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Full Length Article

Phytochemical and Antioxidant Analysis and Antibacterial Efficacy of Methanolic Extracts of Saffron (*Crocus sativus*) from Taliouine Region in Morocco

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Abstract

Saffron (*Crocus sativus* L.) is prized for its distinctive characteristics, which include flavor, color, aroma and therapeutic uses. Although the antioxidant properties of saffron metabolites are well documented, most studies focus on the stigmas, while other parts of the plant, notably the vegetative tissues remain little explored. Extracts from various parts of saffron were obtained by maceration in methanol, followed by filtration and vacuum evaporation. Flavonoids, polyphenols and tannins were quantified by colorimetric methods using aluminum chloride, Folin-Ciocalteu and vanillin reagents, respectively. Antioxidant activity was assessed by 2, 2-diphényl-1-picrylhydrazyl (DPPH) and fluorescence recovery after photobleaching (FRAP) tests and expressed as Half-Maximal Inhibitory Concentration (IC_{50}). Molecular concentration and identification were determined by gas chromatography-mass spectrometry (GC-MS) and antibacterial activity was evaluated with the agar diffusion method against Gram positive and Gram negative strains. Results showed that extracts from several plant locations (stigmas, bulbs, flowers and leaves) had comparable levels of polyphenols (1563, 1098, 1202 and 169 mg EAG/g Dry Matter (DM), respectively), flavonoids (138, 109, 122 and 106 mg EQ/g DM) and tannins (3614, 318, 692 and 181 mg EC/g DM). In terms of antibacterial activity, the bulbs were found to outperform the stigmas, particularly against Gram positive bacteria. For antioxidant activity, methanolic extracts of flowers showed an IC_{50} of 22.91 mg/mL, those of stigmas an IC_{50} of 32.27 mg/mL, those of leaves an IC_{50} of 54.72 mg/mL and those of bulbs an IC_{50} of 61.47 mg/mL. In contrast, leaf and bulb extracts showed lower activity, with IC_{50} of 54.88 and 61.89 mg/mL, respectively, well above the IC_{50} for ascorbic acid (0.082 mg/mL). These results highlight the richness of aerial parts, particularly the flowers and stigmas, in secondary metabolites responsible for their high antioxidant activity. These results underscore that saffron flower and stigma extracts could serve as effective natural alternatives to synthetic antioxidants and antimicrobials in pharmaceutical and cosmetic products.

Keywords: Saffron; Flavonoids; Tannins; GC-MC; Antioxidant activity; Antibacterial activity; Morocco

Introduction

Regular exposure to factors such as air pollution, smoking, unhealthy diet, chronic stress and pathogens, as well as other factors, increases the risk and likelihood of developing non-communicable diseases. At the national level, according to the Ministry of Health, by 2022, these diseases will be the main cause of mortality (80% of deaths). More specifically, cardiovascular diseases account for 38%, cancers for 18%, chronic respiratory diseases and diabetes for 6%. It is generally believed that more than 80% of the world's population uses traditional forms of medicine, mainly plants, to treat a number of diseases (Chiribagula *et al*. 2015). Functional foods of plant origin, rich in bioactive compounds, can offer health benefits that go beyond simple nutrition and are important in preventing chronic illnesses (Xi *et al*. 2007).

Artificial preservatives are commonly employed in meals to ensure product durability and freshness. However, there are many concerns about the effect of these preservatives on food composition and health. Antioxidants of natural origin, known for their usefulness in stabilizing foods without the disadvantages associated with synthetic preservatives, are therefore a

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better alternative. Natural alternatives derived from medicinal and aromatic plants not only replace artificial additives but also these plants possess antimicrobial and antioxidant properties, offering a safe alternative for preserving food without having to use potentially harmful chemicals (Slimani *et al*. 2024).

It has been estimated that 40% of the pharmaceutical industry is based on, metabolites obtained from medicinal plants. Saffron, also referred to as vegetable gold, is also used to extract metabolites from stigmas. The concentration of compounds is determined by the post-harvest dehydration process, which is essential for transforming the stigmas of this plant to gold. Each of these five major saffron-producing nations *i.e*., Iran, Morocco, Spain, Greece and India use a different method of dehydration. Drying is done at ambient humidity in Morocco, Iran and India, either under open air or in sunshine. In Greece, the stigmas are placed on shallow trays at a temperature of 35–45°C for 12 h. In Spain, for 'Mancha' saffron, which benefits from the internationally recognized Protected Designation of Origin (PDO), a variety of heat sources are used, with temperatures ranging from 55 to 85°C (Cid-Pérez *et al*. 2021).

Cool to cold winters and hot, dry summers make the Mediterranean climate an optimum environment for growing saffron. Saffron cultivation in Morocco is concentrated mainly in Taliouine, in the east of the province of Taroudant, with 565 ha and in Taznakht, in the province of Ouarzazate, with 80 ha (Serghini *et al*. 2012). Saffron is widely used in traditional Chinese medicine, in particular to promote blood circulation, eliminate blood stasis, detoxify the body fluids, relieve depression and calm the nerves (Xi *et al*. 2007). Pharmacological studies on saffron showed that this plant reduces the symptoms of depression by regulating neurotransmitter levels and promoting neuroplasticity (Xi *et al*. 2007; Dahchour 2021) and the active elements present in stigma extracts have the ability to reduce blood sugar levels by lowering insulin resistance (Farkhondeh and Samarghandian 2014; Milajerdi *et al*. 2018).

It has also been shown that safranal, picrocrocin, crocetin and crocin, which are components of the stigma, have anti-tumor effects against cancer of the breast, colon, stomach, uterus, lungs, cervix and other cancers (Bhandari 2015). From an economic point of view, saffron is only used for the stigmas, which have a very low yield, thus contributing to the increase in cost. On the other hand, the other parts are often used as fertilizer and fodder, or even simply thrown away, resulting in significant wastage.

This study was aimed to characterize the primary bioactive compounds of Moroccan saffron and evaluate its antibacterial and antioxidant properties through qualitative and quantitative botanical analyses. Furthermore, it investigated strategies for utilizing other parts of the plant to reduce waste and mitigate the high cost of stigmas, which represent the most valuable component due to their extensive applications.

Materials and Methods

Plant material

The saffron species employed in this study was a member of the Iridaceae family and the *Crocus* genus. The Taliouine cultivar, a saffron genotype of Moroccan origin, was used for the *in vitro* experiments. Taliouine (Latitude: 30.5328, Longitude: -7.92556; 30° 31′ 58″ North, 7° 55′ 32″ West) is a town in the south that is regarded as the center of saffron production in Morocco, is where the various parts of the saffron (bulbs, leaves, flowers and stigmas) were planted in August 2022 and harvested in November 2023. The region's soil types, which range from sandy loam to silty, are ideal for saffron growth. The saffron was harvested under specific climatic conditions (temperature between 6°C and 6.7°C, average humidity of around 52%, average monthly rainfall of 30 mm) in the flowering state for 10 days.

Preparation of extracts

To prepare the extracts, 20 g of powder from the saffron sample were prepared using methanol (MeOH). This extraction was carried out by maceration for 72 h, with intermittent stirring. The extracts obtained were then filtered using Whatman filter paper (8 μm). After solvent evaporation at a temperature of 40°C in a rota-steamer (Model R-114, brand Büchi and country of origin Switzerland), the concentrated extracts were preserved at a temperature of 4°C until use.

Qualitative analysis

There are various techniques for detecting the presence of specific compounds in a substance. Tannins can be identified by the formation of a greenish or bluish-black hue after the addition of a few drops of 1% ferric chloride ($FeCl_3$). Their type was distinguished into catechic and gallic tannins using the stiasny test (Alilou *et al*. 2014). Flavonoids were detected by a reaction with cyanidine, which results in a pink-orange coloration for flavones, a purplish-pink coloration for flavonones and a red hue for flavonols (Daoudi *et al*. 2016). Acidification of a basic substance makes it possible to identify anthocyanins, which produce a purplish-blue color, while leucoanthocyanins are characterized by a cherry-red or purplish color (Kallo *et al*. 2018). Saponosides were identified by the formation of a persistent foam greater than 1 cm after 15 min of agitation and proteins by a violet coloration following the addition of a 2% aqueous copper sulphate solution ($CuSO_4$) (Adjatin *et al*. 2018). Essential oils were identified by their odor after evaporation of the ethanolic extract (Fettah 2019). Mucilage produced a flaky precipitate in test tubes after the addition of alcohol and shaking (Daoudi *et al*. 2016). Cardiac glycosides were identified by a red-brown coloration after the addition of sulfuric acid (H_2SO_4) (El-Haoud *et al*. 2018).

Quantitative analysis

Total polyphenols (TPF): According to Nounah *et al*. (2019), the Folin Ciocalteu reagent was used to determine the total amount of phenols. A 0.5 mL of extract was added with 2.5 mL of Folin-Ciocalteu reagent and diluted (1:10 ratio) with distilled water and 4 mL of Na_2CO_3 (7.5%, w/v). After that, the mixture was let stand for 30 min at 45˚C in a water bath. By contrasting the samples with a blank, spectrophotometric measurements were made at 760 nm. Total phenolics contents were expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g extract).

Total flavonoid (TFV): With a colorimetric method modified from Huang *et al*. (2009) recommendations, flavonoid levels were measured. A tube holding 1.25 mL of distilled water was filled with 0.25 mL of the extract solution. After adding 0.075 mL of a 5% sodium nitrite ($NaNO_3$) solution, the combination was allowed to settle for 5 min. A 0.15 mL of 10% aluminum chloride were then added. A 0.5 mL of 1 M sodium hydroxide was added after 6 min. A 0.275 mL of pure water was used to dilute this solution. After that, the combination was left at ambient temperature for 30 min in the dark. The mixture's absorbance was immediately measured at 510 nm and compared to a standard quercetin curve. Total flavonoids were expressed as mg of quercetin equivalent (EQ) per gram of extract (mg EQ/g extract).

Condensed tannins (CT): The technique of Haida and Kribii (2020) was used to measure the tannin concentration. This was done by mixing 1.5 mL of hydrochloric acid, 3 mL of a 4% vanillin-methanol solution and 500 μL of the extract solution. Following a 15-min, a UV-VIS spectrophotometer was used to measure the absorbance at a wavelength of 500 nm in relation to a blank. The amount of tannins was expressed as mg of catechin equivalents (CE) per gram of extract (mg CE/g extract).

Gas chromatography (GC): Gas chromatograph, used to determine different compounds from the saffron extract, consisted of a capillary tube (30 m x 0.25 mm x 0.25 μm, capable of attaining a maximum temperature of 350°C) equipped with a mass spectrometer. The temperature of the ion source, injection and transmit line was kept at 290°C. Seventy eV ionization energy was applied. A self-calibration procedure was used to modify the electron multiplier voltage. The oven was programmed to raise the temperature from 60°C (held for two minutes) to 280˚C, at a rate of increase of 3°C per min. Diluted, particle-free crude samples (1 μL) were injected into the injector at a ratio of 30:1 using a syringe. All data were collected by doing full mass spectral scans in the range 40 to 550 AMU. Total analysis time was 80 min (Zwane *et al*. 2020).

Antibacterial activity of saffron extracts

The antibacterial efficacy of saffron extracts was initially tested using the circular diffusion technique, outlined by Oubihi *et al*. (2020). Using this technique, a bacterial solution with an optical density of 106 CFU/mL was uniformly dispersed onto Petri dishes filled with Müller-Hinton agar medium. After being impregnated with 15 μL of extract, sterile Whatman paper (8 μm) discs measuring 6 mm in diameter were positioned in the middle of each Petri dish. As positive controls, discs containing synthetic antibiotics were also placed there. Following a 24 h incubation period at 37°C, the transparent region that developed surrounding each disc was measured in order to interpret the data (Tarfaoui *et al*. 2022).

Antioxidant activity of saffron extracts

DPPH analysis: The extracts antioxidant activity was assessed utilizing the stable 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH-) scavenging technique, which was modified from Olugbami *et al*. (2015). To put it quickly a DPPH-solution at 76 μM (0.03 mg/mL) was made in methanol. 0.1 mL of each of the extracts evaluated at various concentrations was mixed with 2 mL of this solution. The DPPH solution and the liquid used to dissolve the extracts were obtained separately as a control sample for this purpose. Following an aromatic vortex, the aforementioned combination was allowed to stand at room temperature for 30 min in the dark. At 517 nm, absorbance was measured. The typical positive control molecule was ascorbic acid (Haida *et al*. 2022).

FRAP analysis: Oyaizu (1986) method was used to evaluate the antioxidant reducing power of ferric ions in the preparations. To do this, 2.5 mL of phosphate buffer solution (0.2 *M*, pH 6.6) and 2.5 mL of 1% w/v potassium ferricyanide solution ($K_3Fe(CN)_6$) were combined with 1 mL of extract at various concentrations (0–1 mg/mL). For 20 min, this combination was incubated at 50°C. Following incubation, the reaction was stopped by adding 2.5 mL of 10% w/v trichloroacetic acid and centrifugation was performed for 10 min at 3000 rpm. The resulting solution was then combined with 2.5 mL of distilled water and 0.5 mL of 0.1% w/v $FeCl_3$. At 700 nm, absorbance was measured in comparison to a blank that was made in the same manner. A positive control was ascorbic acid. The IC_{50} concentration was used to express the results (Haida and Kribii 2020).

Statistical analysis

To determine the statistical significance of the findings at a significance level of α = 5%, a Tukey post hoc analysis and a one-way analysis of variance (ANOVA) were conducted with three replications using SPSS version 27. The data was presented as mean ± standard deviation, were used to determine the significance level. All tests were repeated three times to statically validate the results.

Results

Significant variations were found in the extraction yields of the various saffron components, the flowers, stigmas and leaves showed a satisfactory yield of 63.41, 48.75 and 43.38%, respectively on dry weight basis compared with those obtained for the bulbs 15.39% (Table 1). From the point of view of profitability by weight, the methanolic extract of the flowers gave high proportions compared to the other parts of this plant.

Phytochemical screening indicated that flowers and stigmas were particularly rich in secondary metabolites such as tannins, flavonoids, essential oils and proteins. This richness reflected their crucial role in reproduction, attracting pollinators and defending against external aggression. The leaves, although less rich in secondary metabolites, contributed mainly to photosynthesis and have a moderate presence of tannins, lipids and essential oils, providing minimal protection. As for the bulbs, they contain few secondary metabolites but significant quantities of proteins and essential oils, underlining their storage and survival function (Table 2).

Quantitative analysis further revealed wide variations in phenolic compound content depending on the extract analyzed (Table 3). The quality of these compounds ranged from a minimum value of 169.78 ± 5.9 mg EAG/g extract to a maximum value of 1563.50 ± 20.7 mg EAG/g extract. A highest concentration of phenolics was found in the stigmas' methanolic extract, while the extract of the leaves showed the lowest. The quantity of flavonoids was 106.55 ± 0.9 mg EQ/g extract as the minimum value and 138.91 ± 0.3 mg EQ/g extract as the maximum value, leaves had the lowest amount, with a value of 181.30 ± 11 mg EC/g methanolic extract. On the other hand, stigmas had a high amount of TC with a value of 3614.33 ± 4.1 mg EC/g of metanolic extract.

Gas chromatography analyses revealed a remarkable chemical diversity in saffron extracts, with a predominance of volatile and bioactive compounds such as furanones, notably 2(3H)-Furanone, dihydro-4-hydroxy- (24.41%) and 2(5H)-Furanone (23.48%), which play a key role in the aromatic and sensory characteristics. Fatty acids and bioactive lipids, such as n-hexadecenoic acid (1.12%), confer nutritional and therapeutic properties. Functional molecules such as 1, 2, 3-propanetriol, 1-acetate (8.24%) and 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (4.48%) offer prospects for industrial and cosmetic applications. The presence of antioxidant compounds, in particular α-tocopherol (0.15%) and β-sitosterol (0.37%), reinforced the interest of this extract for health uses. Finally, the unidentified fraction (12.13%) suggests a potential for future work. This varied chemical profile highlighted saffron's potential multifarious applications (Table 4–7; Fig. 1–4).

For biological activities we used different bacterial strains, whether Gram positive or Gram negative, to assess the antimicrobial effectiveness of extracts from saffron (Table 8). The results showed the variable antibacterial activity of saffron extracts against different bacterial strains.

Table 1: Extraction yield (%)

Extracts	MeOH
Flowers	63.41
Leaves	43.38
Stigmas	48.75
Bulbs	15.39

Table 2: Chemical groups found in saffron

Chemical groups	Fleurs	Leaves	Stigmas	Bulbes
Tannins	+++	++	+++	+
Catechin tannins	+++	++	+++	+
Gallic tannins	+	---	++	---
Flavonoids	+++	+	+++	---
Flavones	+++	+	+++	++
Flavonones	++	+	+++	+
Flavonols	++	+	++	---
Anthocyanins	++	+	++	--
Leucoanthocyanins	---	---	---	---
Saponosides	---	---	---	---
Proteins	++	+	+++	+
Lipids	+++	+++	+++	--
Essential oils	+++	+++	+++	+++
Mucilages	---	---	+	---
Cardiac glycosides	---	---	---	---
Iridoids	+	---	++	---
Reducing sugars	++	+	++	+

+++ abundant; ++ moderate presence; + weak; -- Trace; --- Absent

Table 3: Total polyphenol, total flavonoid and condensed tannin content

Extracts	TPP (mg EAG/g extract)	TFV (mg EQ/g extract)	TC (mg EC/g extract)
Stigmas	1563.50 ± 20.7a	138.91 ± 0.3a	3614.33 ± 4.1a
Bulbs	1098.93 ± 24.5b	109.20 ± 0.3b	318.38 ± 6.9b
Flowers	1202.54 ± 30.4c	122.15 ± 1.2c	692.21 ± 34.6c
Leaves	169.78 ± 5.9d	106.55 ± 0.9d	181.30 ± 11d

The significant difference ($P < 0.05$) is illustrated by the letters a, b, c and d

Table 4: Gazous phase chromatography coupled with mass spectrometry (GC-MS) of the stigmas of saffron

RT (min)	Peak name	Area (%)
2.469	3,7-Dioxabicyclo [4.1.0] heptane	1.58
4.059	Butanoic acid, 2,2-dimethyl	1.45
4.654	2(5H)-Furanone	3.88
6.657	2,4-Dihydroxy-2,5-diméthyl-3(2H)-furanone	0.85
11.846	2-Cyclohexen-1-one, 3,5,5-trimethyl	0.85
13.871	1,4-cyclohexanedione, 2,2,6-triméthyl-	1.65
15.234	1,3-Cyclohexadiene-1-carbaldehyde	42.18
16.601	2-hydroxy-3,5,5-triméthyl-cyclohex-2-énone	1.36
17.329	1,2,3-propanetriol, 1-acétate	1.32
22.93	4-Hydroxy-2,6,6-trimethyl-3-oxocyclohexa-1,4-dienecarbaldehyde	1.66
48.163	Acide 9,12-octadécadiénoïque (Z, Z)-	3.59
63.6	5-Nonadécén-1-ol	0.96
66.705	Oxirane, tetradecyl-	1.39
	Non Identified	26.65

Bulb extracts exhibited the highest efficacy, with an inhibition zone of 7.5 mm against *S. aureus* and *S. epidermidis*, two Gram positive strains responsible for common infections. Flower extracts also demonstrated significant inhibition zones, reaching 6.6 mm against the same strains. However, the efficacy of leaf and stigma extracts was slightly lower, with values of 6.5 mm and 6.3 mm, respectively, for *S. aureus* and *S. epidermidis.*

Table 5: Gazous phase chromatography coupled with mass spectrometry (GC-MS) of the bulbs of saffron

RT (min)	Peak name	Area (%)
2.457	3,7-Dioxabicyclo [4,1,0] heptane	2.40
4.732	2(5H)-Furanone	12.79
6.624	2 4-dihydroxy-2 5-dimethyl-3(2h)-furanone	0.79
8.253	1,2,3-Propanetriol	1.70
10.197	Acide butanedioïque	2.05
10.476	Éthyl maltol	1.80
12.887	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	5.04
15.115	2(3H)-Furanone, dihydro-4-hydroxy-	31.18
17.005	7-Bromo-1-(tetrahydro-2H-pyran-2-yl)-1H-pyrazolo	1.37
17.997	1,2,3-Propanetriol, 1-acetat	5.70
19.115	Methyl 2,2,4,4-tetramethyl-3,5-dioxo-5-p-tolypentanoate	1.12
23.321	1,2,3-Benzenetriol	1.75
25.881	.□.Lactone	0.97
43.45	Hexadecanoic acid	2.06
48.137	9,12-Octadecadienoic acid (Z)	2.57
	Not Identified	18.90

Table 6: Gazous phase chromatography coupled with mass spectrometry (GC-MS) of saffron flowers

RT (min)	Peak Name	Area (%)
2.462	5-hexyl-2-methyl-2-oxazoline	3.79
3.281	Butanoic acid, 3-hydroxy-	1.44
3.627	3-Furanmethanol	1.61
4.094	Ester méthylique de l'acide butanoïque, 2,2-diméthyl-3-oxo	3.83
4.804	2(5H)-Furanone	23.48
10.5	D-Alanine, N-propargyloxycarbonyl-, propargyl ester	2.09
12.871	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	4.48
14.841	2(3H)-Furanone, dihydro-4-hydroxy-	24.41
15.158	1,3-Cyclohexadiene-1-carboxylic acid	2.09
15.465	Glycérine	2.21
16.817	3,7-Dimethyl-5-oxooc-1-ene	2.47
17.873	1,2,3-propanetriol, 1-acétate	8.24
31.214	5-Octadécène, (E)-	0.06
43.379	n-Hexadecanoic acid	1.12
48.031	Linoelaidic acid	0.99
48.178	9,12-Octadecadienoic acid (Z)	0.84
	Not identified	12.13

Table 7: Gazous phase chromatography coupled with mass spectrometry (GC-MS) of saffron Leaves

RT (min)	Peak Name	Area (%)
2.464	2-Methyl-1-octadecene	2.92
4.707	2(5H)-Furanone	30.20
6.626	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	0.94
7.145	Dimethylamine, N-(neopentyloxy	2.16
10.375	4-(2,4,4-Trimethyl-7-oxabicyclo[4.1.0]hept-2-en-3-yl)butan-2-one	1.87
12.811	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	4.85
14.085	2(3H)-Furanone, dihydro-4-hydroxy-	15.47
17.36	1,2,3-propanetriol, 1-acétate	4.52
43.435	Hexadecanoic acid	4.11
48.326	Acide 9,12,15-octadécatriénoïque	18.98
61.907	9-Octadecenamide	1.16
	Not Identified	10.44

Finally, antioxidant activity was evaluated using the FRAP assay and the DPPH free radical reduction method for our investigation. The reduction of ferric ions (Fe^{3+}) from $K_3Fe(CN)_6$ into ferrous ions (Fe^{2+}) found in $K_3Fe(CN)_6$ is assessed by the second test, a colorimetric assay. Plant extracts generally contain a great diversity of

Table 8: Analysis of the antibacterial activity of saffron

Bacterial strain	Diameter of the inhibition zone in (mm) a				Antibiotics	
	Bulbs	Flowers	Leaves	Stigmas	P	AMP
S. aureus	7.5 ± 0.0a	6.6 ± 0.2b	6.5 ± 0.0b	6.3 ± 0.2b	NA	7
S. epidermidis	7.5 ± 0.0a	6.6 ±0.2ab	6.5 ±0.0ab	6.3 ± 0.2b	NA	NA
S.A.R.M	6.3 ± 0.2b	7.5 ± 0.0a	6.5 ± 0.0b	6.6 ± 0.2b	NA	NA
Klebsiella p.	NA	NA	6.1 ± 02a	Na	NA	NA
E. coli	6.8 ± 0.2a	NA	6.5 ± 0.0a	6.6 ± 0.2a	NA	NA
Acinetobacter b.	6.1 ± 02a	6.3 ± 0.2a	6.6 ± 0.2a	NA	NA	NA
Enterobacter c.	6.3 ± 0.2a	NA	NA	NA	NA	NA

NA: Not active
a, b and ab indicate a significant difference

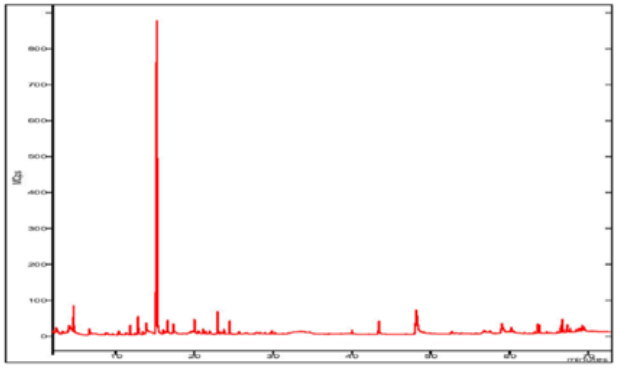

Fig. 1: Stigma chromatogram

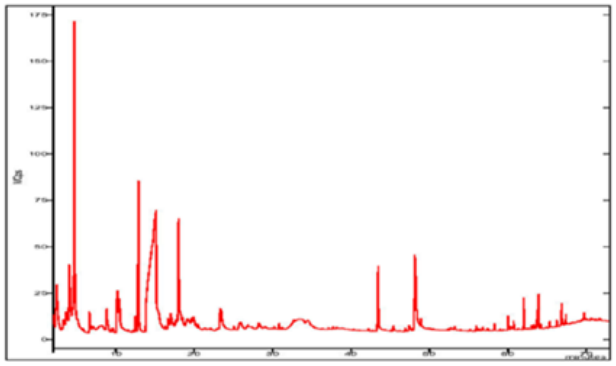

Fig. 2: Bulbs chromatogram

molecules with different structures and functions. These extracts are classified as natural antioxidants due to their ability to reduce and prevent the formation of free radicals. The studied extracts are recognized as natural antioxidants because of their ability to neutralize and prevent the formation of free radicals. According to the results (Fig. 5), saffron extracts demonstrated notable free radical scavenging activity. The methanolic extract of flowers exhibits remarkable antioxidant activity with an IC_{50} of 22.91 mg/mL, followed by stigma extracts with an IC_{50} of 32.27 mg/mL. In contrast, leaf and bulb extracts showed the weakest antioxidant activity, with IC_{50} values of 54.88 mg/mL and 61.89 mg/mL, respectively. In contrast, the IC_{50} of ascorbic acid was significantly lower, with a value of 0.082 mg/mL, highlighting its strong antioxidant potential.

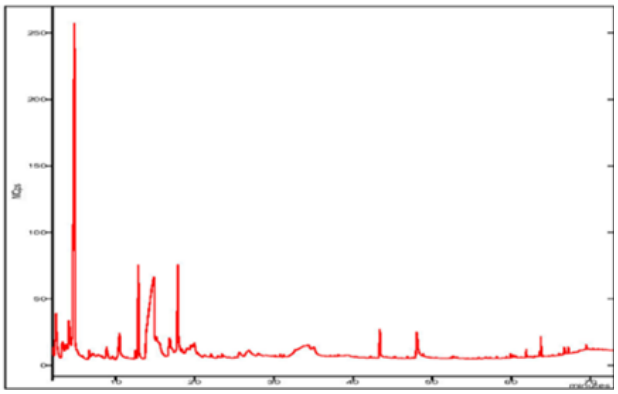

Fig. 3: Flower samples chromatogram

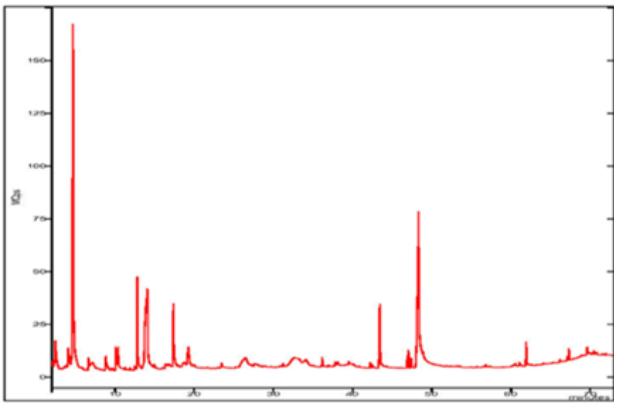

Fig. 4: Leaf samples chromatogram

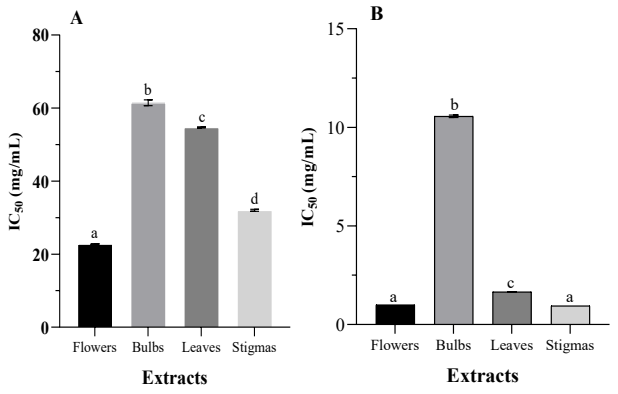

Fig. 5: Antioxidant Activity of Crocus sativus L. Extracts. A: DPPH; B: FRAP Different letters (a, b, c and d) indicate statistically significant differences between groups in antioxidant activity, as determined by one-way ANOVA at a significance level of $P < 0.05$.

a, b, c and d indicate a significant difference

Discussion

Significant variations were observed in the methanolic extraction yields of the different saffron parts, with flowers exhibiting the highest yield, while bulbs recorded the lowest. The yield of natural extracts of the same species can vary according to various factors, as already demonstrated by previous studies (Menghini *et al*. 2018; Mir *et al*. 2022). These factors include, on the one hand, the impact of the extraction method and, on the other, the influence of the vegetative cycle, the harvesting period and the geographical location. In addition, the length of time the plant is dried also plays a role in determining extract yield (Menghini *et al*. 2018; Mir *et al*. 2022).

A higher concentration of secondary metabolites in the flowers and stigmas confirmed their importance in reproduction and interaction with pollinators (Jadouali *et al*. 2018). Conversely, the chemical composition of the leaves illustrates their main function in photosynthesis, with moderate protection against external aggression. The bulbs, because of their role as reserves, prefer proteins and essential oils to the secondary metabolites involved in defense. Finally, the presence of essential oils and lipids in all parts of the plant underlines their fundamental role in protection and adaptation mechanisms.

Comparison of the polyphenol levels in saffron extracts in this study revealed higher levels as compared to previous reports. In this critique, Menghini *et al*. (2018) showed a maximum total polyphenol (13.76 ± 0.13 mg EAG/g DM) in the stigma extract. Rahaiee *et al*. (2015) noted 12.67 ± 0.00 mg EAG/g DM in the methanolic extract, while Farrokhi *et al*. (2021) reported 145.6 ± 19.83 EAG/g DM in the aqueous stigma extract. For bulbs, we observed that the total polyphenol content obtained in our extract was higher than that observed by Baba *et al*. (2015) and Ghanbari *et al*. (2019). For flowers and leaves, Jadouali *et al*. (2018) showed TPF values of 65.34 ± 1.74 mg EAG/g DM for flowers and 81.69 mg EAG/g DM for leaves. Mykhailenko *et al*. (2019) also found a value of 5.62 ± 0.60 mg EAG/g DM for flowers and 3.42 ± 0.2 mg EAG/g DM for leaves. Regarding total flavonoids, Jadouali *et al*. (2018) recorded 60.64 ± 2.71 mg EQ/g and 50.64 ± 1.63 mg EQ/g for leaves and flowers respectively, while Ghanbari *et al*. (2019) and Baba and Malik (2015) reported very low levels in bulb extracts. With regard to TFV content in the stigmas, Rahaiee *et al*. (2015) obtained a value of 0.43 ± 0.03 mg EQ/g DM in the methanolic extract compared with a value of 14.01 ± 0.05 mg EQ/g DM found by Farrokhi in the aqueous extract Farrokhi *et al*. (2021), while Menghini *et al*. (2018) gave a value of 72.06 ± 2.40 mg EQ/g DM in the aqueous extract.

GC-MS analysis of plant parts indicated an existence of many important and recurrent compounds. Among these, 2(5H)-Furanone was detected in stigmas, bulbs, leaves and flowers, highlighting its potential importance in the biological or chemical functions of the plant. Similarly, 2(3H)-Furanone, dihydro-4-hydroxy- appears to be a key molecule, particularly abundant in bulbs and flowers. Leaves also contain significant quantities of 2(5H)-Furanone and 9,12,15-octadecatrienoic acid, while stigmas have a high concentration of 1,3-Cyclohexadiene-1-carbaldehyde. These results show significant chemical diversity within the

different parts of the plant, with specific compounds possibly playing essential biological or ecological roles.

Diglucoside, gammacocetine, beta-carotene, zeaxanthine in glycosidic form and picrocrocine, which gives it a bitter flavor, are among the primary carotenoids (crochets) that make up saffron. It crystallizes during hydrolysis, yielding glucose and safranal (Mykhailenko *et al*. 2019). Significant amounts of the safranal, which gives this spice its aroma (Zwane *et al*. 2020) and is one of the compounds used to assess its quality, were analyzed in this study (Mykhailenko *et al*. 2019). The ISO 3632 standard classified the quality of saffron based on the amount of safranal it contains (Rodríguez et *al*. 2017).

The variation in antibacterial activity could be attributed to the distinct chemical composition of each plant part, with a higher concentration of secondary metabolites in bulbs and flowers, known for their antimicrobial properties. In contrast, *K. pneumoniae* and *E. cloacae* showed notable resistance (Mykhailenko *et al*. 2019). This highlighted the challenge of combating these strains, which are often associated with nosocomial infections and exhibit antibiotic resistance mechanisms. The observed resistance could also be linked to the complexity of Gram-negative bacterial cell walls, which hinder the penetration of active compounds. Interestingly, ampicillin demonstrated activity against *S. aureus*, with an inhibition zone of 7 mm, whereas all strains tested for penicillin were inactive (Akinyemi 2020). This indicated that antibiotic resistance is developing in certain *Staphylococcus* strains, emphasizing the importance of exploring natural alternatives, such as plant extracts, for bacterial infection treatment.

The extracts from flowers and stigmas exhibited high FRAP values, at 0.99 and 0.95 mg/mL, respectively, confirming their ability to act as powerful electron donors. This activity is consistent with their richness in carotenoids and phenolic compounds, which can effectively reduce ferric ions. Leaves, with an activity of 1.64 mg/mL and bulbs, with 10.61 mg/mL, showed moderate antioxidant activity according to the FRAP assay, suggesting that they contain antioxidants but in lower quantities compared to flowers and stigmas (Benkerroum *et al*. 2024). These compounds may include flavonoids or phenolic acids, but their concentration appears to be limited. These findings demonstrated that secondary metabolites (carotenoids, flavonoids and phenols) are concentrated in the plant's aerial portions (flowers and stigma), which give them their potent antioxidant properties. The antioxidant potential of saffron extracts from Taliouine region are consistent with the work of Assimopoulou *et al*. (2005) who attributed the antioxidant activity of saffron to two major bioactive compounds: Crocin and safranal. Several prior studies have examined the antioxidant effects of extracts of saffron flowers, leaves, stigmas and bulbs.

Conclusion

The results show that stigmas are widely valued for their culinary, pharmaceutical and cosmetic applications, the other parts of the plant, often overlooked or intended for disposal or use as fodder, which deserve special attention. The flowers present considerable potential as a source of natural dyes and antioxidant compounds, while the leaves, often rich in flavonoids, polyphenols and other antioxidant compounds, could be used for the production of extracts for the cosmetic industry or for the formulation of dietary supplements aimed at preventing oxidative stress. The bulbs, essential for the plant's propagation, could also be explored for their antimicrobial properties or for applications in cosmetics and agro-industry. These findings highlight the need to valorize all parts of Moroccan saffron, not only to reduce agricultural waste but also to maximize its economic and environmental potential. This circular and sustainable approach opens new perspectives for diverse applications in the fields of health, food, agriculture and industry.

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Author Contributions

This work has been reviewed and approved by all authors for publication.

Conflict of Interest

Each author attests that they have no disclosed conflicts of interest.

Data Availability

The corresponding author can provide any data generated or analyzed in this study upon reasonable request and all data are included in this paper.

Ethics Approval

Not applicable to this paper.

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