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RESEARCH ARTICLE

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Aromatic potential, quality and antioxidant activity of saffron grown in Morocco

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Abstract

Saffron is a spice derived from the flower of *Crocus sativus* L., which has a special aroma, colour and odour influencing positively its economic value. In this context, ten saffron ecotypes were screened for their biochemical composition and antioxidant activity. The samples were also analysed using GC-MS and LC-MS to determine their content of volatile and phenolic compounds, respectively. The results revealed statistically significant differences among samples based on moisture (9.09%-11.23%), total phenols (31.62-62.71 mg EAG/g), total flavonoids (23.02-40.02 mg ER/mg), total carotenoids (66.12-155.05 μg/g), picrocrocin (88.99-121.53), crocin (137.44-228.39) and safranal (26.56-53.04). The radical scavenging activity ranged from 17.09% to 29.53% for DPPH assay, and oscillated from 0.128 mmol AAE/g to 0.239 mmol AAE/g for ABTS test, while the ferric reducing antioxidant potency (FRAP) varied from 0.974 to 1.989 mmol Fe^{2+}/g. Gas chromatography-mass spectrometry (GC-MS) analysis identified 66 volatile compounds, among which the Safranal and Isophorone were the most abondant. The ES1 from Taliouine recorded a very distinct volatile composition compared to the others ecotypes with 22 authentic volatile compounds. Moreover, liquid chromatography- mass spectrometry (LC-MS) analysis revealed 14 phenolic compounds with picrocrocin and crocin were found to be the major compounds. The principal component analysis classified the investigated ecotypes into two mean distinctive sets with ES1 and ES9 were distinguished as a single items. The α-pinene, β-pinene, limonene, anethole, acetic acid, ketoisophorone, isophorone, safranal, thymoquinone, total flavonoids, FRAP and total carotenoids, are the main discriminant variables. The two-dimensional analysis of the clustered heatmaps divided showed a relatively similar patterns as the principal component analysis (PCA) and confirmed the singularity of the sample ES1 based on its particular volatile profile dominated mainly by α-terpinyl acetate, methyleugenol, copaene, anethole, limonene, methylcyclopentane, which were not identified in the other samples even at minor levels. These findings herein found revealed the high quality of Moroccan saffron, which is very important for the species breeding and valorization.

KEYWORDS
antioxidant activity, *Crocus sativus*, GC-MS, LC-MS, phenolic compounds, volatile compounds

1 | INTRODUCTION

Saffron is the spice derived from the plant botanically known as *Crocus sativus* L. belonging to *Iridaceae* family. This plant is cultivated since antiquity for the use of its stigmas (saffron) not only as a spice, but also as a traditional herbal medicine.[1–2] Regarding the origins and domestication of saffron, Vavilov[3] suggests the Middle East, while other authors indicate Central Asia or the islands of south-western Greece.[4] From this area, it would have spread to India, China and the countries of the Middle East. The Arabs propagated saffron throughout the Mediterranean basin,[5] such as in Morocco, where it was probably introduced in the 9th century.[6]

The *Crocus sativus* L. is a herbaceous perennial plant of 25 to 40 cm of height. The corm, the leaf structure and the floral organ with three red-orange stigmas, constitute the main part of the plant. The corms have a diameter from 3 to 5 cm and are covered with tunics.[7] Saffron is recognized as the most expensive spice in the world with a production of about 475 tones/year. The main producing countries are Iran, India and Morocco, but it is also produced in other countries such as Greece, Spain, Argentina, United States, China and Japan.[7] This spice is used as a condiment, colouring agent and as a medicinal plant. It is traditionally considered to be an anodyne, antidepressant, respiratory decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant and sedative. It is used in scarlet fever, small pox, colds, asthma, heart diseases, tumour, cancer, flatulent colic and in menstrual disorders.[8–9]

Morocco is the fourth producer of saffron worldwide with 6.8 tonnes produced in 2018 on an area of about 1800 hectares.[10] The traditional saffron-producing region is located in the south-centre of Morocco, mainly Taliouine and Taznakht region from the provinces of Taroudant and Ouarzazate, respectively.[11] The plan of Green Morocco has paid particular attention to the development of the saffron sector through the use of modern production techniques as well as the search for favourable areas for the introduction of the saffron crop such as Ourika, Chefchaouen, Midelt, Oujda, Sefrou, Bouchaoun, Tinghir, Errachidia.[12]

In order to assess the aromatic potential and quality of saffron cultivated in Morocco, the present study has as specific objectives: (a) the identification of the biochemical composition of ten saffron ecotypes as well as the evaluation of their antioxidant activities, (b) the exploration of the correlations among the parameters analysed (moisture, total phenolic compounds, total flavonoids, total carotenoids, safranal, picrocrocin, crocin, DPPH, FRAP and ABTS) and finally (c) the classification of the ecotypes according to their biochemical composition.

Practical applications

Saffron is a spice used in human food and in the treatment of several diseases, which confers it a very important economic value. In this context, an evaluation of its biochemical composition and its antioxidant activity will allow a beneficial use as well as a good choice of the vegetal material for the farmer. According to the obtained results, the saffron cultivated in Morocco presents a biochemical richness which can be exploited in the program of improvement of this spice.

2 | MATERIAL AND METHODS

2.1 | Plant material

In October 2020, ten saffron samples were provided from five regions of Morocco representing the main cultivation areas, namely Ifrane, Boulmane, Azilal, Taliouine and Taznakht. The geographical location and the main ecological factors of the studied ecotypes are presented in Figure 1 and Table 1. Saffron from ES1 to ES8 were provided from the Cooperative "Dar ZAAFARANE" while the samples ES9 and ES10 were collected from producers of saffron in Boulmane and Azilal.

2.2 | Chemicals and reagents used

DPPH Reagent; ABTS Reagent; TPTZ Reagent; Ciocalteu Folin reagent; $FeCl_3$; $FeSO_4$; Potassium Iodate; potassium acetate; Gallic Acid; Rutin; Ascorbic Acid; Potassium Persulphate; Aluminium Chloride ($AlCl_3$); Sodium Carbonate; Analytical Methanol; Analytical Ethanol; Analytical Hexane; Chloridric Acid; and Acetic Acid. All other products used have an analytical grade.

2.3 | Moisture content

The determination of moisture content of ten saffron ecotypes is carried out following to the international standard ISO/TS 3632-2.[13] The moisture content is expressed as a percentage of the mass fraction of the sample.

2.4 | Phytochemical Composition

2.4.1 | Extraction preparation

The extraction was performed according to the protocol described by Tajik et al.[14] Briefly, 0.1 g of the samples was mixed with 10 mL of methanol 80% (v/v) using an Ultra-Turrax digital homogenizer and Ultrasonic bath for 5 min at 25°C. The extraction was performed three times and the three recovered supernatants were centrifuged for 20 min at 4000 rpm. The resulting supernatant was filtered through Whatman No. 4 filter paper and evaporated with the Rotovapor apparatus (Buchi Suisse R – 210). The residue obtained was recovered in 15 mL of methanol for further analysis.

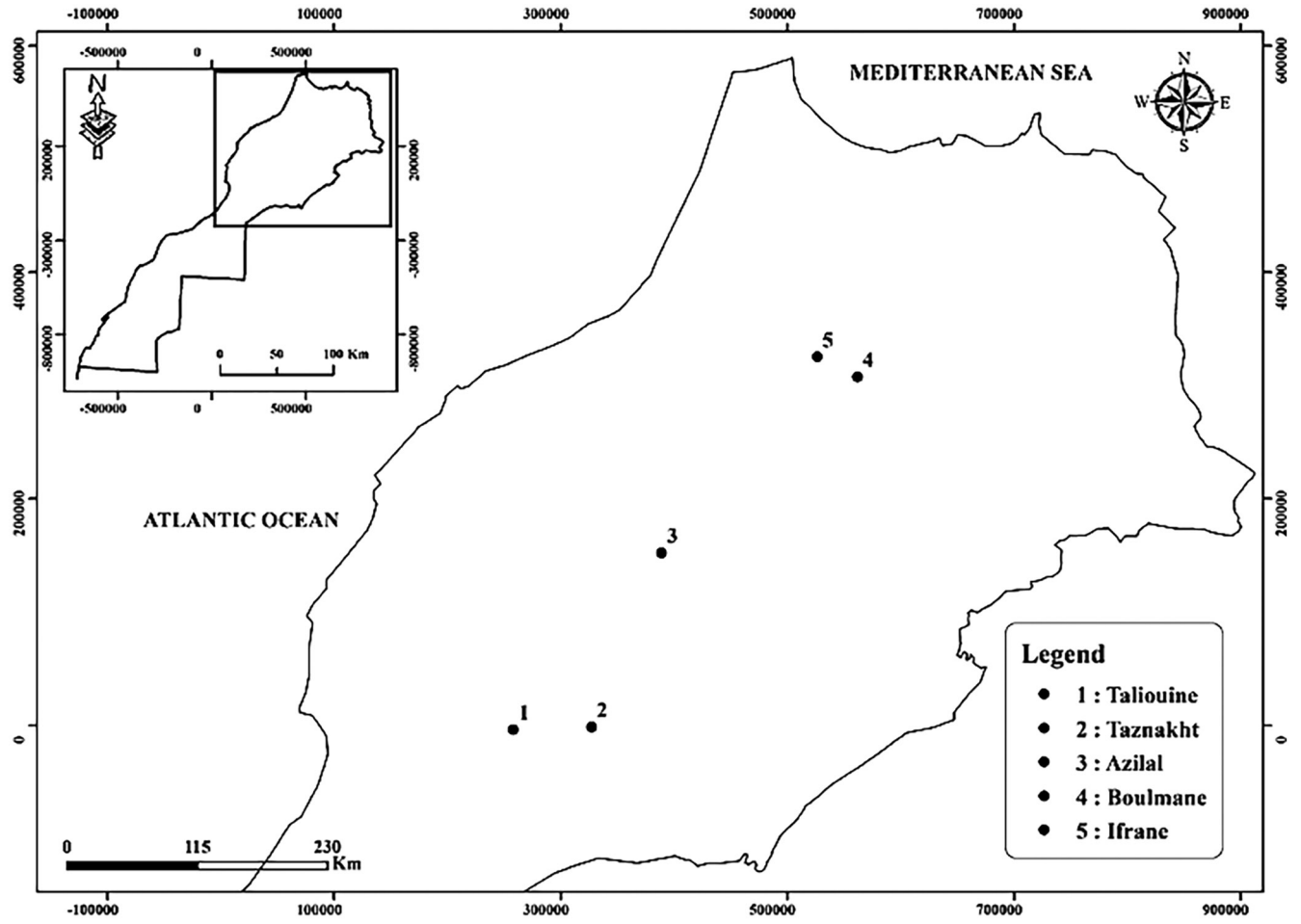

FIGURE 1 Map of Morocco Showing locations of saffron ecotypes analysed.

TABLE 1 Geographic and ecological characteristics of saffron analysed in this study

Region	Ecotypes	Geographic origin	Altitude (m)	Rainfall (mm)	Temperature Min-Max
Taliouine	ES1, ES6	Souss-Massa	1586	140-214	20-40°C
Boulmane	ES2, ES9	Fès-Meknès	2029	800-1000	-2,6 -28,9°C
Ifran	ES3	Fès-Meknès	1664	1041	3,62-18,48°C
Taznakht	ES4, ES7, ES8	Darâa-Tafilalet	968	149,0	-4,7- 38,2°C
Azilal1	ES5, ES10	Beni Mellal Khénifra	1377	550-700	6°C- 35°C

2.4.2 | Determination of total phenols

The method described by Tajik et al.[14] was used to determine total phenols. Approximately 0.5 mL of the diluted extract (1/5 v/v), was added to 2.5 mL of Folin-Ciocalteu reagent (10%). After 5 min at room temperature, 2 mL of sodium carbonate Na_2CO_3 (75 g/L) was introduced to mixture. The absorbance was measured at 765 nm against a blank after 90 min of incubation in the dark at room temperature using a UV-VIS spectrophotometer (Spectra Physics JASCO V-630). A calibration curve was performed applying gallic acid (0-300 µg/mL). The results are expressed as milligramme equivalent of gallic acid per gram of dry plant matter (mg GAE/g DM).

2.4.3 | Determination of total flavonoids

Total flavonoids were determined, as reported by Lamaison and Carnat.[15] Breifly, 1 mL of aluminium chloride ($AlCl_3$, 10%) was mixed with 1 mL of the diluted extract (1/100). The absorbance was measured with a spectrophotometer at 430 nm (Spectra Physics JASCO

V-630) against a blank after 15 min of incubation in the dark and room temperature. A standard curve was constructed using rutin with a concentrations of 0-1 mg/mL. The results obtained are expressed as milligrammes of rutin equivalent per gram of dry plant material (mg RE/g DM).

2.4.4 | The total carotenoids content

The total carotenoids content was performed according to the protocol prescribed by Lahmass et al.[16] In a glass beaker, 100 mg of saffron were added to 6 mL of a mixture of three solvents hexane/acetone/ethanol (v / v; 15/7, 5/7, 5). The extraction was carried out at room temperature using an Ultra-Turax apparatus under stirring. After 10 min, the extract was centrifuged at 4500 rpm for 5 min and the resulting supernatant adjusted to 10 mL with the extraction solvent. The absorbance was measured with a spectrophotometer (Spectra Physics JASCO V-630) at 450 nm. The total carotenoid content was calculated applying the formula below and expressed as μg/g.

$$CT = \frac{\text{DO sample x Volume (mL)}}{\text{EC x sample weight (g)}}$$

where EC: 2592 (extinction coefficient of β-carotene in petroleum ether).

2.4.5 | Determination of safranal, picrocrocin and crocin content

The preparation of the ecotypes extract was carried out following the ISO procedure.[17] In a glass vial, 25 mg of the sample was placed with 45 mL of distilled water. The mixture was agitated with a magnetic stirrer at 1000 rpm for 1 h in the dark. Then, 5 mL of distilled water was added and homogenized by stirring. 1 mL of the homogenate was placed in a glass tube with 9 mL of distilled water. The solution was filtered through a hydrophilic polytetrafluoroethylene (PTFE) filter with a pore diameter of 0.2 μm. The filtrate was analysed directly with a UV-visible spectrophotometer (Spectra Physics JASCO V-630) equipped with a 1 cm path quartz. The amount of Picrocrocin, Crocin and Safranal expressed as direct reading of the absorbance of 1% aqueous solution of dried saffron at 257, 330 and 440 nm, respectively, with the following equation:

$$E^{1\%}_{1cm}\,\lambda\,\text{max} = \frac{D \times 1000}{m(100 - H)}$$

where $E^{1\%}_{1cm}\,\lambda$ max, The absorbance at the respective wavelength; D, The specific absorbance; m, The sample weight (g); H, The moisture content.

2.5 | Determination of Antioxidant Activity

2.5.1 | DPPH test

The capacity of saffron to scavenge the DPPH radical was evaluated with the procedure described by Tuberoso et al.[18] Briefly, 50 μL of diluted extracts (1/2; 1/10; 1/20; 1/50; 1/100) were mixed with 2 mL of DPPH solution (0.04 mmol /l in methanol). After 60 min of incubation in the dark with room temperature, the optical density measurements were determined at 517 nm using a spectrophotometer (Spectra Physics JASCO V-630). The results were expressed as percentage inhibition (%), according to the following formula:

$$RSA(\%) = \frac{A0 - AS}{A0} \times 100$$

where A_0 is the absorbance of control and A_S is the absorbance of the sample.

2.5.2 | ABTS test

The antioxidant activity of the extracts was assessed by ABTS assay according to the method used by Urbani et al.[19] The $ABTS^{+\bullet}$ solution was prepared by combining an equal volume of a stock solution of ABTS (7 mmoL/L) with a solution of potassium persulphate (2.45 mmoL/L). The mixture was then maintained in the dark for 16 hours before being diluted by methanol to an absorbance of 0.70 (±0.02) at 734 nm. Then, 2 mL of $ABTS^{+\bullet}$ solution was added to 30 μL of ecotypes dilutions (1/2; 1/10; 1/20; 1/50; 1/100). The absorbance was measured at 734 nm by a spectrophotometer (Spectra Physics JASCO V-630) after incubation for 6 min in the dark at room temperature. A calibration range on ascorbic acid at concentrations between 0.05 mmoL/L and 4.5 mmoL/L was constructed. The results were expressed as mmol AAE/g DM.

2.5.3 | FRAP test

The FRAP test was performed according the protocol prescribed by Benzie and Strain[20] as modified by Pulido et al.[21] The FRAP reagent, produced by mixing TPTZ (10 mmoL/L) in HCl (40 mmoL/L), $FeCl_3$ (20 mmol/L) and potassium acetate buffer (300 mmoL/L, pH 3.6) with a ratio of 1:1:10, was incubated at 37°C for 10 min. Then, 3 mL of the FRAP reagent was added to 300 μL of distilled water and 100 μL of the sample. The absorbance was measured at 595 nm after 30 min of incubation at 37°C using a spectrophotometer (Spectra Physics JASCO V-630). A standard curve was constructed within the constraction range of 0.1-2 mmoL/L $FeSO_4$. The values were expressed as mmol Fe^{2+}/g DM.

2.6 | Determination of volatile compounds

Static headspace extraction of volatile compounds was performed using solid phase microextraction (SPME) with a 65 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibre. The analysis of saffron volatiles was carried out by gas chromatography-mass spectrometry (GC-MS) applying a gas chromatography Agilent 7890 A with masse selective detector 5975 Network MSD and coupled to an automatic sampling system MPS (Gerstel), a polyethylenglycol capillary column VF- WAXms (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and a split/splitless injector, and the Library pal 600 k. Approximately 1 g of the investigated sample was placed into a 20 mL glass vial sealed with a Teflon-coated screw cap and heated to 60°C for 20 minutes and the fibre was then exposed to the saffron headspace. After 20 min, the SPME fibre was automatically with drawn from the vial and introduced into the GC injector. Working conditions were: splitless mode with injector temperature at 250°C, the oven temperature program was 40°C for 2 min, rising at 4°C/min to 64°C, then increasing at 2°C/min to 100°C and finally at 20°C/min to 300°C (held for 5 min). The flow rate was set to 1 mL/min (helium) was set up. Mass spectra were recorded in EI mode at 70 eV, scanning the 35-395 m/z range. The interface and source temperatures were 230 and 280°C, respectively.

2.7 | Determination of the phenolic compounds

In a Sovirel tube, 50 mg of saffron was placed with 2 mL of methanol/H_2O (80/20). The mixture was heated in an ultrasonic bath for 20 min then centrifuged at 3000 g for 10 min. The supernatant was filtered through a syringe filter (0.2 μm) and placed in a vial. Then, 10 μL of extracts was analysed on a High Performance Liquid Chromatography system coupled to mass spectrophotometry (LC-MS). Phenolic compounds were separated at a set temperature of 40°C and chromatograms were recorded at 250, 280, 320, 370 and 510 nm. The separation gradient was formed by water-methanol.

2.8 | Statistical analysis

The data obtained were normalized to a comparable scale and then ($\mu = 0$ and $\sigma = 1$) subjected to several analyses, including Analysis of variance (ANOVA) with the Duncan's multiple range test (DMRT) to determine the significance of differences between the ecotypes. In addition, the correlation coefficients and their significance levels were determined using Pearson's correlation ($\alpha = 0.05$). All these analyses were performed using IBM SPSS v22 software. Moreover, the ecotypes classification was identified with Principal Component Analysis (PCA) and a two-dimensional hierarchical heatmap. These analyses were performed with R 3.0.2 software.

3 | RESULTS AND DISCUSSION

3.1 | Moisture level

The results showed a significant variation in moisture content, which oscillated from 9.03% for "ES3" to 11.23% for "ES4" (Table 2). The moisture level of the ten ecotypes herein studied is lower than 12%, meaning that Moroccan saffron conforms to the requirements of the ISO/TS 3632 standard. Indeed, a low moisture content is a crucial factor in maintaining the quality and to increase storage time of product.

3.2 | Phytochemical Composition

The results for total phenols, total flavonoids and total carotenoids were presented in Table 2, revealed a significant difference among the ten ecotypes ($P < .001$). In fact, the total polyphenol varied from 31.63 (ES3) to 62.71 mg EAG/g DM for ES4. These values are substantially higher than those reported by Lahmas et al.[16] (16.63 mg EAG/g DM) in saffron cultivated in the region of Oujda (Eastern Morocco). Furthermore, Baba et al.[22] reported a low level of total polyphenols in Indian saffron (8.28 mg EAG/g DM). However, Belyagoubi et al.[23] recorded a high amount of total phenolics in Algerian saffron (97.993 mg EAG/g DM). Recent investigation indicated that agroclimatic conditions and types of extraction lead to a large variation in total phenolic content.[24] The total polyphenol content in saffron is very important compared to other food additives and spices, such as *E. caryophyllata*, *Lavandula spp.*, *C. domestica Val* and *C. longa* L. (0.26, 0.22, 35.6 and 21.4 mg EAG/100 g DM, respectively).[24] For the total flavonoid, the highest level observed in ES7 (40.02 mg ER/g DM) followed by ES8 (39.86 mg ER/g DM), while the lowest recorded in ES1 (23.02 mg ER/g DM). These concentrations are higher of those recorded for Algerian saffron (5.96 mg EC/g DM)[23] and for Indian saffron (3.53 mg ER/g DM).[22] Regarding the total carotenoid content, the highest content was registered in ES9 (155.0 μg/g), while ES3 showed the lowest (66.12 μg/g). These results are higher in comparison to the amount reported by Lahmas et al.[16] (16.132 μg/g) but lower of that found in Iranian saffron (579.67 ± 11.12 μg/g).[25]

The analysis of safranal, picrocrocin and crocin allows for a global assessment of the aromatic, taste and colouring quality of saffron.[26] The amount of picrocrocin, crocin and safranal in the ten ecotypes are significantly different ($P < .001$) (Table 3). The level of safranal in the ecotypes ranged from 26.56 (ES2) to 53.04 (ES3). According to the ISO/TS 3632 standard,[17] Saffron under investigation falls into category I (between 20 and 50), indicating that Moroccan saffron is of superior quality. Concerning the picrocrocin, the values varied from 88.99 to 121.53. The ES1 from Taliouine recorded the maximum value, while ES4 from Taznakht showed the minimum value. All ecotypes contained a picrocrocin level exceeding 70, which means that they are classified as Category I.[17] The crocin content (137.44-228.39) allowed to classify the ten ecotypes in the three categories. The ecotypes of category I with a decreasing order are ES5,

TABLE 2 Total phenolic, total flavonoids, total carotenoid and moisture of ten saffron ecotypes

Ecotypes	Total phenols (mg EAG/g DM)	Total flavonoids (mg ER/g DM)	Total Carotenoid (µg/g)	Moisture (%)
ES1	45.07 ± 7.58 c. d	**23.02 ± 0.14 a**	71.12 ± 10.44 a	9.82 ± 0.076 c
ES2	57.13 ± 1.90 b	36.79 ± 0.20 e. f	91.2 ± 2.18 b	11.00 ± 0.1 e. f
ES3	**31.63 ± 2.72 b**	27.76 ± 2.35 b	**66.12 ± 1.85 a**	**9.03 ± 0.064 a**
ES4	**62.71 ± 4.24 e. f**	32.81 ± 0.62 c	93.82 ± 0.05 b. c	**11.23 ± 0.057 f**
ES5	46.48 ± 0.66 a	38.78 ± 0.63 f. g	115.16 ± 8.89 d. e	10.93 ± 0.11 e
ES6	48.63 ± 4.58 f	35.97 ± 1.29d. e	112.71 ± 9.24 c. d. e	9.81 ± 0.051 c
ES7	41.35 ± 4.10 b. c	**40.02 ± 0.74 g**	131.21 ± 0.49 e	10.42 ± 0.275 d
ES8	56.86 ± 1.06 b. c	39.86 ± 2.55 g	100.36 ± 3.24 b. c. d	9.86 ± 0.086 c
ES9	53.18 ± 2.28 b	38.19 ± 0.87 e. f. g	**155.05 ± 2.07 f**	10.46 ± 0.321 d
ES10	41.53 ± 5.17 d. e	34.27 ± 0.53 c. d	101.31 ± 2.67 b. c. d	9.31 ± 0.209 b
F	16.222	57.207	17.790	60.991
ANOVA mean square	256.181***	92.719***	2116.694***	1.640***

Note: Significance level ***$P < .001$; bold values represent minimum and maximum; different letters (a-i) in the columns represent statistically significant differences between ecotypes at $P < .05$.

ES7, ES6 and ES8, belonging to the regions of Azilal, Taliouine and Taznakht, respectively. The ES1, ES2, ES4, ES9 and ES10 ecotypes, from Taliouine, Boulmane, Taznakht, Boulmane and Azilal1, respectively, with values equal or higher than 150, are classified in category II. The ES3 from the region of Ifran, belongs to category III.[17] The results obtained are comparable to the values published by Nazarian et al.,[27] for saffron from different regions of the Herat province in Afghanistan (picrocrocin:104.50, safranal:34.95 and crocin:236.95). The Crocin, picrocrocin and safranal contents differ significantly among countries, as well as among saffrons grown in the same country and belonging to similar edapho-climatic conditions. These variations are probably due to other factors, such as genotype, cultivation and drying condition.[19] Indeed, the typologies of drying methods, such as shade, heating system, electric ovens and sun exposure, greatly influence the phytochemical composition of saffron.[28]

3.3 | Antioxidant activities

The results of antioxidant activity assessment of ten Moroccan saffron ecotypes using DPPH, ABTS and FRAP tests indicated a significant difference ($P < .001$) between the ecotypes (Table 4). In general, the samples showed a weak scavenging properties against the DPPH radical. In fact, the percentages of inhibition of ten saffron ecotypes ranged from 17.09% (ES3) to 29.52% (ES4) with an average of 22.17%. The decreasing order of DPPH scavenging capacity is ES4 > ES6 > ES10 > ES5 > ES2 > ES7 > ES9 > ES8 > ES1 > ES3. These results are lower than others showed by Samaha et al.[29] studying the Lebanese saffron (42.3%-59.9%). Several studies reported that antioxidant activity of saffron stigmas is less than other parts of the plant.[25]

Furthermore, the values obtained for the scavenging capacity of the ABTS radical varied from 0.128 mmol AAE/g DM for the ecotype ES10 to 0.239 mmol AAE/g DM for the ecotype ES4. The decreasing order of ABTS scavenging capacity is as follows: ES4 > ES8 > ES5 > ES9 > ES6 > ES1 > ES7 > ES2 > ES3 > ES10 meaning that the latter has the highest ABTS radical scavenging capacity. These values are in agreement with those found by Urbani et al.[18] in Italian saffron (61.2-161.4 µmol TE/g), but lower of the result registered in Greek saffron methanolic extract (12.90 mmol TE/g).[30]

TABLE 4 Antioxidant activity with DPPH, ABTS and FRAP tests

Ecotypes	DPPH (%)	ABTS (mmol AAE/g DM)	FRAP (mmol Fe^{2+}/g DM)
ES1	17.094 ± 0.189 a	0.183 ± 0,0161 b	**0.974 ± 0,006 a**
ES2	22.651 ± 0.567 c	0.178 ± 0,071 b	1.693 ± 0,026 f
ES3	**17.002 ± 0.199 a**	0.134 ± 0,002 a	1.452 ± 0,012 d
ES4	**29.525 ± 0.258 f**	**0.239 ± 0,007 d**	1.969 ± 0,012h
ES5	23.425 ± 0.079 d	0.236 ± 0,004 d	**1.989 ± 0,022 i**
ES6	26.707 ± 0.338 e	0.199 ± 0,001 c	1.721 ± 0,006g
ES7	22.467 ± 0.358 c	0.183 ± 0,007 b	1.368 ± 0,006 c
ES8	19.312 ± 0.139 b	0.236 ± 0,024 d	1.199 ± 0,002 b
ES9	20.045 ± 0.577 b	0.202 ± 0,004 c	1.465 ± 0,001 e
ES10	23.587 ± 0.179 d	**0.128 ± 0,007 a**	1.447 ± 0,019 d
Mean	22.17	0.190	1.527
F	290.349***	223886***	44 589,497***

Note: Significance level ***$P < .001$; bold values represent minimum and maximum; different letters (a-i) in the columns represent statistically significant differences between ecotypes at $P < .05$.

Moreover, the FRAP test revealed a capacity of scavenging ranging from 0.974 to 1.989 mmol Fe^{2+}/g DM, with an overall average of 1.527. According to the results obtained, the ten ecotypes are ranked in descending order in the following order: ES5 > ES4 > ES6 > ES2 > ES9 > ES3 > ES10 > ES7 > ES8 > ES1. The most powerful saffron sample in terms of antioxidant activity is ES5, while the weakest is ES1. The antioxidant activity of Moroccan saffron is lower in comparison to the antioxidant activity of the floral by-products of Italian saffron.[31]

TABLE 3 Safranal, Picrocrocin and Crocin content

	ES1	ES2	ES3	ES4	ES5	ES6	ES7	ES8	ES9	ES10	F
Safranal $E^{1\%}_{1cm}$ 330	42.06	**26.56**	**53.04**	42.12	36.92	34.63	35.12	36.23	27.09	28.46	755.570***
Picrocrocin $E^{1\%}_{1cm}$ 257	**121.53**	89.58	92.4	**88.99**	111.189	112.23	109.38	99.88	110.78	95.43	267.706***
Crocin $E^{1\%}_{1cm}$ 440	163.02	183.52	**137.44**	177.65	**228.39**	218.63	225.2	200.02	178.6	160.96	4517.870***

Note: ***significant difference at the level of 0.001, bold values represent minimum and maximum.

3.4 | Volatile compound Identification

The results of the study of volatile compounds of ten saffron ecotypes using gas chromatography-mass spectrometry (GC-MS) are summarized in Table 5. The total number of compounds found was 66, which is similar to the total reported by Sevindik[32] in a study on Turkish saffron (64). According to values obtained, the ecotypes showed a great content of safranal, varying from 19.17 (ES7) to 66.7% (ES5) with an average of 36.67%. The most abundant volatile compounds detected by Karabagias et al.[33] in saffron collected from Moroccan, Greek, Iranian and Spanish was safranal with 79.04, 71.35, 76.42 and 64.71% of saffron's total volatile fraction. In addition, the highest concentration of Isophorone (45.7%) recorded in ES8, while the lowest (1.10%) revealed in ES2, with an average of 22.89%. This finding is higher than the isophorone contribution to the total volatile fraction of Moroccan, Greek and Spanish saffron (1.94, 8.26 and 13.26%, respectively)[33]. Moreover, the concentration of 2.2.6-trimethyl-1.4 -Cyclohexanedione registered in this study varied from 3.79% (ES5) to 20.68% (ES2) with an average of 9.68%. While, the concentration of 2,2,6-trimethyl- 1.4-cyclohexanedione in Spanish, Iranian, Greek and Moroccan saffron was 0.90%, 0.57%, 0.48% and 0.11%, respectively.[33] Regarding Ketoisophorone, the highest level (23.31%) and the lowest (3.77%) are indicated by ecotypes ES9 and ES2, respectively, with an average of 9.68%. Similarly, the presence of acetic acid is observed at modest amounts in all ecotypes except ES1. The highest concentration recorded in ES8 (2.39%), whereas, the lowest concentration registered in ES5 (0.75%) with an average of 1.55%. In addition, 2(5H)-Furanone, 2.2.6-trimethylcyclohexane-1.4-dione and 4-Hydroxy-2.6.6-trimethyl-3-oxocyclohexa-1.4-dienecarbaldehyde are identified in all samples, except ES1 and ES2, with weak concentrations (0.11% (ES5)- 2.16% (ES8), 1.52% (ES7)- 0.79% (ES10) and 0.83% (ES8)- 6.95% (ES10), respectively). The results obtained by Azarabadi and Özdemir[34] in all saffron marketed in Iran indicated that the Acetic acid, 2-(5H)-furanone, isophorone, 4-ketoisophorone, 2,6,6-trimethyl-1,4-cyclohexanedione were the main volatile compounds. Based on the results obtained, ES1 from Taliouine recorded a very distinct volatile composition compared to the other samples. It revealed high concentrations of α-Terpinyl acetate (34.82%), followed by Anethole (19.93%), Methyleugenol (11.87%), Caryophyllene (7.45%), Limonene (4.39%), β-Pinene (1.31%) and α-pinene (0.30%). Other chemicals, with modest amounts but crucial roles in the aroma of saffron,[35] were recorded namely: Lanierone, which occurred only in two ecotypes "ES4" and "ES10" with concentrations of 1.10% and 0.01%, respectively, and also 4-Hydroxy-2.6.6-trimethylcyclohex-1-enecarbaldehyde (HTCC), which recorded in ES5 (0.52%) and ES2 (0.75%). The HTCC is present in greater quantities in humide ecotypes because it is synthesized by enzymatic hydrolysis.[36] Indeed, the moisture content and the drying process have a great impact on the concentration and composition of volatile compounds of saffron. A previous study on Taliouine saffron, conducted in 2017 by Atyane et al.[28] found that the content of safranal obtained by gas chromatography analysis was significantly influenced by the drying process of saffron. In general, these variations in volatile profiles of ten ecotypes may be due to abiotic environmental factors and biotic factors. In the case an authentication between samples is needed. Therefore, the volatiles identified as discriminators could serve as biomarker. Indeed, ES1 is characterized by 22 compounds like α-Terpinyl acetate, Anethole, Methyleugenol and Copaene. The ES2 distinguished by six compounds as Thymoquinone, ES5 and ES9 by four compounds, ES4 by two compounds, while ES3 and ES6 characterized by one compound (Table 6).

3.5 | Phenolic compounds profile

The results of assessment of the phenolic compounds of the six ecotypes are presented in Figure 2 and Table 7. Fourteen phenolic compounds were identified in Moroccan saffron. Indeed, the mass spectrum detected the presence of picrocrocin, which is one of the three major compounds determining the quality of saffron, with a ratio m/z of 336.6 to 337.9. The sample ES3 from Ifrane, showed the highest intensity, while the sample ES6 from Taliouine area recorded the lowest. The m/z ratio of crocin−4 was found to be 532.4-554.4 with the highest values recorded especially in ecotype ES2 followed by sample ES6, while the lowest value is revealed by sample ES1. This latter is lacking the crocin-2 and Kaempferol-di-O-glycoside, which are detected in all the analysed ecotypes with a m/z of 744.0-750 and 456.4-456.5, respectively. The crocin-3 compound, with a m/z of 652.1-652.2, was found to be abundant in ES4 but rare in ES2. Similarly, the mass spectrum allowed us to identify kaempferol, which is detected in all the ecotypes studied with m/z of 287 except in "ES6". Regarding Kaempferol-3-gentiobioside-7-glucoside and Kaempferol 3-sophoroside-7-glucoside, are also found in all ecotypes except "ES2" and "ES1," which are revealed rich in Quercetin (m/z = 488,9). For the case of catechin, it is detected only in sample "ES5" with an m/z ratio of 292.4-296.7. Additionally, the chromatograms recorded other phenolic compounds such as phenylethyl butanoate and ergot-7,22-diene-3β,5α,6β-triol. These results are consistent with those of Aiello et al.,[37] who detected kaempferol, Kaempferol-3-gentiobioside-7-glucoside, 3-sophoroside-7-glucoside, Kaempferol-di-O-glycoside, Quercetin and Catechin in Greek saffron.

TABLE 5 Volatile compounds of the ten Moroccan saffron ecotypes

Compound name	Ecotypes (%)									
	ES1	ES2	ES3	ES4	ES5	ES6	ES7	ES8	ES9	ES10
2.3-Butanedione	–	–	–	–	–	0.09	0.16	–	0.27	0.03
Methyl-Cyclopentane	0.99	–	–	–	–	–	–	–	–	–
Acetic acid	–	**0.96**	**1.16**	**1.03**	**0.75**	**1.98**	**1.68**	**2.39**	**1.75**	**2.23**
1-Butanol	–	0.26	0.03	–	–	–	0.18	0.1	-	0.37
Acetic acid. Methoxy-. ethyl ester	–	–	–	0.44	–	–	0.3	–	–	–
1-hydroxy-2-Propanone	–	–	–	–	–	0.1	–	–	0.1	–
Formic acid. 2-methylpropyl ester	–	0.18	–	–	–	–	–	–	–	–
Formic acid. Butyl ester	–	0.77	_	–	–	–	–	–	–	–
Acetoin	–	–	–	–	–	1.15	–	0.46	1.55	1.06
Butyrolactone	–	–	–	–	0.23	–	–	–	–	–
4-hydroxy-butanoic acid.	–	–	–	–	–	0.3	0.21	–	–	0.43
2(5H)-Furanone	–	–	**1.76**	**1.12**	**0.11**	**0.41**	**0.23**	**2.16**	**1.46**	**0.37**
α-pinene	**0.3**	–	–	–	–	–	–	–	–	–
β-Pinene	**1.31**	–	–	–	–	–	–	–	–	–
2.2.3-trimethyl-Nonane	–	0.3	–	–	–	–	–	–	–	–
Limonene	**4.39**	–	–	–	–	–	–	–	–	–
3-Carene	0.04	–	–	–	–	–	–	–	–	–
β-Isophorone	–	–	**0.29**	**3.07**	–	**1.01**	**16.49**	**1.22**	**4.63**	**5.34**
2-Isopropylidene-3-methylhexa-3.5-dienal	–	–	–	–	–	–	–	–	0.19	–
α-Terpinene	0.58	–	–	–	–	–	–	–	–	–
2.6.6-Trimethyl-1.4-cyclohexadiene-1-carboxaldehyde	–	–	**1.15**	**1.34**	**1.08**	**1.01**	**1.52**	**1.14**	**1.34**	**0.79**
Isophorone	–	**1.1**	**11.75**	**24.28**	**11.58**	**41.04**	**18.54**	**45.7**	**29.27**	**22.75**
γ-Diosphenol	–	–	–	0.28	–	0.27	0.44	–	–	–
Dehydroacetic Acid	–	–	–	–	–	–	–	–	0.36	–
D-Camphor	–	–	–	–	–	–	–	–	0.15	–
Ketoisophorone	–	**3.77**	**14.07**	**14.82**	**11.4**	**16.1**	**22.39**	**13.03**	**23.31**	**19.73**
Lanierone	–	–	–	1.1	–	–	–	–	–	0.01
2.2.6-trimethylcyclohexane-1.4-dione	–	**20.68**	**6.48**	**12.24**	**3.79**	**7.11**	**9.72**	**6.57**	**5.47**	**15.1**
Safranal	–	**45.8**	**60.76**	**34.07**	**66.07**	**23.15**	**19.17**	**30.88**	**27.18**	**22.96**
Eucarvone	–	–	–	–	1.25	–	–	–	–	–
4-Hydroxy-2.6.6-trimethylcyclohex-1-enecarbaldehyde	–	**0.75**	**0.21**	**0.03**	**0.52**	–	–	–	–	–
2-Hydroxy-3.5.5-trimethylcyclohex-2-ene-1.4-dione	–	–	–	–	1.13	–	–	–	–	–
Benzaldehyde. 4-(1-methylethyl) -	1.62	–	–	–	–	–	–	–	–	–
2-Hydroxy-3.5.5-trimethylcyclohex-2-ene-1.4-dione	–	2.16	–	–	–	–	–	–	–	–
Anethole	**19.93**	–	–	–	–	–	–	–	–	–
cis. cis-Nepetalactone	–	–	0.99	0.69	–	0.18	0.59	–	0.44	0.66
Mint furanone	–	–	–	–	0.93	–	–	0.16	–	–
Thymoquinone	–	**11.17**	–	–	–	–	–	–	–	–
Carbonic acid. Monoamide. N-octyl-. propyl ester	–	–	–	–	0.29	–	–	–	–	–
δ-Elemene	0.51	–	–	–	–	–	–	–	–	–
1-Cyclohexanone. 2-methyl-2-(3-methyl-2-oxobutyl)	–	–	–	0.58	–	0.2	0.83	0.2	0.47	1.08
α-Terpinyl acetate	**34.82**	–	–	–	–	–	–	–	–	–
Copaene	**10.49**	–	–	–	–	–	–	–	–	–
Car-3-ene-2.5-dione	–	1.3	–	–	–	–	–	–	–	–
Cedr-9-ene	–	–	–	0.01	–	–	–	–	–	–

TABLE 5 (Continued)

Compound name	Ecotypes (%)									
	ES1	ES2	ES3	ES4	ES5	ES6	ES7	ES8	ES9	ES10
β-Elemene	0.77	–	–	–	–	–	–	–	–	–
4-Hydroxy-2.6.6-trimethyl-3-oxocyclohexa-1.4-dienecarbaldehyde	–	–	**1.25**	**3.14**	**0.89**	**1.04**	**6.05**	**0.83**	**1.43**	**6.95**
Methyleugenol	**11.87**	–	–	–	–	–	–	–	–	–
Cedr-8-ene	–	–	–	0.15	–	–	–	–	–	–
Caryophyllene	**7.45**	–	–	–	–	–	–	–	–	**0.08**
cis-α-Bergamotene	0.48	–	–	–	–	–	–	–	–	–
α.β-Dihydro-β-ionone	–	–	–	–	–	0.02	–	–	0.1	0.03
Elemicin	–	–	0.1	–	–	–	–	–	–	–
Isoelemicin	–	–	–	–	–	0.06	–	–	0.21	–
Humulene	1.13	–	–	–	–	–	–	–	–	–
trans-Geranylacetone	–	–	–	–	–	0.04	–	–	–	–
5-Hydroxy-6-methoxy-8-[(4-amino-1-methylbutyl) amino] quinoline trihydrobromide	0.72	–	–	–	–	–	–	–	–	–
α-Curcumene	0.27	–	–	–	–	–	–	–	–	–
β-Selinene	0.06	–	–	–	–	–	–	–	–	–
α-Muurolene	0.8	–	–	–	–	–	–	–	–	–
6-Methoxy-4-methylcoumarin	–	–	–	–	–	0.01	–	–	_	0.03
β-Bisabolene	0.44	–	–	–	–	–	–	–	–	–
γ-Cadinene	0.14	–	–	–	–	–	–	–	–	–
Cadina-1(10).4-diene	0.9	–	–	–	–	–	–	–	–	–
5.8-Diethyldodecane	–	–	–	–	–	–	–	–	0.01	–
Benzaldehyde. 6-hydroxy-4-methoxy-2.3-dimethyl-	–	–	–	–	–	0.36	_–	0.16	0.14	–

The compounds in bold are the most important volatiles in the ten saffron ecotypes analyzed.

TABLE 6 Authentic volaliles compounds of some ecotypes studied

Eotypes	Authentic volatiles
ES1	Methyl-Cyclopentane, α-pinene, β-Pinene, Limonene, 3-Carene, α-Terpinene, Benzaldehyde. 4-(1-methylethyl) –, Anethole, δ-Elemene, α-Terpinyl acetate, Copaene, β-Elemene Methyleugenol, cis-α-Bergamotene, Humulene, 5-Hydroxy-6-methoxy-8-[(4-amino-1-methylbutyl) amino] quinoline trihydrobromide, α-Curcumene, β-Selinene, α-Muurolene β-Bisabolene, γ-Cadinene, Cadina-1(10).4-diene
ES2	Formic acid. 2-methylpropyl ester, Formic acid. Butyl ester, 2.2.3-trimethyl-Nonane, 2-Hydroxy-3.5.5-trimethylcyclohex-2-ene-1.4-dione, Thymoquinone, Car-3-ene-2.5-dione
ES3	Elemicin
ES4	Cedr-9-ene, Cedr-8-ene
ES5	Butyrolactone, 4-Hydroxy-2.6.6-trimethylcyclohex-1-enecarbaldehyde, 2-Hydroxy-3.5.5-trimethylcyclohex-2-ene-1.4-dione, Carbonic acid. Monoamide. N-octyl-. propyl ester
ES6	trans-Geranylacetone
ES9	2-Isopropylidene-3-methylhexa-3.5-dienal, Dehydroacetic Acid, D-Camphor, 5.8-Diethyldodecane

3.6 | Correlation analysis

The correlation between the moisture, total phenolics, total flavonoids, total carotenoids, safranal, picrocrocin, crocin, DPPH, FRAP and ABTS assay is presented in Table 8. The results obtained registered a significant positive correlation between crocin and the following parameters: Moisture ($r = 0.57^{**}$), total flavonoids ($r = 0.71^{**}$), total carotenoids ($r = 0.54^{**}$), ABTS ($r = 0.60^{**}$), FRAP (0.48^{*}) and picrocrocin (0.39^{*}). However, no significant correlation was found between the total polyphenol and the antioxidant activity tests (DPPH, ABTS and FRAP). The total phenolic content and antioxidant activity of several plant extracts were previously reported to have no significant association.[38] This finding can be explained as the antioxidant activity of the ecotypes can be influenced by the

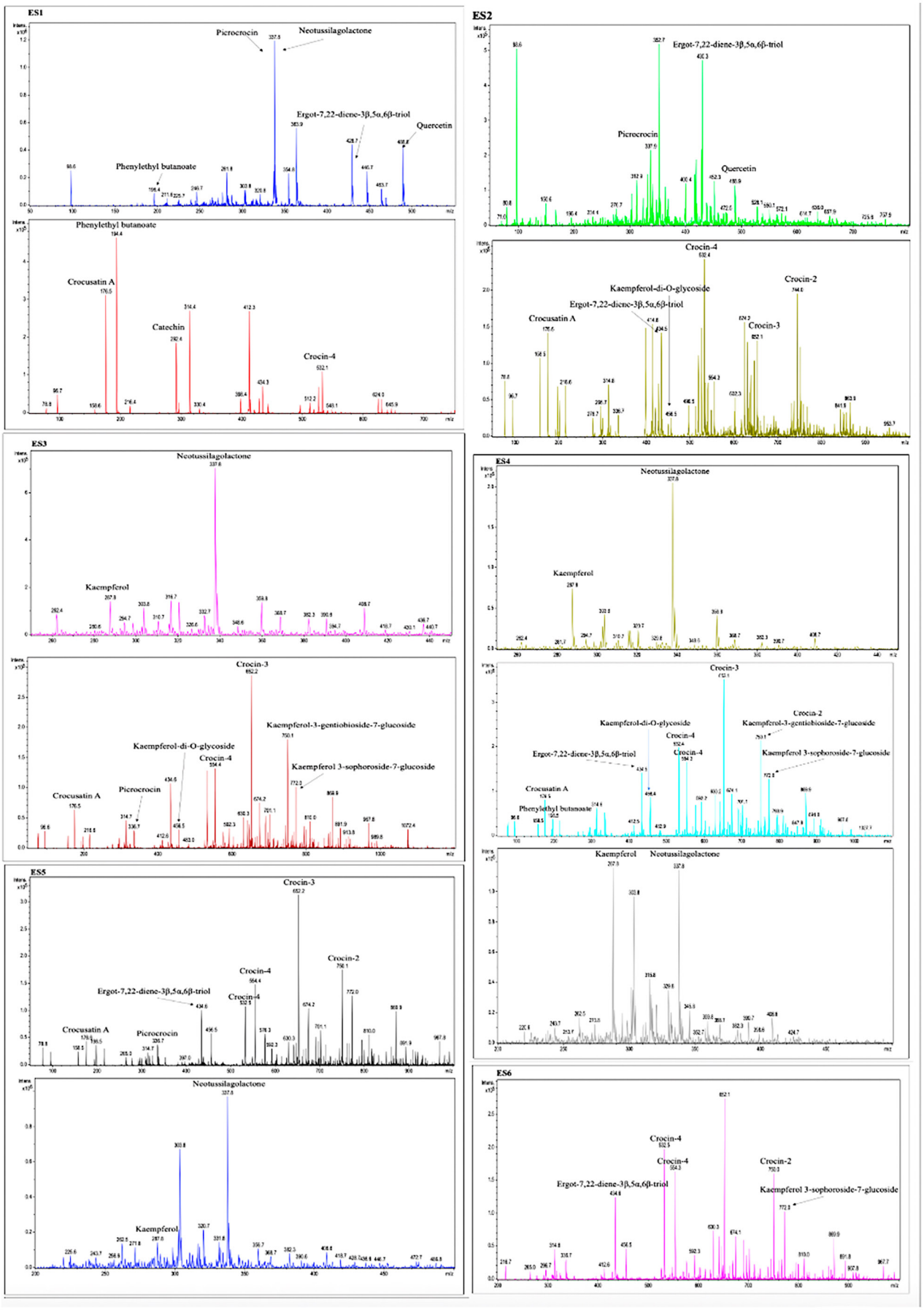

FIGURE 2 LC chromatograms of the six Moroccan saffron ecotypes.

TABLE 7 Phenolic compounds of the six Moroccan saffron ecotypes

Compounds Number	Compound name	m/z	Ecotypes
1	Kaempferol	287	ES2, ES3, ES4, ES5
2	Kaempferol-3-gentiobioside-7-glucoside	744-750.1	ES3, ES4, ES5, ES6
3	Kaempferol-di-O-glycoside	456.4-456.5	ES2, ES3, ES4, ES5, ES6
4	Kaempferol 3-sophoroside-7-glucoside	772	ES3, ES4, ES5, ES6
5	Quercetin	488.9	ES1, ES2
6	Crocusatin A	176.5-176.6	ES1, ES2, ES3, ES4, ES5
7	Neotussilagolactone	337.8	ES1, ES2, ES3, ES4, ES5
8	Crocin-2	744.0-750.1	ES2, ES3, ES4, ES5, ES6
9	Crocin-3	652.1-652.2	ES2, ES3, ES4, ES5
10	Crocin-4	532.5-554.4	ES1, ES2, ES3, ES4, ES5, ES6
11	Picrocrocin	336.6-337.9	ES1, ES2, ES3, ES4, ES5, ES6
12	Ergot-7.22-diene-3β.5α.6β-triol	430.3-434.6	ES1, ES2, ES3, ES4, ES5, ES6
13	Phenylethyl butanoate	194.4-198.5	ES1, ES2, ES4, ES5
14	Catechin	292.4-296.7	ES1, ES2, ES3, ES4, ES6

TABLE 8 Correlation among biochemical parameters analysed

	Moisture	PT	FT	CT	DPPH	ABTS	FRAP	Safranal	picrocrocin	Crocin
Moisture	1									
PT	−0.374	1								
FT	0.421*	−0.364	1							
CT	0.334	−0.311	0.690**	1						
DPPH	0.472*	0.295	0.443	0.217	1					
ABTS	0.719**	−0.285	0.417	0.293	0.376	1				
FRAP	0.570**	−0.176	0.41	0.327	0.769**	0.358	1			
Safranal	−0.333	0.144	0.603**	−0.608**	−0.257	−0.061	0.372	1		
Picrocrocin	0.04	−0.059	−0.088	0.299	−0.257	0.221	−0.098	−0.062	1	
Crocin	0.572**	−0.265	0.710**	0.545**	0.42	0.606**	0.482*	−0.353	0.397*	1

Abbreviations: CT, Total carotenoids; FT, Total flavonoids; PT, Total polyphenols.Significance level: *: $P < .05$; **: $P < .01$.

extraction technique, the solvent and the presence of metal ions.[39] Moreover, synergistic and antagonistic interactions between antioxidants in extracts may interfere with the correlation, or there may be non-phenolic molecules that may react with the Folin-Ciocalteu reagent without being free radical scavengers.[40]

3.7 | Multivariate analysis

3.7.1 | Principal component analysis

The principal component analysis (PCA) based on correlation coefficients was performed to determine the main factors contributing to the classification of saffron ecotypes based on their biochemical parameters. The first two components explained 51.94% of the total variation with 36.71% of the information provided by the first component (PC1), against 15.23% given by the second component (PC2) (Figure 3). The PC1 was strongly influenced, in particular,

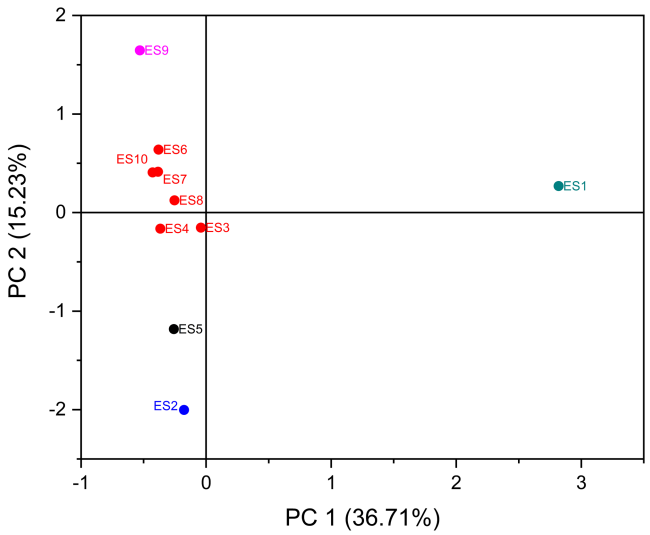

FIGURE 3 Scatter plot for the first two principal components of ten saffron samples based on their biochemical composition.

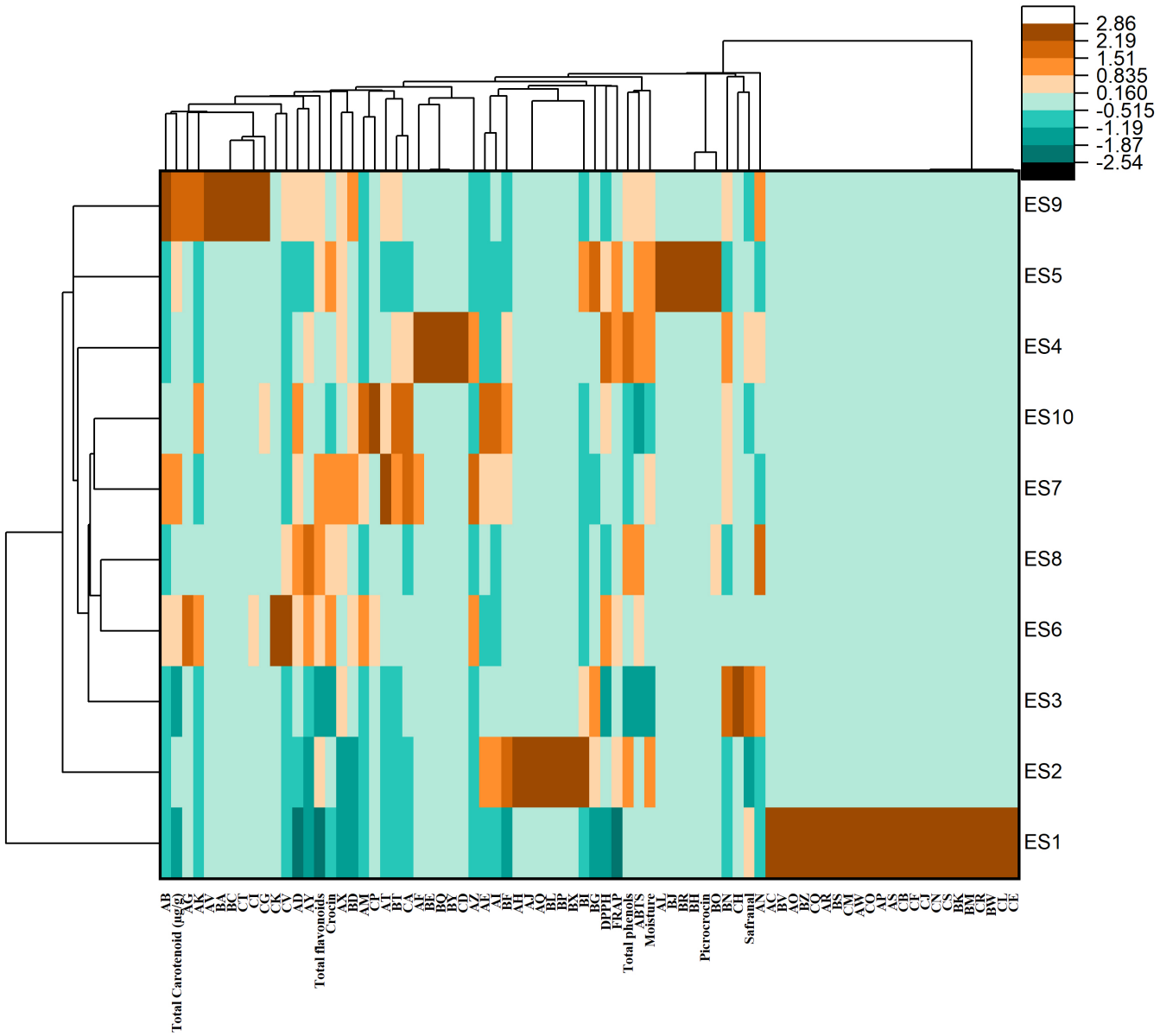

FIGURE 4 Two-dimensional hierarchical heatmap of the ten saffron ecotypes based on biochemical composition. 2.3-Butanedione: AB; Methyl-Cyclopentane: AC; Acetic acid: AD; 1-Butanol: AE; Acetic acid. Methoxy-. ethyl ester: AF; 1-hydroxy-2-Propanone: AG; Formic acid. 2-methylpropyl ester: AH; Formic acid. Butyl ester: AJ; Acetoin: AK; Butyrolactone: AL; 4-hydroxy-butanoic acid: AL; 2(5H)-Furanone: AM; α-pinene: AN; β-Pinene: AO; 2.2.3-trimethyl-Nonane: AQ; Limonene: AR; 3-Carene: AS; β-Isophorone: AT; 2-Isopropylidene-3-methylhexa-3.5-dienal: AV; α-Terpinene: AW; 2.6.6-Trimethyl-1.4-cyclohexadiene-1-carboxaldehyde: AXE; Isophorone: AY; γ-Diosphenol: AZ; Dehydroacetic Acid: BA; D-Camphor: BC; Ketoisophorone: BD; Lanierone: BC; 2.2.6-trimethylcyclohexane-1.4-dione: BD; Safranal: BG; Eucarvone: BH; 4-Hydroxy-2.6.6-trimethylcyclohex-1-enecarbaldehyde: BI; 2-Hydroxy-3.5.5-trimethylcyclohex-2-ene-1.4-dione: BJ; Benzaldehyde. 4-(1-methylethyl)-: BK; 2-Hydroxy-3.5.5-trimethylcyclohex-2-ene-1.4-dione: BL; Anethole: BM; cis.cis-Nepetalactone: BN; Mint furanone: BO; Thymoquinone: BP; Carbonic acid. Monoamide. N-octyl-. propyl ester: BR; δ-Elemene: BS; 1-Cyclohexanone. 2-methyl-2-(3-methyl-2-oxobuty): BT; α-Terpinyl acetate: BV; Copaene: BW; Car-3-ene-2.5-dione: BX; Cedr-9-ene: BY; β-Elemene: BZ; 4-Hydroxy-2.6.6-trimethyl-3-oxocyclohexa-1.4-dienecarbaldehyde: CA; Methyleugenol: CB; Cedr-8-ene: CD; Caryophyllene: CE; cis-α-Bergamotene: CF; α.β-Dihydro-β-ionone: CG; Elemicin: CH; Isoelemicin: CI; Humulene: CJ; trans-Geranylacetone: CK; 5-Hydroxy-6-methoxy-8-[(4-amino-1-methylbutyl) amino] quinoline trihydrobromide: CL; α-Curcumene: CM; β-Selinene: CN; α-Muurolene: CO; 6-Methoxy-4-methylcoumarin: CP; β-Bisabolene: CQ; γ-Cadinene: CR; Cadina-1(10).4)-diene: CS; 5.8-Diethyldodecane: CT; Benzaldehyde. 6-hydroxy-4-methoxy-2.3-dimethyl: CV.

by 2.3-Butanedione, α-pinene, β-pinene, limonene, anethole, α-terpinyl acetate, copaene, methyleugenol, caryophyllene, acetic acid, ketoisophorone, total flavonoids and FRAP. Whereas, the PC2 is mainly explained by isophorone, ketoisophorone, safranal, thymoquinone and total carotenoids. The biplot displays the sampled ecotypes distribution, where two main subclusters were identified independently of their origins. Thus the first one regroups more than the half of sample and was composed of ES3, ES4, ES6, ES7, ES8

and ES10, whereas the second was only composed of two ecotypes ES6 and ES2. It is noteworthy that, ES1 was largely distinguished from all other samples, particularly due to its unique volatile profile dominated mainly by α-Terpinyl acetate, Methyleugenol, Copaene, Anethole, Limonene, Methyl-Cyclopentane, which were not identified in the other samples even at minor levels. Likewise, the sample ES9 was clustered as a single item but not too far from the other ecotypes. This sample had a high proportion of Thymoquinone. The cluster composed of ES2, ES5, ES3, ES4, ES6, ES7, ES8 and ES10 was mainly characterized with great level of Isophorone, safranal and Thymoquinone. This distribution showed a very distinctive profile of the sampled ecotypes indicating a high variability of biochemical attribute, which were shown to be efficient markers for saffron identification and discrimination.

3.7.2 | Hierarchical two-dimensional heatmap

A hierarchically clustered heatmap was conducted to obtain a simplified and holistic representation of the saffron biochemical variability within the dataset. Indeed, a colour-coded two-dimensional heatmap constructed with two clusters using Euclidean distance following Ward's method, the horizontal cluster is ecotype-oriented, whereas the vertical is variable-oriented (Figure 4). In the coloured data matrix, the strong effect is represented by a brown colour with high intensity, while a weak effect is represented by a green colour with high intensity. According to the heatmap, the analysed variables showed a large effect in ecotype clustering, especially for moisture, ABTS, DPPH, total phenols, total flavonoids, corin, Safranal, Isophorone, 1-Butanol, Acetic acid, Anethole, Ketoisophorone and γ-Diosphenol. The ecotype-oriented cluster displayed the same classification pattern as the PCA, which same to confirm the large chemodiversity within the sampled saffron ecotypes. Thus, the sample ES1 was classified separately from the other ecotypes due to its distinct composition, mainly volatile compounds. ES2 was also classified as a distinguished ecotype even being relatively close to the group formed by ES3, ES6, ES8, ES7 and ES10. Finally, the last subgroup was formed by ES9, ES5 and ES4. Regarding the differences in the identification of discriminant variables using the heatmap and PCA methods, they are related to the explained variance, which is total with the heatmap analysis compared to the PCA.[41] Nevertheless, both methods identified acetic acid, ketoisophorone, isophorone, safranal, total flavonoids as potential discriminant variables.

4 | CONCLUSION

The aim of present study was to deepen the knowledge on the biochemical composition and the antioxidant activity of Moroccan saffron, which plays a very important socio-economic role, particularly in rural regions. The phytochemical analyses carried out on saffron, revealed a high content of total phenols, total flavonoids, total carotenoids and a medium antioxidant activity. In addition, the contents of crocin, picrocrocin and safranal, of saffron cultivated in Morocco showed that all the analysed ecotypes have good quality. These data, are consolidated by the GC-MS and LC-MS that showed its richness in aromatic and phenolic composition that differs according to the geographical origin.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data is not available, but if the manuscript advanced to the next stage, you may be asked to provide additional information from the corresponding author.

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