0.950	-0.910	-0.299	**1**									
Ca	**1.000***	0.040	-0.040	0.070	**-0.999***	0.350	**1**								
Mg	0.571	0.845	-0.845	-0.781	-0.524	0.970	0.569	**1**							
P	0.230	0.982	-0.982	-0.956	-0.174	0.992	0.227	0.930	**1**						
Zn	0.567	-0.798	0.798	0.860	-0.613	-0.571	0.570	-0.352	-0.671	**1**					
Fe	0.748	0.695	-0.695	-0.612	-0.709	0.885	0.746	0.972	0.818	-0.122	**1**				
Mn	0.286	0.970	-0.970	-0.937	-0.231	**0.998***	0.283	0.950	**0.998***	-0.627	0.850	**1**			
Cu	0.785	0.654	-0.654	-0.566	-0.748	0.857	0.783	0.957	0.784	-0.066	**0.998***	0.819	**1**		
Mo	0.557	0.854	-0.854	-0.792	-0.508	0.974	0.554	**1.000***	0.937	-0.369	0.968	0.955	0.952	**1**	
B	0.997	-0.038	0.038	0.147	**-1.000***	0.276	**0.997***	0.503	0.151	0.632	0.692	0.208	0.732	0.488	**1**

**Values in bold are different from 0 at a significance level α = 0.05*

Table 4:- Eigenvalues, % of variability and cumulative proportion explained for each PCA factor.

	F1	**F2**
Value own	9.92	5.08
Variability (%)	66.16	33.84
Cumulative %	66.16	100.00

Table 5:- Contribution of different elements (%) to the main factors in the PCA.

	F1	**F2**
Moisture	2.733	14.357
Ash	7.723	4.601
C	7.723	4.601
N	6.728	6.547
Na	2,238	15.324
K	9.728	0.682
Ca	2.704	14.413
Mg	10.04	0.072
P	9.108	1.895
Zn	1.676	16.422
Mn	9.211	1.693
Fe	9.423	1.279
Cu	8.865	2.369
Mo	10.059	0.036
B	2.041	15.710

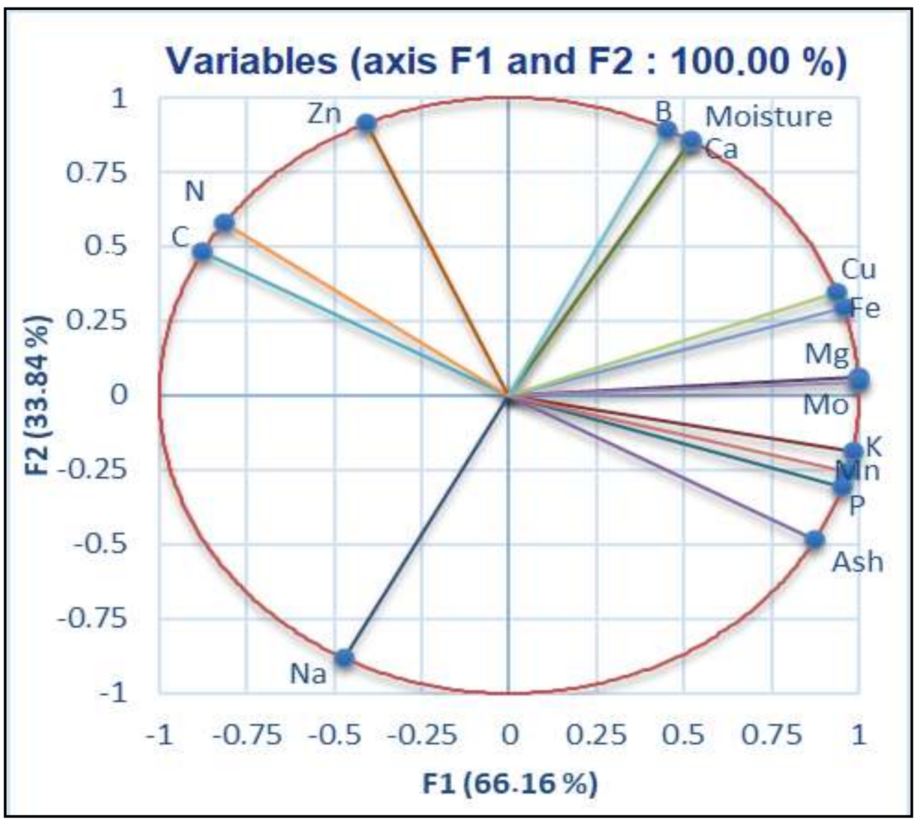


Figure 2:- Correlation circle obtained with PCA for the constituent elements of *Crocus sativus L.*

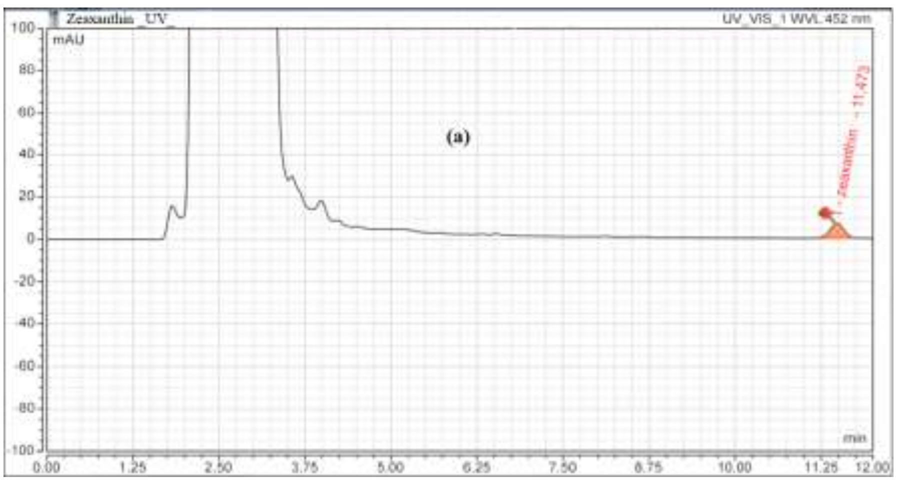
Zeaxanthin, vitamin E and folic acid

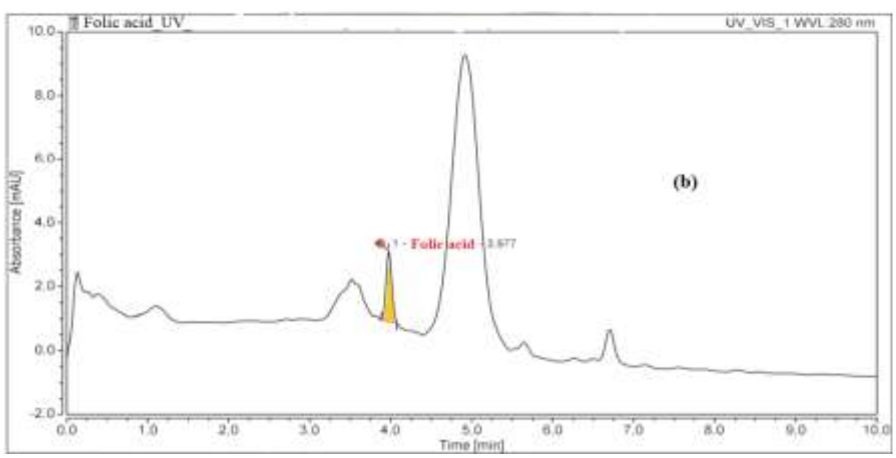
Table 6 indicates the contents of zeaxanthin (15.561 ppm), vitamin E (1.048 ppm) and folic acid (103.382 ppm) found after analysis by UHPLC for one gram of the dried stigmas of saffron as well as their respective retention times 11.473 min, 4.588 min and 3.977 min as shown in the chromatograms in Figure 3-a, 3-b and 3-c. The level of zeaxanthin found in *Crocus sativus L.* from Morocco (15.561 ppm) is higher than that cited by NordiyanahAnuar et al. (2018) for the *Crocus sativus L.* from Malaysia (11.33 ppm) [36]. Zeaxanthin is a pigment of the xanthophyll family (carotenoid) and isomer of lutein. Vitamin B9 or folic acid is found at a higherlevel in saffron (103.382 ppm), which makes it a good source of folate especially for pregnant women thanks to its intervention in the prevention of the nervous system and immune system in the fetus [37]. Thus, the vitamin E content in saffron is remarkable (1.048 ppm). Indeed, the properties of vitamin E or alpha-tocopherol are the subject of a large majority of studies [38, 39]. It is the major antioxidant of lipid media (oils, biological membranes, lipoproteins). It has a good-free radicals scavenging properties involved in the prevention of cardiovascular disease and cancer, limiting peroxydation of lipids and beneficial effects on the immune system.

Table 6:- UHPLC results of the stigmas of *Crocus sativus L.*

No.	Compound	Formula	Molecular weight (g mol^{-1})	Retention time (min)	Height mAU	Amount ppm*
1	Zeaxanthin	$C_{40}H_{56}O_2$	568.88	11.473	6.826	15.561
2	Vitamin E	$C_{29}H_{50}O$	430.71	4.588	0.284	1.048
3	Vitamin B9	$C_{19}H_{19}N_7O_6$	441.40	3.977	2.198	103.382

** The contents for one gram of the stigmas of Crocus sativus L.*





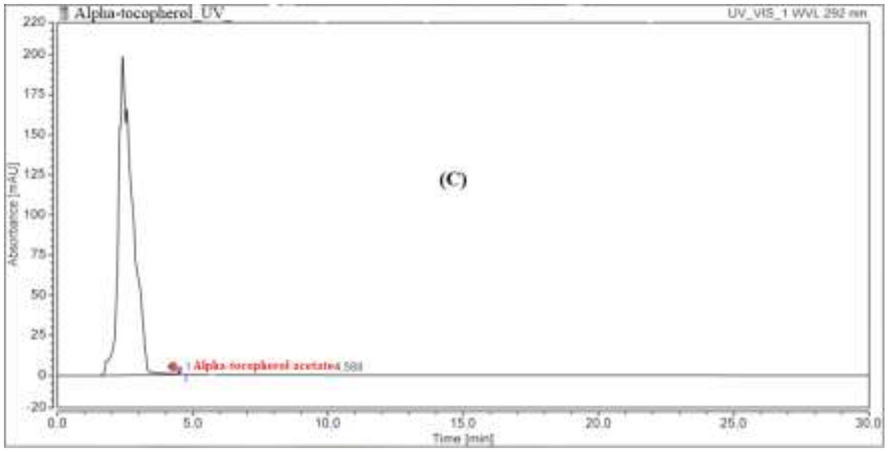


Figure 3:- HPLC chromatograms of zeaxanthin (a), vitamin B9 (b) and vitamin E (c) detected in the stigmas of *Crocus sativus L.*

Total phenolic content, total carotenoids and antioxidant activity of saffron

Results on the phenolic and carotenoids contents of saffron stigmas with antioxidant activity obtained using different methods are presented in Table 7 and expressed as means ± standard deviation of triplicate analyses. Total phenolic content of the extracts obtained from saffron stigmas was determined using Folin–Ciocalteu method using gallic acid calibration curve (Figure 4). The total phenolic content in *Crocus sativus L.* was estimated as 9.51 ± 1.10 mg GAE/g DW. This result is higher than that obtained by Karimi et al. (2010) [40] for methanolic extract of Iranian saffron (6.5 ± 0.02 mg GAE/g DW).

The antioxidant activity of saffron stigmas was evaluated by the DPPH method and the table 7 regrouped the results obtained of IC50 and DPPH. scavenging activity. The extract under test had higher antioxidant activity when the IC50 value was lower [41]. IC 50 was estimated for each sample extract from a series of its concentrations prepared with 80% methanol according to their percentages of inhibition. The values obtained are compared to those of ascorbic acid taken as reference (0.02 mg/ml). The average value of the IC50 obtained for Moroccan saffron was estimated as 1.37 ± 0.14 mg/ml. Gismondi et al. [42] evaluated the antioxidant activity of the Italian saffron aqueous extract as IC50 and they found a value of 3.76 mg DW. At a concentration of 3 mg/ml, the DPPH. scavenging activity of Moroccan saffron methanol extract measured and expressed as % inhibition of dpph had a value of 56.41%. Assimopoulou et al. (2005) reported a relatively strong DPPH scavenging activity of methanolic extract (51.02% inhibition at 2500 ppm), which was in accordance with the result of this study [43].

Carotenoids, also called tetraterpenoids, are natural pigments of intense color, produced mainly by plants and synthesized in subcellular structures (plastids, i.e. chloroplasts and chromoplasts) of plants [44]. Carotenoids are divided into two classes: (i) carotenes (ii) xanthophylls. β-carotene, which belongs to the first class, is the most common in foods [45]. The content of total carotenoids in Moroccan saffron was estimated at 28.53 ± 2.27 mg β-carotene /g DW.

Table 7:- Total phenolic, antioxidant activity and total carotenoids of *Crocus sativus L.*

Total phenolic content [a]	**DPPH. scavengingactivity** [b]	**IC50** [c]	**Total carotenoids** [d]
9.51 ± 1.10*	56.41 ± 1.10*	1.37 ± 0.14*	28.53 ± 2.27*

*[a] mg GAE / g DW; [b] % inhibition; [c] inhibition concentration is defined as the concentration of product required to reduce 50% of the DPPH radical (mg/ml); [d] mg ß- carotene/g DW; * Value ± SD.*

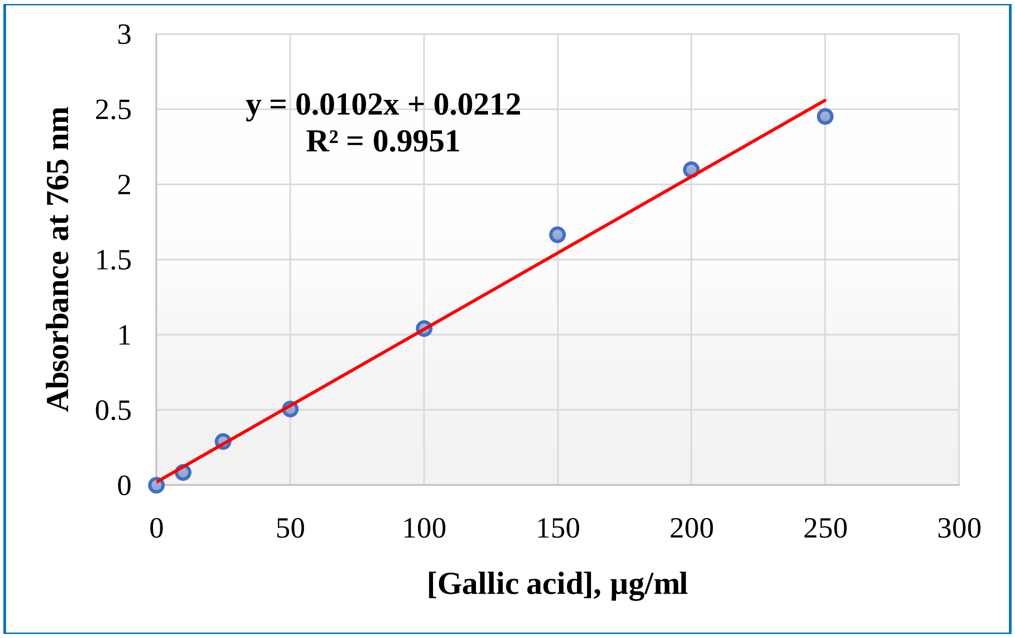


Figure 4:- Gallic acid calibration curve.

Correlation between ash content, total phenolic content, total carotenoids, zeaxanthin, vitamins and antioxidant activity of saffron

Table 8 regrouped the correlations between the total mineral content (ash) and the other parameters analyzed (total phenolic content, total carotenoids, zeaxanthin, vitamin E, vitamin B9 and antioxidant activity) for the *Crocus sativus L*.to better understand these different links, a bivariate Pearson correlation was carried out.

Pearson'scorrelation analysis revealed a perfect correlation (r = 1) between total phenolic and antioxidant activity. Positive correlations were observed between total carotenoids and zeaxanthin content (r = 0.998). These correlations are significant at a level of significance $\alpha = 0.05$. It is important to notice the existence of other correlations but not significant, such as the correlation between ash and vitamin B9 (r = - 0.984).

Makhlouf et al. [46] showed that saffron extract obtained from the flower of *C. sativus L.* had high amounts of polyphenolic components that can reduce free radical activity and provide strong protection for different organs (liver, kidney, lung, and heart) against certain oxidative damage.

Chen et al. [5] highlighted that crocins in saffron extract have strong antioxidant effects and that these properties are influenced by the sugars attached to the crocetin part. They also added that the antioxidant activity of saffron methanolic extract was higher than that of tomatoes and carrots.

Sulaiman et al. [47] have found a moderate correlation between total phenolic content and antioxidant activity for dried pulps of banana. They added that the most significant antioxidants in plant materials are thought to be phenolic chemicals. The phenolic compounds also have optimal structural characteristics for free radical scavenging abilities.The antioxidant activity of the extracts from the pulps and peels of the eight banana cultivars may also be influenced by the type and quantity of phenolic components. Vitamins C, E, and β-carotene, which are nonphenolic antioxidants found in bananas, may also have a role in boosting antioxidant activity.

The positive correlation found between carotenoids and zeaxanthin was explained by the fact that the zeaxanthin is a xanthophyll (class of carotenoids). Many authors have described the role of the carotenoids and zeaxanthin, in protecting against age-related macular degeneration [12,13]. In saffron stigmas, the bio-oxidative cleavage of zeaxanthin allowed to produce two bioactive molecules: crocetin dialdehyde and picrocrocin.

No significant correlation was observed between the mineral content of *Crocus sativus L.* and the other parameters analyzed (total phenolic content, total carotenoids, zeaxanthin, vitamin E, vitamin B9 and antioxidant activity) at a level of significance α = 0.05. Sulaiman et al. have also confirmed no correlation between the antioxidant activity and mineral content except between DPPH activity and Mn content which can be due to the role of Manganese in activating enzymes that improve the biosynthesis of flavonoids.

Table 8:- Correlation matrix.

	Ash	**Total phenolic content**	**Antioxidantactivity**	**Total carotenoids**	**Zeaxanthin**	**Vitamin E**	**Vitamin B9**
Ash	1						
Totalphenolic content	0.239	1					
Antioxidantactivity	0.239	**1.000**	1				
Total carotenoids	-0.134	0.930	0.930	1			
Zeaxanthin	-0.068	0.952	0.952	**0.998**	1		
Vitamin E	-0.747	0.466	0.466	0.758	0.714	1	
Vitamin B9	-0.984	-0.408	-0.408	-0.044	-0.110	0.618	1

Conclusion:-

These various correlations obtained in this study with a proximate analysis and mineral composition open a new window for saffron control use as a micronutrient supplement in favor of the active molecules carried by its stigmas such as the zeaxanthine. The results herein presented are of utmost importance to further promote. Itsused for nutraceutical purposes (food supplements), which can also broaden the therapeutic options available to medical professionals to deal with major diseases that threaten human health, such as cancer, cardiovascular disease, depression, and age-related macular degeneration (AMD). This potential and this wealth of *Crocus sativus L.* will make it possible to prepare an extract rich in minerals, antioxidants, vitamins and zeaxanthine. The preparation could be the subject of an innovative nutraceutical solution which will certainly enrich the offer of food supplements to support the experts in their prevention strategies and to fight against eye diseases.

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Statements And Declarations

The authors declare that they have no competing interests.

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