Food Analytical Methods (2026) 19:264
https://doi.org/10.1007/s12161-026-03180-y

RESEARCH

Enhancing the Extraction Efficiency of Phenolic Compounds from Moroccan *Crocus sativus* L. Using a Simplex-Centroid Mixture Design

Chaimae Rais[1] · Chaimae Slimani[2,3,4] · Farid Mansouri[5] · Boutayna Rhourri-Frih[6] · Laila Elhanafi[4] · Meryem Benjelloun[4] · Miroslava Kačániová[7,8] · Stefania Garzoli[9] · Abderrahim Lazraq[4]

Received: 3 April 2026 / Accepted: 12 June 2026 / Published online: 20 June 2026
© The Author(s) 2026

Abstract

This study aimed to improve the extraction efficiency of three solvents and their combinations on phenolic compounds from Moroccan *Crocus sativus* L. stigmas and corms. A simplex-centroid mixture was used with a solvent system containing water, ethanol, methanol, and their mixtures to optimize the response of total phenolic compounds (TPC). According to the validated model for each part, the optimal solvent system was the ternary mixture (0.5; 0.25; 0.25 v/v/v) water, ethanol, and methanol for stigmas, while the optimal value of phenolic compounds was 97.43 mg GAE/g DM. Also, the optimal mixture for corms was (0.75:0.25 v/v) water and ethanol with phenolic compounds of 12.87 mg GAE/g DM. The optimal extracts showed a noticeable antioxidant potential especially for stigmas (0.072 mg/mL DPPH IC_{50}; 101.72 mg AAE/g DM) and important antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Furthermore, based on LC–MS/MRM profile of the two extracts, we observed that vanillin, salicylic acid, and gentisic acid were identified as major phenolic compounds. This study shows that the combination of the solvent mixture may provide better biological activities than those given by some commercial antioxidants and may subsequently be useful as natural food preservatives.

Keywords *Crocus sativus* L. · Stigmas · Corms · Simplex-centroid design · Phenolic compounds · Biological activities · LC–MS/MRM

Introduction

Crocus sativus L. is a perennial plant belonging to the Iridaceae family (Nemati et al. 2019). It is mainly cultivated for its dried stigmas called saffron, which exhibit anti-inflammatory, antioxidant, analgesic, antihypertensive, anticancer, hypolipidemic, antitussive, anticonvulsant, anxiolytic, antidepressant, and antinociceptive properties (Slimani et al. 2022; Amatto et al. 2024; Ara et al. 2024; Shadab et al. 2024).

Dried stigmas of *C. sativus* contain more than 150 volatile compounds, primarily terpenes and their esters, which exhibit important biological activities that may be useful for designing different functional products in the food, medical, and cosmetic industries (Gohari et al. 2013; Colucci Cante et al. 2022; Delam et al. 2023; Abu Safe et al. 2023).

In addition, several researchers have shown that various parts of *C. sativus*, including the corms, contain bioactive compounds, especially anthocyanins, carotenoids, phenolic compounds, flavonoids, saponins, and terpenoids (Loizzo et al. 2016; Mykhailenko et al. 2019; Ghaderi et al. 2020). These compounds are commonly used in the food industry as antimicrobial agents, antioxidants, and food stabilizers (Sarfarazi et al. 2022; Memarzia et al. 2023).

Saffron is valued not only as a culinary spice but also for its promising therapeutic potential. Its rich content of flavonoids and phenolic acids has been associated with beneficial effects in neurodegenerative disorders, diabetes, cardiovascular diseases, and cancer prevention. These compounds display strong antioxidant and anti-inflammatory activities, which may mitigate oxidative stress, limit β-amyloid aggregation, and contribute to metabolic homeostasis. Moreover, saffron constituents have been reported to enhance endothelial function, regulate vascular tone and blood pressure, and exert anticancer activity by promoting apoptosis and suppressing tumor progression, particularly

Extended author information available on the last page of the article

when acting in concert with crocin and safranal (Anaeigoudari et al. 2023; Mahood et al. 2023; Muduli et al. 2024; Slimani et al. 2025a, 2025b).

Given the importance of these bioactive compounds, the choice of the appropriate extraction solvent becomes crucial for obtaining an efficient and quantitative extraction of these precious constituents (Tomšik et al. 2016). For this purpose, the combination of solvents with different polarities has been recommended to achieve extraction of bioactive compounds (Martins et al. 2013; Tormena et al. 2019; Sarfarazi et al. 2022).

Response surface methodology (RSM) is a powerful statistical tool that can be used to optimize complex processes (Slimani et al. 2021; Fadil et al. 2022; Samimi 2023). This tool has been widely used in various fields (Slimani et al. 2022). A mixture design was chosen to determine the optimal values of the variables using mathematical models and quantitative data, effectively optimizing extraction procedures. This approach allows for efficient optimization while minimizing the cost required for experimentation, providing valuable information for the extraction of bioactive compounds (Marcheafave et al. 2019; Alani et al. 2024).

However, despite extensive research on saffron stigmas, several critical knowledge gaps remain. First, most extraction optimization studies have focused exclusively on stigmas and have relied primarily on one-factor-at-a-time approaches, with limited use of systematic mixture design methodologies capable of identifying synergistic solvent interactions. Second, corms which are produced in quantities 5–10 times greater than stigmas per plant remain largely unexplored as a source of phenolic compounds and are routinely discarded as agricultural waste. Third, no comparative studies have simultaneously optimized extraction conditions for both stigmas and corms under identical experimental settings, limiting our understanding of tissue-specific extraction requirements. Finally, the relationship between optimized phenolic extracts and their biological activities, particularly antioxidant and antimicrobial properties, has not been systematically investigated for Moroccan *C. sativus*.

Therefore, the main objective of this study was to address these gaps by (1) systematically optimizing solvent mixtures (water, ethanol, and methanol) for phenolic extraction from both stigmas and corms using a simplex-centroid mixture design; (2) comparing optimal extraction conditions between these two plant organs; (3) evaluating the antioxidant and antibacterial activities of the optimized extracts; and (4) identifying major phenolic constituents using LC–MS/MRM analysis. This work represents the first comparative optimization of phenolic extraction from saffron stigmas and corms and provides a scientific basis for the valorization of saffron agricultural waste into value-added natural antioxidants.

Materials and Methods

Plant Material Collection and Preparation

Plant material used in the present study is composed of stigmas and corms of *C. sativus* from Taliouine region (province of Taroudant, Morocco; 30.5323° N, 7.9243° W), provided by Fogoug Agricultural Cooperative in October–November 2019. Stigmas and corms were carefully sorted and dried at 40 °C. The drying operation was maintained until the weight stabilized. The dried samples were ground using an electric grinder and sifted. The 300 µm fine powder was stored at − 28 °C until use. The botanical identity of the material as *C. sativus* was authenticated by a botanist of the National Agency for Medicinal and Aromatic Plants (Taounate, Morocco) on the basis of the characteristic morphological features of the species. *C. sativus* is the only *Crocus* species cultivated by the supplying cooperative, which further excludes confusion with related taxa.

Ultrasonic-Assisted Extraction

In order to study the effect of solvent polarity on phenolic compound yield, a mixture design was used. The extraction was carried out using an ultrasonic bath (35 kHz, nominal power 160 W) for 30 min in a dark, temperature-controlled room maintained at 25 ± 1 °C, using a solid-to-solvent ratio of 0.1 g/10 mL of solvent. The mixture was then centrifuged at 3000 rpm for 10 min. The supernatant was recovered and analyzed for its TPC. All extractions were carried out in triplicate, and 12 experimental runs, randomly ordered, are listed in Table 1, with the center point performed in triplicate.

Table 1 Three-factor experimental matrix

	Independent variable		
	X_1 (water)	X_2 (ethanol)	X_3 (methanol)
1	1	0	0
2	0	1	0
3	0	0	1
4	1/2	1/2	0
5	1/2	0	1/2
6	0	1/2	1/2
7	2/3	1/6	1/6
8	1/6	2/3	1/6
9	1/6	1/6	2/3
10	1/3	1/3	1/3
11	1/3	1/3	1/3
12	1/3	1/3	1/3

Experimental Design

The experimental matrix was performed using a three-factor, single-response approach, adopting the simplex-centroid model. The three factors chosen were the solvents water, ethanol, and methanol (rated X_1, X_2, and X_3) with proportions ranging from 0 to 100%, and the response was the phenolic compound content.

In the simplex-centroid design, the pure components at the vertices represent 100% of each solvent. The mixture performance was defined as follows: the sides represent the binary mixtures (1/2:1/2:0); the axial points represent the ternary mixtures (2/3:1/6:1/6), and the three central points represent the ternary mixtures (1/3:1/3:1/3).

Regression models including linear (Eq. 1), quadratic (Eq. 2), and special cubic (Eq. 3) were assessed to relate the response to the independent variables.

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + \varepsilon \quad (1)$$

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{11}X_1X_1 + a_{22}X_2X_2 + a_{33}X_3X_3 + \varepsilon \quad (2)$$

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3 + a_{123}X_1X_2X_3 \quad (3)$$

Y: phenolic compound content (response); X_1, X_2, X_3: considered factors; a_i (a_0, a_1, a_2): factorial coefficients.

After validation of the design, the best possible solvent combination, leading to the highest phenolic compound content, was chosen. Thus, the optimal extracts obtained were used to carry out the phytochemical assays, the evaluation of various biological activities, and LC–MS/MRM analysis.

Evaluation of Phytochemical Compounds

Total Phenolic Compounds

Total phenolic compounds were determined according to the Folin–Ciocalteu method (Singleton and Rossi 1965). A volume of 200 µL of the extract was mixed with 1.5 mL of 10% (v/v) Folin–Ciocalteu reagent. After 5 min, 1.5 mL of 5% (w/v) sodium carbonate was added. The absorbance was measured at 725 nm after 2 h of incubation. The TPC was determined using a gallic acid calibration curve (0–100 mg/L; $R^2 = 0.959$). Results were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM). All spectrophotometric measurements reported in this study were carried out on a UV–visible spectrophotometer.

Total Flavonoid Content

Total flavonoid content in different extracts was determined as described by Barros et al. (Barros et al. 2011). Briefly, 1 mL of extract was mixed with 0.3 mL of 5% sodium nitrite ($NaNO_2$). After 5 min, 0.3 mL of 10% $AlCl_3$ was added. After 6 min, 2 mL of 1 M NaOH was added, and the volume of the mixture was made up to 10 mL with distilled water. The absorbance was then measured at 510 nm. Total flavonoid content was quantified using a quercetin calibration curve (0–100 mg/L; $R^2 = 0.953$) and was expressed as mg quercetin equivalents per gram of dry matter (mg QE/g DM).

Condensed Tannin Content

Condensed tannins were determined using the Bate-Smith reaction (Ribéreau-Gayon and and Stonestreet 1966). Briefly, 1 mL of extract was mixed with 0.5 mL of distilled water and 1.5 mL of 37% hydrochloric acid. The mixture was heated for 30 min in a water bath at 100 °C. The optical density "OD" was measured at 550 nm. The difference in OD between the hydrolyzed tube and the control (tube at room temperature) corresponds to the number of tannins present in the extract by the following relation:

$$T(\mathrm{mg/g}) = (\mathrm{DO_{S2}} - \mathrm{DO_{S1}}) \times 19.33.$$

with OD_{S2}: after heating and OD_{S2}: control.

Evaluation of Antioxidant Activity

DPPH Free Radical-Scavenging Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging method was used to evaluate the antioxidant activity of *C. sativus* extracts (Brand-Williams et al. 1995). A two-fold serial dilution series was prepared from each extract stock solution (4 mg/mL). Then, 1 mL of each dilution was mixed with 1 mL of an ethanolic DPPH solution (0.004%). The mixture was left in the dark for 30 min and the discoloration, compared to the negative control containing only the DPPH solution, was measured at 517 nm. Also, butylated hydroxytoluene (BHT) was used as a positive control. The percentage of the antioxidant activity was calculated according to the following equation:

$$\text{Antioxidant activity}(\%) = \frac{\text{Abs DPPH} - \text{Abs final}}{\text{Abs DPPH}} \times 100$$

Antioxidant activity of these extracts was determined from their half-maximal inhibitory concentration (IC_{50}). It is the concentration necessary to reduce 50% of DPPH radicals (Hebi and Eddouks 2016).

Ferric Reducing Antioxidant Power Assay

Analysis of the ferric reducing power (FRAP) of the two parts of the *C. sativus* plant was based on the protocol of Udayaprakash et al. (2015). A phosphate buffer (0.2 M; pH = 7) was prepared by mixing Na_2HPO_4 and NaH_2PO_4 in an appropriate ratio. A volume of 2.5 mL of the phosphate buffer was mixed with 1 mL of the extract and with 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 30 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 6500 rpm for 10 min. Next, 2 mL of the supernatant was mixed with 2 mL of distilled water and 0.4 mL of 0.1% $FeCl_3$. The absorbance of the solution was measured at 700 nm. The results were expressed as milligrams of ascorbic acid equivalents per gram of dry matter (mg AAE/g DM), calculated from an ascorbic acid calibration curve (0–300 µg/mL; $R^2 = 0.819$).

Antibacterial Activity

Bacterial strains used in this study were two Gram-negative bacteria: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853); and two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 29213) and *Bacillus subtilis* (ATCC 3366).

From a 24-h pure culture grown on LB agar, one to two colonies were transferred into sterile saline for each strain. The suspensions were vortexed thoroughly and adjusted to 0.5 McFarland (1.5×10^8 CFU/mL). The plant extracts were dissolved in 5% (v/v) DMSO. A DMSO control (5% v/v) was included to verify that this concentration did not inhibit strain growth. The minimal inhibitory concentration (MIC) was defined as the lowest extract concentration that inhibited visible microbial growth. MIC determination was performed using the broth microdilution method in 96-well microplates containing Mueller–Hinton broth. Microplates were incubated for bacteria at 37 °C for 24 h. After incubation, 10 µL of resazurin solution (0.1% v/w) was added to indicate microbial viability (Bouhdid et al. 2009). To determine the minimal bactericidal concentration (MBC), 3 µL from wells showing no visible growth was plated into LB agar and incubated at 37 °C for 24 h; the MBC was defined as the lowest extract concentration yielding no colony growth.

After incubation, the MBC was recorded as the lowest concentration yielding no visible growth. To highlight the nature of the antibacterial effect of the extracts tested, the MBC/MIC ratio was calculated (Levison 2004).

LC–MS/MRM Analysis

The different extracts were suspended in a 1 mL 80/20 (v/v) water/methanol mixture in order to get analyzed using LC–MS instrumentation. Then, 2 µL of this suspension was diluted in a 20-mL volumetric flask with mobile phase, before the phytochemical analysis by LC–MS. A volume 5 µL was injected into the LC–MS.

LC–ESI–MS/MS analysis was carried out on a Shimadzu gradient UHPLC system (CBM-20A) coupled to a QTRAP 5500 mass spectrometer fitted with a TurboV™ electrospray ionization source (SCIEX API 5500). Chromatographic separation was achieved on a reversed-phase C_{18} column (150 × 1 mm, 3 µm particle size) at a flow rate of 0.100 mL/min. The mobile phase consisted of solvent A, water acidified with 2% (v/v) acetic acid (900 mL of water mixed with 18 mL of acetic acid; final pH 2.57), and solvent B, methanol.

The gradient program chosen is from Termentzi and Kokkalou (Termentzi and Kokkalou 2008) and is described in Table 2.

Mass spectrometry was operated over the m/z 50–500 at a scan speed 1000 Da/s. The ion spray voltage was set at 5500 V in the positive ion mode. The trap field time was set at 3 ms. MRM mode was used to quantify the analytes of interest. The detection of precursor ion and major fragment ion of the standards and MRM acquisition was done for every individual compound.

For each analyte, the precursor ion and its principal product ion were optimized by direct infusion of the corresponding standard, and the resulting MRM transitions were used for detection in the extracts. As external calibration curves were not established for each analyte, the analysis is targeted and semi-quantitative; relative abundances are therefore reported as peak signal (cps) and as relative intensity expressed as a percentage of the maximum signal rather than as absolute concentrations.

Table 2 Gradient program chosen in the present experiment

Time (min)	(%) Solvent A	(%) Solvent B
0 to 2	90	10
37	0	100
42	0	100
45	90	10
50	90	10

Statistical Analyses

This study was carried out using Statistica software (version 13.1; StatSoft Inc., USA). For each answer, the linear, quadratic, and cubic models were tested. The most appropriate model, with a confidence level of 95% ($p < 0.05$), was adopted.

The data obtained were subjected to a statistical analysis to find the variability existing between the optimum extracts. The data were processed using the SYSTAT 12 software. Statistical significance was identified at 95% confidence level ($p < 0.05$). A mean comparison test was performed whenever a significant effect of the studied factor was detected by ANOVA.

Results

Efficacy of the Solvent's Formulation on TPC

The simplex-centroid design, including different combinations of the studied solvents and the observed response of each experiment, is listed in Table 3. TPC extracted from stigmas and corms varied from 17.23 to 100.85 and 1.4 to 10.25 mg GAE/g of DM, respectively. We clearly observed that the combination $1/3\ X_1 + 1/3\ X_2 + 1/3\ X_3$ showed the highest phenolic compound value for the stigmas, while for the corms, the optimal combination was the one obtained with water–ethanol mixture (0.5:0.5).

Statistical Validation of the Postulated Model

Linear, quadratic, and special cubic mixture models were fitted and compared. The ANOVA showed that the special cubic model was acceptable for stigmas (p value: 0.03; R^2: 0.938). In contrast, the quadratic model was the one that satisfactorily reproduces the response for corms with a p value of 0.048 and an R^2 of 0.945 (Table 4). For corms, the quadratic model was selected as the best supported model ($p = 0.048$; $R^2 = 0.945$), while the special cubic model was rejected because its additional cubic term was not significant ($p = 0.205$), despite a higher R^2 (0.965).

Table 4 Analysis of variance of regression models from mixture design

ANOVA source	Sum of squares	DF	F value	p value	R^2
Stigmas					
Linear	2237.836	2	1.862	0.217	0.318
Quadratic	3177.196	3	3.249	0.119	0.769
Special cubic	1194.167	1	10.968	0.030	0.938
Corms					
Linear	77.442	2	13.109	0.003	0.766
Quadratic	18.130	3	5.493	0.048	0.945
Special cubic	2.001	1	2.286	0.205	0.965

Solvent Components' Effects and Fitted Model

The analysis of the effects enabled us to estimate the contribution of each solvent to the optimization of phenolic compound yields (Table 5). The lack-of-fit test was not significant for either plant part ($p = 0.073$ for stigmas and $p = 0.079$ for corms), indicating an adequate fit of the model to the experimental data.

Based on the fitted models, the water–ethanol interaction (X_1X_2) was identified as the most favorable combination for maximizing phenolic extraction from corms. In contrast, the ternary interaction water–ethanol–methanol ($X_1X_2X_3$) provided the optimal solvent domain for stigmas. In both matrices, the water–ethanol–methanol interaction (X_2X_3) exhibited negative coefficients (−55.66 for stigmas and −1.48 for

Table 3 Different combinations generated by the chosen mixing design and the response recorded for each experiment

Run number	Independent variable			Phenolic compounds (mg GAE/g DM)	
	X_1 (water)	X_2 (ethanol)	X_3 (methanol)	Stigmas	Corms
1	1	0	0	71.47 ± 0.85	9.41 ± 0.4
2	0	1	0	17.23 ± 0.43	1.40 ± 0.01
3	0	0	1	61.63 ± 0.78	3.31 ± 0.10
4	1/2	1/2	0	81.00 ± 1.31	10.25 ± 0.45
5	1/2	0	1/2	74.97 ± 0.67	9.26 ± 0.22
6	0	1/2	1/2	23.47 ± 0.12	2.93 ± 0.15
7	2/3	1/6	1/6	77.20 ± 0.61	9.52 ± 0.22
8	1/6	2/3	1/6	73.33 ± 1.59	4.83 ± 0.05
9	1/6	1/6	2/3	75.57 ± 0.93	3.34 ± 0.02
10	1/3	1/3	1/3	99.15 ± 1.73	7.24 ± 0.11
11	1/3	1/3	1/3	100.85 ± 1.45	7.06 ± 0.04

Table 5 ANOVA results for model adjustment

ANOVA source	Sum of squares	DF	*F* value	*p* value	Equation
Stigmas					
Model	6609.200	6	10.117	0.021	$y = 66.40\,x_1 + 20.72\,x_2 + 62.24\,x_3 + 143.45\,x_1\,x_2 + 24.76\,x_1\,x_3 - 55.66\,x_2\,x_3 + 978.98\,x_1\,x_2\,x_3$ $r^2 = 0.938$
Total error	435.517	4			
Lack of fit	434.072	3	100.131	0.073	
Pure error	1.445	1			
Corms					
Model	95.571	5	17.373	0.003	$y = 9.58\,x_1 + 1.60\,x_2 + 3.02\,x_3 + 16.53\,x_1\,x_2 + 7.78\,x_1\,x_3 - 1.48\,x_2\,x_3$ $r^2 = 0.945$
Total error	5.500	5			
Lack of fit	5.485	4	89.560	0.079	
Pure error	0.015	1			

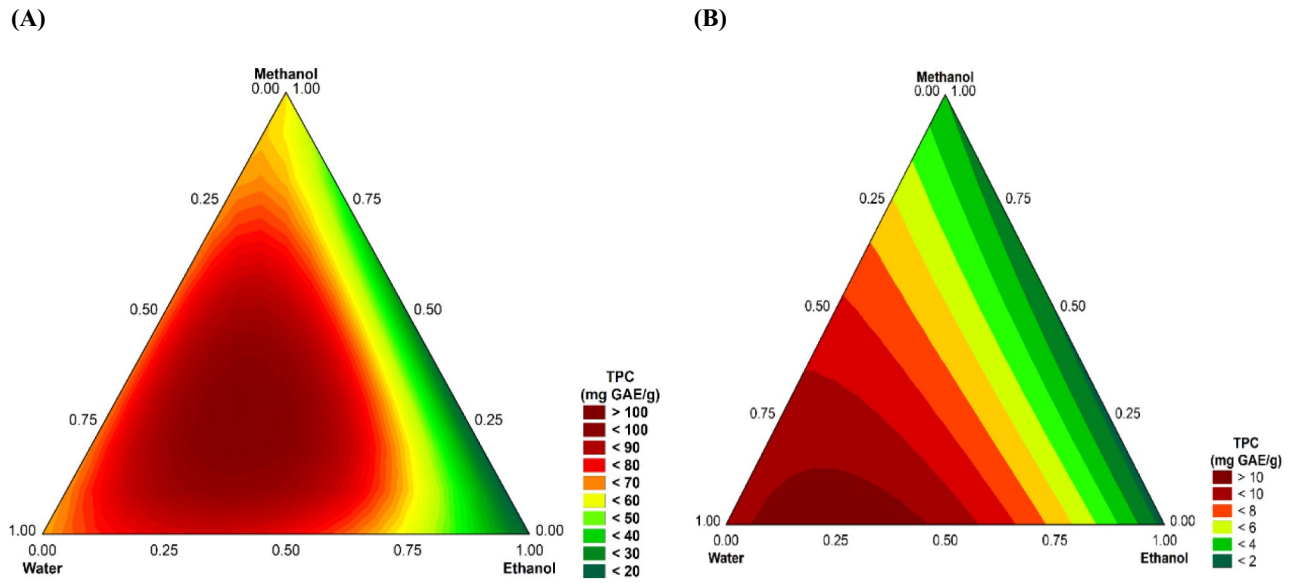

Fig. 1 2D representation of the variations in phenolic compounds obtained from the mixture design. **A** Stigmas; **B** corms

stigmas), indicating an unfavorable (antagonistic) contribution to phenolic recovery; therefore, this binary mixture was not retained as a desirable extraction condition.

Formulation Optimization and Desirability Study

The 2D contour plots indicated that binary/ternary solvent mixtures provided higher extraction yields than the corresponding pure solvents (100%). In Fig. 1, the optimum region was identified by the critical point, whose coordinates represent the solvent proportions that maximize the predicted response. For stigma extracts, the highest phenolic yield was achieved with a mixture of 50% water, 25% ethanol, and 25% methanol (Fig. 1A). For corm extract, the maximum response was obtained with 75% water and 25% ethanol (Fig. 1B).

Table 6 Predicted and observed phenolic compound values for the optimal mixture

Response	Independent variables			Predicted values	Observed values
	Water	Ethanol	Methanol		
Stigmas	1/2	1/4	1/4	102.08	97.43
Corms	3/4	1/4	0	10.41	12.87

The design optimum corresponds to the solvent composition predicted by the model to maximize the response. A validation (point-test) experiment is then carried out at this optimum to compare the experimental value with the predicted one. The close agreement between both values confirms the adequacy of the model and the reproducibility

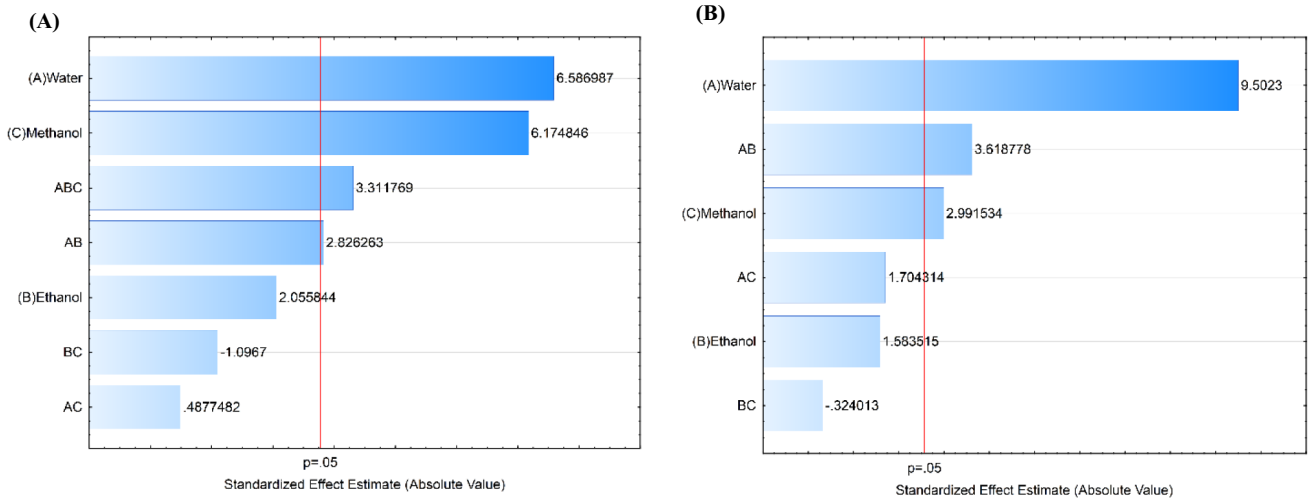

Fig. 2 Pareto charts. **A** Stigmas; **B** corms

Table 7 Phenolic compounds, flavonoid, and condensed tannin content of *C. sativus* extracts

Molecules	Stigmas	Corms
Phenolic compounds (mg GAE/g DM)	$97.43^{a***} \pm 3.85$	$12.87^{b***} \pm 0.37$
Flavonoids (mg QE/g DM)	$59.17^{a***} \pm 1.27$	$9.17^{b***} \pm 1.82$
Condensed tannins (mg/g DM)	$2.27^{a***} \pm 0.34$	$0.13^{b***} \pm 0.05$

***Very highly significant

of the optimum conditions. As shown in Table 6, no significant differences were observed between predicted and measured responses, indicating that the response surface methodology optimized solvent mixture is reliable for predicting polyphenol yield in our extracts.

Pareto Charts

Pareto charts allowed to visualize the importance of the different combinations used in a hierarchical form (Fig. 2). However, the ethanol–methanol mixture decreases the desired response for both stigmas and corms.

Phytochemical Dosage of Extracts from the Optimal Mixture

TPC, flavonoids, and condensed tannins of the extracts from the optimal mixture of each part are shown in Table 7. We noticed that the stigmas have the highest values for TPC, flavonoids, and condensed tannins. Results showed that there were very highly significant differences between the two parts of *C. sativus* for TPC ($F = 480.158$; df = 1; $p \leq 0.001$), flavonoids ($F = 509.735$; df = 1; $p \leq 0.001$), and condensed tannins ($F = 600.3$; df = 1; $p \leq 0.001$).

Table 8 IC_{50} of optimal extracts of *C. sativus* for the DPPH

Methods	IC_{50} (mg/mL)		
	Stigmas	Corms	BHT
DPPH	$0.072^{a***} \pm 0.0009$	$2.947^{b***} \pm 0.070$	$0.032^{c***} \pm 0.0002$

***Very highly significant

Evaluation of Antioxidant Activity

Efficacy of the Solvent's Formulation on DPPH

The antioxidant capacity of the different *C. sativus* extracts was determined from the median inhibitory concentration IC_{50} (Table 8). Based on our results, the IC_{50} values recorded for the different tests (DPPH) showed good activity. Thus, the optimal extract of saffron stigmas showed higher antioxidant activity than that of corms for both analyses.

Statistical analysis of results showed that the organ factor ($F = 1707.088$; df = 2) had a very highly significant effect on DPPH IC_{50} ($p \leq 0.001$).

Ferric Reducing Antioxidant Power Assay

Reducing power assay is a method used to measure the ability of an antioxidant to donate an electron to an oxidizing agent. In this assay, the ability of the extracts to reduce Fe^{3+} to Fe^{2+} was determined. Figure 3 clearly shows that the stigma extract had the highest reducing power (101.72 ± 0.75 mg AAE/g DM), followed by the corms with a content of 36.60 ± 1.16 mg AAE/g DM.

The variance analysis of results relating to ferric reducing power revealed a highly significant effect between the

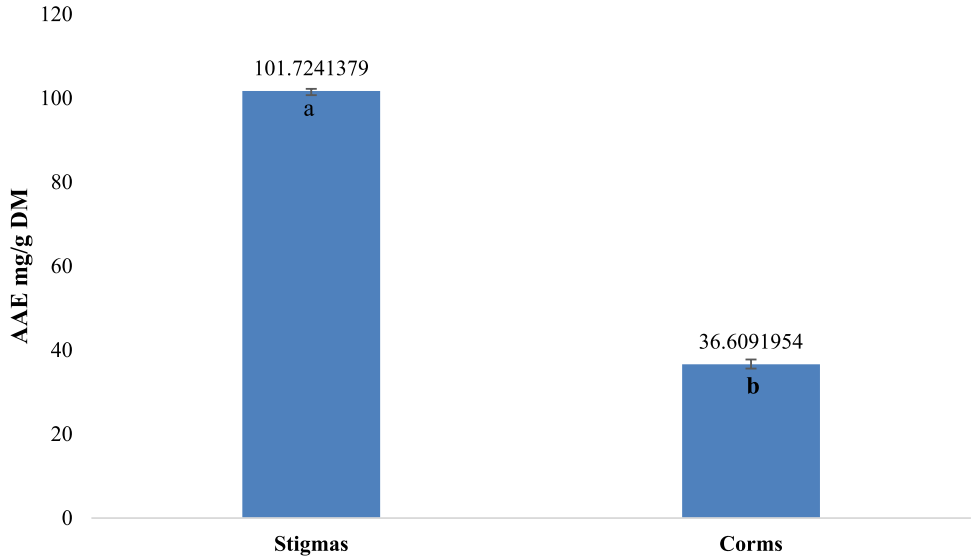

Fig. 3 Ferric reducing antioxidant of *C. sativus* stigmas and corms

Table 9 Minimum inhibitory concentration of optimal extracts

	Minimum inhibitory concentration (mg/mL)	
	Stigmas	Corms
E. coli	1.6	15
S. aureus	1.6	15
P. aeruginosa	0.8	1.6
B. subtilis	0.4	1.6

two parts of the studied plant and the reference molecule ($F = 3138.604$; df = 1; $p \leq 0.001$).

Antibacterial Activity of the Optimal Mixture Extracts

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of the tested extracts against the different bacterial strains are summarized in Table 9. MIC values varied between 0.4 and 15 mg/mL of extract. In both extracts, the highest MICs were consistently observed for *Escherichia coli*, whereas the lowest MICs were recorded against *Bacillus subtilis*, indicating that *B. subtilis* was the most sensitive strain under our experimental conditions.

Minimal Bactericidal Concentration

Minimal bactericidal concentration (MBC) is presented in Table 10. For stigma extract, the lowest MICs were observed for *B. subtilis* (0.4 mg/mL, G^+) and *P. aeruginosa* (0.8 mg/mL, G^-), whereas *E. coli* (G^-) and *S. aureus* (G^+) showed higher MICs (1.6 mg/mL). For corm extracts, higher MICs were recorded for *E. coli* and *S. aureus* (15 mg/mL), while *P. aeruginosa* and *B. subtilis* were more susceptible (MIC 1.6 mg/mL). Overall, antibacterial susceptibility was strain-dependent rather than Gram-dependent, since both Gram-negative and Gram-positive bacteria exhibited high and low MIC values depending on the species.

LC–MS-MRM Analyses

MRM analysis enabled the targeted quantification of each molecular class by monitoring diagnostic fragment ions.

Table 10 Antibacterial parameters of extracts and their interpretation

Strains	Stigmas				Corms			
	MIC	MBC	MBC/MIC	Inter	MIC	MBC	MBC/MIC	Inter
E. coli	1.6	1.6	1	Bd	15	15	1	Bd
S. aureus	1.6	1.6	1	Bd	15	60	4	Bd
P. aeruginosa	0.8	1.6	2	Bd	1.6	15	>4	Bs
B. subtilis	0.4	1.6	4	Bd	1.6	15	>4	Bs

Inter, interpretation; *Bd*, bactericide; *Bs*, bacteriostatic

Peak signal (cps) and relative intensity (percent of the maximum signal) were used to estimate the abundance of the different molecular families across *C. sativus* extracts. Among phenolic acids, vanillin was the predominant compound, followed by salicylic and gentisic acids (Table 11).

When the intensity is expressed as a percentage of maximum intensity, we found that the two extracts seem to be more or less equivalent in their proportion of phenolic acids with high values for vanillin, p-coumaric acid, and gallic acid.

In the case of anthocyanins, we highlighted that 3,5-cyanidin diglucoside is the most present. More specifically, the fragment m/z 287.1 cps has the highest intensity with a value of 3317 cps in the stigma extract.

Discussion

The simplex-centroid mixture design was applied to evaluate the effects of solvent composition on phenolic yield in *C. sativus*. The observed differences among solvent mixtures were primarily attributable to solvent polarity (Table 3), confirming that phenolic extraction is largely

Table 11 Molecules identified in stigma and corm extracts from *C. sativus* by LC–MS/MRM

	Tentative assignment	Formula	MW	$[M-H]^-$ found (M/Z)	MS/MS fragment ions (M/Z) and relative intensity (%) stigmas	MS/MS fragment ions (M/Z) and relative intensity (%) corms
1	Vicenin 2	$C_{27}H_{30}O_{15}$	594.5	593.2	12.695 (0.295); 25.4 (1.391); 79.352 (4.347)	3.171 (0.236); 60.323 (4.492); 69.838 (5.1)
2	Rutin	$C_{27}H_{30}O_{16}$	610.5	609.2	3.171 (0.173); 15.866 (0.869); 47.628 (2.609)	3.171 (0.236); 15.876 (1.182); 38.095 (2.836)
3	Vitexin	$C_{21}H_{20}O_{10}$	432.38	433.2	101.581 (2.197); 53.971 (1.197); 79.361 (1.717)	114.295 (2.951); 276.181 (7.131); 101.590 (2.623)
4	Schaftoside	$C_{26}H_{28}O_{14}$	564.5	567.2	15.866 (0.343); 9.523 (0.206)	25.4 (0.655); 3.171 (0.081)
5	Kaempferol	$C_{15}H_{10}O_6$	286.2	287	3.171 (0.06)	–
6	Quercetin	$C_{15}H_{10}O_7$	302.2	317.1	288.87 (6.249); 57.133 (1.236); 31.742 (0.686)	1355.533 (34.999); 92.066 (2.377); 6.342 (0.163)
7	Luteolin	$C_{15}H_{10}O_6$	286.2	287.1	228.561 (4.944); 396.857 (8.585); 663.485 (14.354)	133.352 (3.443); 930.161 (24.016); 815.876 (21.065)
8	Hesperidin	$C_{28}H_{34}O_{15}$	610.1	611.2	69.857 (1.511); 6.352 (0.137); 3.171 (0.068); 1.685 (0.274)	73.028 (1.885); 6.342 (0.163); 15.876 (0.409)
9	Salicylic acid	$C_7H_6O_3$	138.1	137.1	82.533 (4.521); 1825.438 (100)	60.323 (4.492); 1342.828 (100)
10	Syringic acid	$C_9H_{10}O_5$	198.1	197	152.419 (8.349); 17.047 (1.043)	203.190 (15.315); 19.047 (1.418); 60.333 (4.493)
11	p-Coumaric acid	$C_9H_8O_3$	164	163.1	831.742 (45.564); 60.314 (3.304); 79.342 (4.346)	165.076 (12.293); 41.266 (3.073); 73.019 (5.437)
12	Gallic acid	$C_7H_6O_5$	170.1	169	3.171 (0.173); 593.666 (32.521); 142.383 (7.824)	6.352 (0.473); 1161.942 (86.529); 298.409 (22.222)
13	Ferulic acid	$C_{10}H_{10}O_4$	194	193	41.276 (2.261); 596.790 (32.693); 98, 409 (5.391)	374.6 (27.896); 269.809 (20.092); 311.114 (23.168)
14	Vanillin	$C_8H_8O_3$	152.1	153.2	196.809 (4.257); 4622.2 (100); 495.257 (10.714)	101.581 (2.622); 3872.981 (100); 431.752 (11.147)
15	Gentisic acid	$C_7H_6O_4$	154.1	155.1	1507.942 (32.623); 441.285 (9.547); 57.142 (1.236)	1657.114 (42.876); 288.847 (7.458); 57.152 (1.475)
16	Caffeic acid	$C_9H_8O_4$	180.1	181.1	187.285 (4.051); 355.561 (7.692); 95.228 (2.060)	1101.6 (28.443); 946.057 (24.427); 355.561 (9.180)
17	Pelargonidin 3 glucoside	$C_{21}H_{21}O_{10}^+$	433.4	431.1	622.228 (34.086); 60.323 (3.304); 38.104 (2.087)	241.276 (17.967); 114.285 (8.510); 165.085 (2.293)
18	Pelargonidin 3,5 diglucoside	$C_{27}H_{31}ClO_{15}$	631	593.2	34.914 (1.912); 31.742 (1.738); 28.517 (1.565)	38.104 (2.837); 34.914 (206); 44.447 (3.31)
19	3,5-Cyanidin diglucoside	$C_{27}H_{31}O_{16}^+$	611.2	611.2	47.638 (1.030); 3317.476 (71.772); 19.047 (0.412)	73.019 (1.885); 111.44 (2.869); 66.676 (1.721)
20	Delphinidin 3 glucoside	$C_{21}H_{21}O_{12}^+$	465.1	465.1	98.409 (2.129); 139.685 (3.022); 184.114 (3.983)	101.6 (2.950); 641.285 (16.557); 511.104 (13.196)
21	Petunidin	$C_{16}H_{13}O_7+(Cl\text{-})$	317.1	317.1	53.961 (1.167); 82.552 (1.786); 34.552 (0.755); 44.447 (0.961)	114.276 (2.950); 117.457 (3.032); 199.990 (5.163); 142.847 (3.688)

polarity-driven. It is well established that total phenolic content (TPC) extraction efficiency is influenced by several parameters, among which solvent polarity is particularly critical (Butsat and Siriamornpun 2016; Rauf et al. 2018). In the present study, our objective was to identify an optimal solvent formulation comprising water, ethanol, and methanol that maximizes TPC yield from both stigmas and corms.

Our results demonstrate that aqueous–organic solvent systems (water combined with ethanol and/or methanol) consistently produced higher phenolic yields than the corresponding pure solvents. This enhancement can be attributed to the intermediate polarity achieved by combining water with less polar alcohols, which improves solute–solvent interactions and facilitates the dissolution and diffusion of a wider range of bioactive compounds (Wijekoon et al. 2011).

The synergistic effect of water–alcohol mixtures likely arises from multiple concurrent mechanisms: water disrupts hydrogen-bonding networks within the plant cell wall matrix and solubilizes highly polar glycosylated phenolics, while alcohols enhance penetration into lipophilic cellular components and promote extraction of semi-polar aglycones (Dai and Mumper 2010; Do et al. 2014). Moreover, the intermediate dielectric constants of aqueous–alcoholic mixtures (ε_water = 80; ε_ethanol = 24.3; ε_methanol = 32.6) create an optimal solvent environment for phenolic compounds spanning a broad polarity range, from polar phenolic acids to moderately polar flavonoid glycosides (Koraqi et al. 2023).

The negative interaction coefficient observed for the ethanol–methanol binary mixture ($X_2X_3 = -55.66$ for stigmas; -1.48 for corms) warrants mechanistic consideration. This antagonistic effect likely reflects the similar intermediate polarities and comparable hydrogen-bonding capacities of ethanol and methanol, which result in overlapping extraction windows that are suboptimal for recovering either highly polar or less polar phenolic compounds (Teh and Birch 2013). In contrast, solvent systems containing water either binary or ternary span a broader polarity spectrum, enabling more comprehensive extraction of the chemically diverse phenolic classes present in saffron tissues.

The pronounced difference in optimal solvent composition between stigmas (50% water, 25% ethanol, 25% methanol) and corms (75% water, 25% ethanol) reflects fundamental anatomical and biochemical distinctions between these organs. Stigmas, as specialized reproductive tissues exposed to environmental stressors such as UV radiation and pathogen attack, accumulate a diverse array of phenolic compounds including flavonoid glycosides, anthocyanins, phenolic acids, and condensed tannins for photoprotection and chemical defense (Winterhalter and Straubinger 2000; Carmona et al. 2006). This chemical complexity necessitates a ternary solvent system to maximize extraction efficiency. Conversely, corms function primarily as carbohydrate storage organs and exhibit simpler phenolic profiles dominated by highly polar, water-soluble glycosides, which are adequately extracted using binary aqueous–ethanol mixtures (Baba et al. 2015). The observed reduction in phenolic recovery upon methanol addition to corm extracts (Esmaeelian et al. 2020) further supports the conclusion that corm phenolics are predominantly polar rather than semi-polar.

These mechanistic considerations are reinforced by the chemical profiles obtained here. The stigma extract contained, in addition to phenolic acids, the anthocyanin 3,5-cyanidin diglucoside and a range of flavonoid glycosides spanning a broad polarity window, which is consistent with the need for a ternary water–ethanol–methanol system whose intermediate and complementary polarities cover this range. In contrast, the corm profile was dominated by more polar, water-soluble phenolic acids and glycosides, for which the addition of the less polar methanol provided no benefit and even reduced recovery, in agreement with the negative ethanol–methanol interaction coefficient and with the binary water–ethanol optimum identified for this organ.

Using an optimized solvent mixture consisting of 50% water, 25% ethanol, and 25% methanol, we obtained a phenolic concentration of approximately 97.43 mg GAE/g DM from saffron stigmas. Comparable results were reported by Belyagoubi et al. (2021), who recorded a TPC value of 97.99 mg GAE/g DM from Algerian stigmas using a water–methanol (7:3) solvent system. The close agreement between these values suggests that optimized aqueous–alcoholic solvent systems consistently achieve high phenolic yields from saffron stigmas irrespective of geographic origin.

Nevertheless, substantial variability in reported TPC values exists in the literature, largely attributable to differences in extraction methodology. Zaazaa et al. (2021), working with stigmas from Taliouine (Morocco), reported a TPC of 72.47 mg GAE/g DM using a decoction method. Similarly, Saeed et al. (2021) reported 45.01 ± 2 mg GAE/g DM for pooled samples from Spain, Saudi Arabia, and Afghanistan extracted by decoction. Azghandi et al. (2021) obtained an even lower value (~27.2 mg GAE/g DM) using a water–ethanol mixture for Iranian saffron. These reduced yields can be attributed to thermal degradation of heat-sensitive phenolics during boiling (Spigno et al. 2007) and to suboptimal solvent polarity that limits comprehensive phenolic solubilization (Naczk and Shahidi 2004).

In contrast, our ultrasound-assisted extraction performed at room temperature with optimized solvent mixtures achieved substantially higher yields. This improvement is likely due to acoustic cavitation, which generates localized high-pressure microenvironments that disrupt plant cell

walls without inducing thermal degradation (Vilkhu et al. 2008; Chemat et al. 2017). The combination of physical disruption (ultrasound) and chemical optimization (solvent polarity) produces synergistic effects that maximize phenolic recovery while preserving thermolabile compounds (Tiwari 2015). Collectively, these findings confirm that ultrasound-assisted extraction coupled with a ternary water–ethanol–methanol solvent system represents one of the most effective strategies for TPC extraction from saffron stigmas.

For corms, application of the optimized binary solvent mixture (75% water, 25% ethanol) yielded a TPC of 12.87 mg GAE/g DM. Comparable values were reported by Sánchez-Vioque et al. (2012), who obtained approximately 10 mg GAE/g DM using an ethanol–water (7:3) mixture, while Baba et al. (2015) reported 7.07 mg GAE/g DM using maceration with pure ethanol. The higher yields observed in our study can be attributed to both optimized solvent composition and ultrasound assistance, which more effectively overcome the dense anatomical structure of storage organs than conventional maceration (Roselló-Soto et al. 2015). Consistent with Esmaeelian et al. (2020), our results further demonstrate that water–ethanol mixtures outperform water–methanol systems for corm extraction, reinforcing the conclusion that corm phenolics are predominantly polar glycosides with high water solubility.

The extracts obtained in this study exhibited strong antioxidant activity, exceeding or matching values reported in previous studies (Samaha et al. 2021; Ouahhoud et al. 2022). The stigma extract displayed markedly higher antioxidant capacity (DPPH $IC_{50} = 0.072$ mg/mL; FRAP = 101.72 mg AAE/g DM) than the corm extract, which correlates with its substantially higher TPC (97.43 vs. 12.87 mg GAE/g DM), flavonoid content, and condensed tannin levels. However, the relationship between phenolic concentration and antioxidant activity was not strictly linear. Although stigmas contained approximately 7.6-fold higher TPC than corms, the difference in DPPH scavenging activity (as reflected by IC_{50} values) was proportionally greater, indicating that specific phenolic structures contribute disproportionately to antioxidant efficacy (Leopoldini et al. 2011).

This enhanced activity is attributable to the presence of phenolic acids and flavonoids with structural features favorable for antioxidant action. LC–MS/MRM analysis identified salicylic acid, gentisic acid, and vanillin as predominant phenolic acids. Salicylic and gentisic acids possess ortho-dihydroxy configurations that enable effective chelation of pro-oxidant transition metals (e.g., Fe^{2+}, Cu^{2+}) and stabilization of phenoxyl radicals via intramolecular hydrogen bonding (Cai et al. 2004; Leopoldini et al. 2011). Moreover, the detection of 3,5-cyanidin diglucoside exclusively in stigma extracts is particularly significant, as anthocyanins exhibit exceptional radical-scavenging capacity due to extended π-conjugation, multiple hydroxyl groups, and efficient electron or hydrogen donation (Castañeda-Ovando et al. 2009; Saini et al. 2024).

The high FRAP values observed reflect strong reducing power, indicative of the ability of phenolic hydroxyl groups to donate electrons and reduce Fe^{3+}–TPTZ complexes to Fe^{2+} (Antolovich et al. 2002). This electron-donating capacity is critical for terminating lipid peroxidation chain reactions in food systems, positioning saffron phenolic extracts as potential natural alternatives to synthetic antioxidants such as BHA and BHT (Gülçin 2012; Sridhar and Charles 2019). Nevertheless, it should be emphasized that in vitro antioxidant assays, while informative, do not fully predict in vivo efficacy or performance in complex food matrices, where interactions with proteins, lipids, and other components may modulate bioactivity (Prior et al. 2005).

The antibacterial activity results obtained in this study demonstrated that both stigma and corm extracts exhibited moderate antimicrobial effects against all tested bacterial strains, with minimum inhibitory concentration (MIC) values ranging from 0.4 to 15 mg/mL. Notably, the stigma extract showed no clear differential susceptibility between Gram-positive and Gram-negative bacteria (Table 9). Both *B. subtilis* (Gram-positive) and *P. aeruginosa* (Gram-negative) exhibited low MIC values (0.4–0.8 mg/mL), whereas *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) required higher extract concentrations (1.6 mg/mL). These findings are consistent with those reported by Zara et al. (Zara et al. 2021), who investigated Moroccan and Italian saffron stigmas and similarly observed strain-dependent rather than Gram-dependent antibacterial responses.

The absence of Gram-selective activity suggests that saffron phenolic extracts do not primarily exert their antimicrobial effects through mechanisms strongly dependent on cell envelope architecture, such as outer membrane permeability differences between Gram-negative and Gram-positive bacteria. Instead, these extracts likely act through more universal mechanisms, including membrane depolarization, induction of oxidative stress, inhibition of essential metabolic enzymes, or interference with nucleic acid synthesis (Cushnie and Lamb 2005; Daglia 2012). The particularly high susceptibility of *B. subtilis* and *P. aeruginosa* may reflect species-specific vulnerabilities related to stress response systems, membrane composition, or efflux pump efficiency (Nazzaro et al. 2013).

The MBC/MIC ratios, which were predominantly between 1 and 2 for most strains, indicate that the extracts exert primarily bactericidal rather than bacteriostatic effects. This distinction is particularly relevant for food preservation applications, where irreversible microbial inactivation is generally preferred over temporary growth inhibition (Pankey and Sabath 2004). However, despite their bactericidal nature, the relatively high MIC values (0.4–15 mg/

mL) compared with conventional antibiotics or commercial preservatives limit the feasibility of direct application as standalone antimicrobial agents. At such concentrations, practical use in food systems would likely be constrained by economic considerations and potential adverse sensory effects (Saleem 2014).

Nevertheless, saffron phenolic extracts may be highly effective when applied within combination strategies. Sub-inhibitory concentrations of phenolics have been shown to disrupt biofilm formation, increase membrane permeability, and potentiate the activity of other antimicrobial agents, resulting in synergistic effects (Xu et al. 2012; Langeveld et al. 2014). Furthermore, the dual functionality of these extracts providing both antioxidant protection against lipid oxidation and antimicrobial activity positions them as multifunctional ingredients rather than single-purpose preservatives. Such multifunctionality is particularly attractive for minimally processed foods, where the use of mild, complementary preservation hurdles is preferred (Tiwari et al. 2011; Calo et al. 2015).

LC–MS/MRM analysis enabled the identification and semi-quantitative profiling of phenolic acids and flavonoids present in both stigma and corm extracts. Belyagoubi et al. (2021) reported that methanolic extracts of *C. sativus* stigmas from Algeria contained a wide range of phenolic acids and flavonoids identified by HPLC–DAD, including gallic acid, vanillin, caffeic acid, syringic acid, *p*-coumaric acid, salicylic acid, quercetin, and kaempferol. In the present study, LC–MS/MRM analysis identified vanillin as the predominant phenolic acid, followed by salicylic acid and gentisic acid, while 3,5-cyanidin diglucoside was detected as the major anthocyanin. These findings are consistent with previous reports describing a characteristic saffron phenolic profile dominated by simple phenolic acids and flavonoid glycosides (Mykhailenko et al. 2019; Sun 2023).

The predominance of vanillin is noteworthy. Although vanillin exhibits relatively modest direct antioxidant activity compared with polyhydroxylated phenolics, it can contribute indirectly to antioxidant capacity through synergistic interactions, including aldehyde-mediated stabilization of cellular structures and potential chelation of pro-oxidant metal ions (Tai et al. 2011). The high abundance of salicylic acid is particularly relevant to the observed antimicrobial activity, as salicylates are known to inhibit bacterial respiration, disrupt membrane integrity, and interfere with quorum-sensing pathways involved in bacterial communication and virulence (Price et al. 2000; El-Mowafy et al. 2014).

When phenolic acid signal intensities were expressed as percentages of maximum intensity, both stigma and corm extracts exhibited similar relative proportions of major phenolic acids, including vanillin, *p*-coumaric acid, and gallic acid. This observation suggests that tissue-specific differences in total phenolic content primarily reflect quantitative rather than qualitative variations in phenolic composition. However, the detection of anthocyanins exclusively in stigma extracts specifically 3,5-cyanidin diglucoside (m/z 287.1; 3317 cps) represents a clear qualitative distinction with functional implications. Anthocyanins contribute uniquely to antioxidant activity and pigmentation through mechanisms unavailable to simple phenolic acids, including enhanced radical scavenging and resonance stabilization (He and Giusti 2010; Khoo et al. 2017).

It should be noted that the LC–MS/MRM analysis performed in this study was semi-quantitative, relying on signal intensity (counts per second) and relative percentages rather than absolute quantification using calibration curves with authentic standards. Consequently, precise determination of individual phenolic concentrations was not possible. Future studies should incorporate external standard calibration or standard addition approaches to enable absolute quantification and more rigorous structure–activity relationship analyses (Robbins 2003; Gruz et al. 2008).

Taken together, these observations indicate that the superior antioxidant (DPPH IC_{50} = 0.072 mg/mL; FRAP = 101.72 mg AAE/g DM) and antibacterial performance of the stigma extract relative to the corm extract is attributable both to its substantially higher total phenolic content and to its richer qualitative profile, in particular the presence of salicylic and gentisic acids, whose ortho-dihydroxy and metal-chelating features favor radical scavenging and bacterial-membrane disruption, and of the anthocyanin 3,5-cyanidin diglucoside, detected exclusively in stigmas, which contributes additional radical-scavenging capacity unavailable to the simple phenolic acids predominating in corms.

From an applied perspective, this study demonstrates that saffron corms currently discarded as agricultural waste represent a viable source of phenolic antioxidants. Given that each saffron plant produces 5–10 daughter corms annually compared with a single set of three stigmas, corm utilization could provide substantial quantities of bioactive extracts at near-zero raw material cost, thereby addressing both waste valorization and sustainable natural product development (Cardone et al. 2020). Although corm extracts exhibited lower phenolic content and bioactivity than stigma extracts, their abundance and current waste status make them economically attractive for large-scale production of natural antioxidants.

However, several limitations should be acknowledged. First, this study focused exclusively on optimizing solvent composition using ultrasound-assisted extraction, while other influential parameters including extraction temperature, time, solid-to-liquid ratio, ultrasound power, and frequency were kept constant. Comprehensive optimization incorporating these additional variables through experimental designs such as Box–Behnken or central composite designs could further enhance extraction efficiency (Chemat et al. 2017; Koraqi et al. 2023).

Second, all bioactivity assays were conducted in vitro under controlled laboratory conditions, which do not fully reflect the complexity of real food matrices. Phenolic compounds may undergo oxidation, polymerization, or interactions with proteins and lipids, potentially altering their stability, bioavailability, and efficacy (Ozdal et al. 2013). Therefore, shelf-life studies and validation in real food systems (e.g., oils, meat products, or dairy matrices) are required to confirm practical applicability (Huber et al. 2009).

Third, the relatively high MIC values observed limit the feasibility of direct application as standalone antimicrobial agents, suggesting that incorporation into hurdle technology approaches—combining multiple mild preservation strategies or synergistic combinations with conventional preservatives—may represent more realistic application pathways (Alzamora et al. 2003). In addition, the characteristic color and flavor imparted by saffron extracts may restrict their use to specific food products where such sensory attributes are acceptable or desirable.

Finally, the LC–MS/MRM analysis conducted in this study was semi-quantitative and did not include absolute quantification using calibration curves with authentic standards. This limitation restricts precise determination of individual phenolic concentrations and precludes robust correlation analyses between specific compounds and observed biological activities. Moreover, factors such as geographic origin, cultivation practices, harvest time, and post-harvest processing are known to influence saffron phytochemical composition (Carmona et al. 2006). As this study examined a single batch from Taliouine, Morocco, further validation across multiple origins and harvest seasons is required to assess the general applicability of the optimized extraction protocols.

This study successfully optimized solvent systems for phenolic extraction from *C. sativus* stigmas and corms using a simplex-centroid mixture design, demonstrating that a ternary solvent system (water–ethanol–methanol, 50:25:25) for stigmas and a binary system (water–ethanol, 75:25) for corms provide superior extraction yields compared with pure solvents. The optimized stigma extract exhibited strong antioxidant activity (DPPH IC_{50} = 0.072 mg/mL; FRAP = 101.72 mg AAE/g DM) and moderate antibacterial effects (MIC 0.4–1.6 mg/mL against susceptible strains). LC–MS/MRM analysis identified vanillin, salicylic acid, gentisic acid, and 3,5-cyanidin diglucoside as major phenolic constituents.

The demonstrated bioactivities, particularly the strong antioxidant capacity, suggest potential applications of these extracts as multifunctional natural ingredients. Nevertheless, translation from laboratory-scale optimization to practical application requires further investigation, including validation in real food matrices, shelf-life assessment, sensory evaluation, techno-economic analysis, and regulatory compliance evaluation.

Future research should focus on (1) comprehensive process optimization incorporating additional extraction parameters such as temperature, time, and ultrasound intensity; (2) evaluation of extract performance in real food systems, particularly with respect to lipid oxidation inhibition; (3) targeted metabolomic analyses with absolute quantification to establish structure–activity relationships; (4) investigation of synergistic effects with conventional preservatives or other hurdle technologies; (5) pilot-scale extraction and feasibility studies for corm valorization; and (6) assessment of bioavailability and in vivo antioxidant effects using appropriate cell culture or animal models.

Conclusions

This study successfully optimized solvent systems for phenolic extraction from Moroccan *C. sativus* stigmas and corms using a simplex-centroid mixture design, demonstrating that ternary (water:ethanol:methanol, 1/2:1/4:1/4) and binary (water:ethanol, 3/4:1/4) solvent mixtures achieve superior yields (97.43 and 12.87 mg GAE/g DM, respectively) compared with pure solvents. This work represents the first comparative optimization of phenolic extraction from both stigmas and corms under identical methodological conditions, thereby providing tissue-specific extraction protocols. Despite comparable qualitative phenolic profiles, the substantial differences in yield and bioactivity between stigmas and corms highlight predominantly quantitative, tissue-specific variations with direct implications for extract functionality. The optimized stigma extract exhibited substantial antioxidant capacity (DPPH IC_{50} = 0.072 mg/mL; FRAP = 101.72 mg AAE/g DM) and moderate antibacterial activity (MIC 0.4–0.8 mg/mL against susceptible strains). LC–MS/MRM analysis identified vanillin, salicylic acid, and gentisic acid as the major phenolic constituents. From a practical perspective, these findings support the valorization of saffron corms currently discarded as agricultural waste as a sustainable source of natural antioxidants for food preservation applications, although their moderate antimicrobial potency suggests that integration with hurdle technology approaches may be required. However, translation of these laboratory-scale optimizations into practical applications requires validation in real food matrices, assessment of potential sensory impacts, and techno-economic feasibility analyses. Future research should prioritize comprehensive process optimization incorporating ultrasound parameters, evaluation of extract performance in food systems with particular emphasis on lipid oxidation inhibition, and pilot-scale extraction studies to establish the commercial viability of corm valorization as a circular economy strategy within saffron production systems.

Author Contributions Chaimae Rais: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Chaimae Slimani: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Farid Mansouri: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Boutayna Rhourri-Frih: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Laila Elhanafi: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Meryem Benjelloun: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. Miroslava Kačániová: Writing – review & editing, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. Stefania Garzoli: Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. Abderrahim Lazraq: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization.

Funding Open access funding provided by Università degli Studi di Roma La Sapienza within the CRUI-CARE Agreement.

Data Availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process During the preparation of this manuscript, the authors used an AI-assisted language model (ChatGPT, OpenAI) to support language editing and improve clarity and readability of the text. After using this tool, the authors carefully reviewed, edited, and validated the content and take full responsibility for the integrity and originality of the published work.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abu Safe FA, Badr AN, Shehata MG, El-Sayyad GS (2023) Antimicrobial and anti-aflatoxigenic activities of nanoemulsions based on *Achillea millefolium* and *Crocus sativus* flower extracts as green promising agents for food preservatives. BMC Microbiol 23:289. https://doi.org/10.1186/s12866-023-03033-2

Alani AH, Johari MAM, Bunnori NM, Majid TA (2024) Application of response surface methodology for optimizing the compressive strength of green ultra high strength concrete using silica fume. J Build Rehabil 9:5. https://doi.org/10.1007/s41024-023-00361-8

Alzamora S, Lopez-Malo A, Palou E, Guerrero S (2003) Plant antimicrobials combined with conventional preservatives for fruit products, in: S. Roller (Ed.), Natural antimicrobials for the minimal processing of foods, CRC Press. https://doi.org/10.1201/9780203508244.ch11

Amatto PDPG, Chaves L, Braga GG, Carmona F, Pereira AMS (2024) Effect of Crocus sativus L. (saffron) and crocin in the treatment of patients with type-2 diabetes mellitus: a systematic review and meta-analysis. J Ethnopharmacol 319:117255. https://doi.org/10.1016/j.jep.2023.117255

Anaeigoudari F, Anaeigoudari A, Kheirkhah-Vakilabad A (2023) A review of therapeutic impacts of saffron (Crocus sativus L.) and its constituents. Physiol Rep 11:e15785. https://doi.org/10.14814/phy2.15785

Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K (2002) Methods for testing antioxidant activity. Analyst 127:183–198. https://doi.org/10.1039/b009171p

Ara SA, Akhlaq S, Ahmad B, Haque M, Fazil M, Akram U, Sayeed A, Ali Khan A (2024) The perpetual impact of COVID-19 on the geriatric population's quality of life and potential preventive and therapeutic measures in Unani medicine: an evidence-based review. CTM 10:e120423215714. https://doi.org/10.2174/2215083810666230412130045

Azghandi Fardaghi A, Eshaghi A, Feizy J, Lakshmipathy R (2021) Antioxidant capacity and chemical composition of different parts of saffron flowers. J Food Bioprocess Eng. https://doi.org/10.22059/jfabe.2021.316326.1078.

Baba SA, Malik AH, Wani ZA, Mohiuddin T, Shah Z, Abbas N, Ashraf N (2015) Phytochemical analysis and antioxidant activity of different tissue types of *Crocus sativus* and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. S Afr J Bot 99:80–87. https://doi.org/10.1016/j.sajb.2015.03.194

Barros L, Carvalho AM, Ferreira ICFR (2011) Exotic fruits as a source of important phytochemicals: improving the traditional use of *Rosa canina* fruits in Portugal. Food Res Int 44:2233–2236. https://doi.org/10.1016/j.foodres.2010.10.005

Belyagoubi L, Loukidi B, Belyagoubi-Benhammou N, Gismondi A, Di Marco G, D'Agostino A, Canini A, Benmahieddine A, Rouigueb K, Ben Menni D, Atik-Bekkara F (2021) Valorization of Algerian saffron: stigmas and flowers as source of bioactive compounds. Waste Biomass Valor 12:6671–6683. https://doi.org/10.1007/s12649-021-01454-6

Bouhdid S, Abrini J, Zhiri A, Espuny MJ, Manresa A (2009) Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. J Appl Microbiol 106:1558–1568. https://doi.org/10.1111/j.1365-2672.2008.04124.x

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Butsat S, Siriamornpun S (2016) Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of *Amomum chinense* C., International Food Research Journal. https://www.semanticscholar.org/paper/Effect-of-solvent-types-and-extraction-times-on-and-Butsat-Siriamornpun/140091435750d1af0ca23ea98bf278bf16854795 (Accessed January 9, 2024)

Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 74:2157–2184. https://doi.org/10.1016/j.lfs.2003.09.047

Calo JR, Crandall PG, O'Bryan CA, Ricke SC (2015) Essential oils as antimicrobials in food systems – a review. Food Control 54:111–119. https://doi.org/10.1016/j.foodcont.2014.12.040

Cardone L, Castronuovo D, Perniola M, Cicco N, Candido V (2020) Saffron (Crocus sativus L.), the king of spices: an overview. Sci Hortic 272:109560. https://doi.org/10.1016/j.scienta.2020.109560

Carmona M, Zalacain A, Sánchez AM, Novella JL, Alonso GL (2006) Crocetin esters, picrocrocin and its related compounds present in

Crocus sativus stigmas and Gardenia jasminoides fruits. Tentative identification of seven new compounds by LC-ESI-MS. J Agric Food Chem 54:973–979. https://doi.org/10.1021/jf052297w

Castañeda-Ovando A, Pacheco-Hernández MaDL, Páez-Hernández MaE, Rodríguez JA, Galán-Vidal CA (2009) Chemical studies of anthocyanins: a review. Food Chem 113:859–871. https://doi.org/10.1016/j.foodchem.2008.09.001

Chemat F, Rombaut N, Sicaire A-G, Meullemiestre A, Fabiano-Tixier A-S, Abert-Vian M (2017) Ultrasound assisted extraction of food and natural products Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem 34:540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035

Colucci Cante R, Gallo M, Varriale L, Garella I, Nigro R (2022) Recovery of carotenoids from tomato pomace using a hydrofluorocarbon solvent in sub-critical conditions. Appl Sci 12:2822. https://doi.org/10.3390/app12062822

Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26:343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Daglia M (2012) Polyphenols as antimicrobial agents. Curr Opin Biotechnol 23:174–181. https://doi.org/10.1016/j.copbio.2011.08.007

Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15:7313–7352. https://doi.org/10.3390/molecules15107313

Delam H, Keshtkaran Z, Shokrpour N, Eidi A, Bazrafshan M-R (2023) The effect of Crocus sativus L. (saffron) herbal tea on happiness in postmenopausal women: a randomized controlled trial. BMC Complement Med Ther 23:176. https://doi.org/10.1186/s12906-023-04014-8

Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y-H (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 22:296–302. https://doi.org/10.1016/j.jfda.2013.11.001

El-Mowafy SA, Abd El Galil KH, El-Messery SM, Shaaban MI (2014) Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in *Pseudomonas aeruginosa*. Microb Pathog 74:25–32. https://doi.org/10.1016/j.micpath.2014.07.008

Esmaeelian M, Jahani M, Einafshar S, Feizy J (2020) Optimization of experimental parameters in subcritical water extraction of bioactive constituents from the saffron (Crocus sativus L.) corm based on response surface methodology. Food Measure 14:1822–1832. https://doi.org/10.1007/s11694-020-00429-w

Fadil M, Lebrazi S, Aboulghazi A, Guaouguaou F-E, Rais C, Slimani C, Es-safi NE (2022) Multi-response optimization of extraction yield, total phenols-flavonoids contents, and antioxidant activity of extracts from moroccan *Lavandula stoechas* leaves: predictive modeling using simplex-centroid design. Biocatal Agric Biotechnol 43:102430. https://doi.org/10.1016/j.bcab.2022.102430

Ghaderi A, Asbaghi O, Reiner Ž, Kolahdooz F, Amirani E, Mirzaei H, Banafshe HR, Maleki Dana P, Asemi Z (2020) The effects of saffron (Crocus sativus L.) on mental health parameters and C-reactive protein: a meta-analysis of randomized clinical trials. Complement Ther Med 48:102250. https://doi.org/10.1016/j.ctim.2019.102250

Gohari A, Saeidnia S, Mahmoodabadi M (2013) An overview on saffron, phytochemicals, and medicinal properties. Phcog Rev 7:61. https://doi.org/10.4103/0973-7847.112850

Gruz J, Novák O, Strnad M (2008) Rapid analysis of phenolic acids in beverages by UPLC–MS/MS. Food Chem 111:789–794. https://doi.org/10.1016/j.foodchem.2008.05.014

Gülçin İ (2012) Antioxidant activity of food constituents: an overview. Arch Toxicol 86:345–391. https://doi.org/10.1007/s00204-011-0774-2

He J, Giusti MM (2010) Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol 1:163–187. https://doi.org/10.1146/annurev.food.080708.100754

Hebi M, Eddouks M (2016) Évaluation de l'activité antioxydante de Stevia rebaudiana. Phytothérapie 14:17–22. https://doi.org/10.1007/s10298-015-0999-y

Huber GM, Vasantha Rupasinghe HP, Shahidi F (2009) Inhibition of oxidation of omega-3 polyunsaturated fatty acids and fish oil by quercetin glycosides. Food Chem 117:290–295. https://doi.org/10.1016/j.foodchem.2009.04.007

Khoo HE, Azlan A, Tang ST, Lim SM (2017) Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res 61:1361779. https://doi.org/10.1080/16546628.2017.1361779

Koraqi H, Petkoska AT, Khalid W, Sehrish A, Ambreen S, Lorenzo JM (2023) Optimization of the extraction conditions of antioxidant phenolic compounds from strawberry fruits (Fragaria x ananassa Duch.) using response surface methodology. Food Anal Methods 16:1030–1042. https://doi.org/10.1007/s12161-023-02469-6

Langeveld WT, Veldhuizen EJA, Burt SA (2014) Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol 40:76–94. https://doi.org/10.3109/1040841X.2013.763219

Leopoldini M, Russo N, Toscano M (2011) The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem 125:288–306. https://doi.org/10.1016/j.foodchem.2010.08.012

Levison ME (2004) Pharmacodynamics of antimicrobial drugs. Infect Dis Clin North Am 18:451–465. https://doi.org/10.1016/j.idc.2004.04.012

Loizzo MR, Marrelli M, Pugliese A, Conforti F, Nadjafi F, Menichini F, Tundis R (2016) Crocus cancellatus subsp. damascenus stigmas: chemical profile, and inhibition of α-amylase, α-glucosidase and lipase, key enzymes related to type 2 diabetes and obesity. J Enzyme Inhib Med Chem 31:212–218. https://doi.org/10.3109/14756366.2015.1016510

Mahood HE, Dahham AA, Sarropoulou V, Tzatzani T-T (2023) Extraction of phenolic and flavonoid compounds and evaluation of their antioxidant activity in saffron anthers (Crocus sativus L.). Not Sci Biol 15:11640. https://doi.org/10.55779/nsb15411640

Marcheafave GG, Tormena CD, Pauli ED, Rakocevic M, Bruns RE, Scarminio IS (2019) Experimental mixture design solvent effects on pigment extraction and antioxidant activity from Coffea arabica L. leaves. Microchemical J 146:713–721. https://doi.org/10.1016/j.microc.2019.01.073

Martins CR, Lopes WA, Andrade JBD (2013) Solubilidade das substâncias orgânicas. Quím Nova 36:1248–1255. https://doi.org/10.1590/S0100-40422013000800026

Memarzia A, Ghasemi SZ, Behrouz S, Boskabady MH (2023) The effects of *Crocus sativus* extract on inhaled paraquat-induced lung inflammation, oxidative stress, pathological changes and tracheal responsiveness in rats. Toxicon 235:107316. https://doi.org/10.1016/j.toxicon.2023.107316

Muduli N, Aparna S, Patri M, Sahoo KK (2024) Saffron stigma extract and crocin play an important neuroprotective role in therapeutic measures against benzo[a]pyrene-induced behavioral alterations in zebrafish. Drug Chem Toxicol 47:131–142. https://doi.org/10.1080/01480545.2023.2250576

Mykhailenko O, Kovalyov V, Goryacha O, Ivanauskas L, Georgiyants V (2019) Biologically active compounds and pharmacological activities of species of the genus *Crocus*: a review. Phytochemistry 162:56–89. https://doi.org/10.1016/j.phytochem.2019.02.004

Naczk M, Shahidi F (2004) Extraction and analysis of phenolics in food. J Chromatogr A 1054:95–111. https://doi.org/10.1016/S0021-9673(04)01409-8

Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V (2013) Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6:1451–1474. https://doi.org/10.3390/ph6121451

Nemati Z, Harpke D, Gemicioglu A, Kerndorff H, Blattner FR (2019) Saffron (*Crocus sativus*) is an autotriploid that evolved in Attica (Greece) from wild *Crocus cartwrightianus*. Mol Phylogenet Evol 136:14–20. https://doi.org/10.1016/j.ympev.2019.03.022

Ouahhoud S, Khoulati A, Kadda S, Bencheikh N, Mamri S, Ziani A, Baddaoui S, Eddabbeh F-E, Lahmass I, Benabbes R, Addi M, Hano C, Asehraou A, Saalaoui E (2022) Antioxidant activity, metal chelating ability and DNA protective effect of the hydro-ethanolic extracts of Crocus sativus stigmas, tepals and leaves. Antioxidants 11:932. https://doi.org/10.3390/antiox11050932

Ozdal T, Capanoglu E, Altay F (2013) A review on protein–phenolic interactions and associated changes. Food Res Int 51:954–970. https://doi.org/10.1016/j.foodres.2013.02.009

Pankey GA, Sabath LD (2004) Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of gram-positive bacterial infections. Clin Infect Dis 38:864–870. https://doi.org/10.1086/381972

Price CTD, Lee IR, Gustafson JE (2000) The effects of salicylate on bacteria. Int J Biochem Cell Biol 32:1029–1043. https://doi.org/10.1016/S1357-2725(00)00042-X

Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53:4290–4302. https://doi.org/10.1021/jf0502698

Rauf A, Nawaz H, Shad MA (2018) Effect of solvent polarity and extraction time on in vitro antioxidant properties of Brassica oleracea Convar Capitata Var L. seeds. Pak J Pharm Sci 31:1889–1897

Ribéreau-Gayon P, Stonestreet E (1966) Le dosage des tanins dans le vin rouge et détermination de leur structure. Anal Chim Acta 48:188–192

Robbins RJ (2003) Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem 51:2866–2887. https://doi.org/10.1021/jf026182t

Roselló-Soto E, Galanakis CM, Brnčić M, Orlien V, Trujillo FJ, Mawson R, Knoerzer K, Tiwari BK, Barba FJ (2015) Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing modeling approaches to optimize processing conditions. Trends Food Sci Technol 42:134–149. https://doi.org/10.1016/j.tifs.2015.01.002

Saeed Alkaltham M, Hayat K, Asif Ahmed M, Mohammad Salamatullah A, Sami R, Khojah E, Al-Mushhin AAM (2021) Bioactive compounds, high performance liquid chromatography screening of phenolic compounds, and antioxidant potential activity of saffron (Crocus sativus L.). J Biobased Mat Bioenergy 15:700–704. https://doi.org/10.1166/jbmb.2021.2106

Saini RK, Khan MI, Shang X, Kumar V, Kumari V, Kesarwani A, Ko E-Y (2024) Dietary sources, stabilization, health benefits, and industrial application of anthocyanins—a review. Foods 13:1227. https://doi.org/10.3390/foods13081227

Saleem M (2014) Natural products as antimicrobial agents – an update, in: D.A. Phoenix, F. Harris, S.R. Dennison (Eds.), Novel antimicrobial agents and strategies, 1st ed., Wiley, pp. 219–294. https://doi.org/10.1002/9783527676132.ch9

Samaha H, Chahine N, Sobolev AP, Menghini L, Makhlouf H (2021) 1H-NMR metabolic profiling and antioxidant activity of saffron (*Crocus sativus*) cultivated in Lebanon. Molecules 26:4906. https://doi.org/10.3390/molecules26164906

Samimi M (2023) Efficient biosorption of cadmium by Eucalyptus globulus fruit biomass using process parameters optimization. Global J Environ Sci Manage. https://doi.org/10.22034/gjesm.2024.01.03

Sánchez-Vioque R, Rodríguez-Conde MF, Reina-Ureña JV, Escolano-Tercero MA, Herraiz-Peñalver D, Santana-Méridas O (2012) In vitro antioxidant and metal chelating properties of corm, tepal and leaf from saffron (Crocus sativus L.). Ind Crops Prod 39:149–153. https://doi.org/10.1016/j.indcrop.2012.02.028

Sarfarazi M, Rajabzadeh Q, Tavakoli R, Ibrahim SA, Jafari SM (2022) Ultrasound-assisted extraction of saffron bioactive compounds; separation of crocins, picrocrocin, and safranal optimized by artificial bee colony. Ultrason Sonochem 86:105971. https://doi.org/10.1016/j.ultsonch.2022.105971

Shadab S, Rao GK, Paliwal D, Yadav D, Alam A, Singh A, Sultana MJ (2024) A comprehensive review of herbal medicines for the treatment of Alzheimer's disease. CTM 10:e080623217816. https://doi.org/10.2174/2215083810666230608151821

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Slimani C, El Goumi Y, Rais C, El Ghadraoui L, Benjelloun M, Lazraq A (2021) Optimization of callogenesis/caulogenesis induction protocol in saffron plant (Crocus sativus L.) using response surface methodology. Biointerface Res Appl Chem 12:4731–4746. https://doi.org/10.33263/BRIAC124.47314746

Slimani C, El Goumi Y, Rais C, El Ghadraoui L, Benjelloun M, Lazraq A (2022) Micropropagation and potential of bioactive compounds of saffron (Crocus sativus L.) for nutrition and health. Not Sci Biol 14:11278. https://doi.org/10.55779/nsb14311278

Slimani C, Rais C, Mansouri F, El Hanafi L, Rhourri-Frih B, Benjelloun M, Ullah R, Shahat AA, Acquaticci L, Caprioli G, Bouyahya A, Lazraq A (2025a) LC-MS/MRM analysis of bioactive compounds from saffron (Crocus sativus L.) by-products: optimization of phenolic compounds recovery using simplex centroid mixture design. Microchem J 209:112714. https://doi.org/10.1016/j.microc.2025.112714

Slimani C, Fadil M, Rais C, El-hanafi L, Benjelloun M, Shin H-M, Giesy JP, Lazraq A, Abulmeaty MMA, Almutairi KM, Aboul-Soud MAM (2025b) Extraction of natural antioxidants from Moroccan saffron (Crocus sativus L.) using ultrasound-assisted extraction: an optimization approach with Box-Behnken design. Ultrason Sonochem 120:107448. https://doi.org/10.1016/j.ultsonch.2025.107448

Spigno G, Tramelli L, De Faveri DM (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J Food Eng 81:200–208. https://doi.org/10.1016/j.jfoodeng.2006.10.021

Sridhar K, Charles AL (2019) In vitro antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: estimation methods for EC50 using advanced statistical programs. Food Chem 275:41–49. https://doi.org/10.1016/j.foodchem.2018.09.040

Sun X (2023) Research progress on cannabinoids in cannabis (Cannabis sativa L.) in China. Molecules 28:3806. https://doi.org/10.3390/molecules28093806

Tai A, Sawano T, Yazama F, Ito H (2011) Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays. Biochimica Et Biophysica Acta (BBA) - Gen Subj 1810:170–177. https://doi.org/10.1016/j.bbagen.2010.11.004

Teh S-S, Birch J (2013) Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. J Food Compos Anal 30:26–31. https://doi.org/10.1016/j.jfca.2013.01.004

Termentzi A, Kokkalou E (2008) LC-DAD-MS (ESI^+) analysis and antioxidant capacity of *Crocus sativus* petal extracts. Planta Med 74:573–581. https://doi.org/10.1055/s-2008-1074498

Tiwari BK (2015) Ultrasound: a clean, green extraction technology. TrAC, Trends Anal Chem 71:100–109. https://doi.org/10.1016/j.trac.2015.04.013

Tiwari BK, Valdramidis VP, Bourke P, Cullen P (2011) Application of plant-based antimicrobials in food preservation., in: M. Rai, M. Chikindas (Eds.), Natural antimicrobials in food safety and quality, 1st ed., CABI, UK, pp. 204–223. https://doi.org/10.1079/9781845937690.0204

Tomšik A, Pavlić B, Vladić J, Ramić M, Brindza J, Vidović S (2016) Optimization of ultrasound-assisted extraction of bioactive compounds from wild garlic (Allium ursinum L.). Ultrason Sonochem 29:502–511. https://doi.org/10.1016/j.ultsonch.2015.11.005

Tormena CD, Marcheafave GG, Rakocevic M, Bruns RE, Scarminio IS (2019) Sequential mixture design optimization for divergent metabolite analysis: enriched carbon dioxide effects on Coffea arabica L. leaves and buds. Talanta 191:382–389. https://doi.org/10.1016/j.talanta.2018.09.002

Udayaprakash NK, Ranjithkumar M, Deepa S, Sripriya N, Al-Arfaj AA, Bhuvaneswari S (2015) Antioxidant, free radical scavenging and GC–MS composition of Cinnamomum iners Reinw. ex Blume. Ind Crops Prod 69:175–179. https://doi.org/10.1016/j.indcrop.2015.02.018

Vilkhu K, Mawson R, Simons L, Bates D (2008) Applications and opportunities for ultrasound assisted extraction in the food industry — a review. Innov Food Sci Emerg Technol 9:161–169. https://doi.org/10.1016/j.ifset.2007.04.014

Wijekoon MMJO, Bhat R, Karim AA (2011) Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (Etlingera elatior Jack.) inflorescence. J Food Compos Anal 24:615–619. https://doi.org/10.1016/j.jfca.2010.09.018

Winterhalter P, Straubinger M (2000) Saffron—renewed interest in an ancient spice. Food Rev Intl 16:39–59. https://doi.org/10.1081/FRI-100100281

Xu X, Zhou XD, Wu CD (2012) Tea catechin epigallocatechin gallate inhibits Streptococcus mutans biofilm formation by suppressing gtf genes. Arch Oral Biol 57:678–683. https://doi.org/10.1016/j.archoralbio.2011.10.021

Zaazaa L, Naceiri Mrabti H, Ed-Dra A, Bendahbia K, Hami H, Soulaymani A, Ibriz M (2021) Determination of mineral composition and phenolic content and investigation of antioxidant, antidiabetic, and antibacterial activities of Crocus sativus L. aqueous stigmas extracts. Adv Pharmacol Pharm Sci 2021:1–7. https://doi.org/10.1155/2021/7533938

Zara S, Petretto GL, Mannu A, Zara G, Budroni M, Mannazzu I, Multineddu C, Pintore G, Fancello F (2021) Antimicrobial activity and chemical characterization of a non-polar extract of saffron stamens in food matrix. Foods 10:703. https://doi.org/10.3390/foods10040703

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Chaimae Rais[1] · Chaimae Slimani[2,3,4] · Farid Mansouri[5] · Boutayna Rhourri-Frih[6] · Laila Elhanafi[4] · Meryem Benjelloun[4] · Miroslava Kačániová[7,8] · Stefania Garzoli[9] · Abderrahim Lazraq[4]

✉ Chaimae Slimani
chaimae.slimani@usmba.ac.ma

✉ Stefania Garzoli
stefania.garzoli@uniroma1.it

Chaimae Rais
c.rais@ueuromed.org

Farid Mansouri
f.mansouri@ump.ac.ma

Boutayna Rhourri-Frih
boutayna.frih@u-bordeaux.fr

Laila Elhanafi
lailaelhanafi4@gmail.com

Meryem Benjelloun
Mery_benjelloun@hotmail.com

Miroslava Kačániová
m.kacaniova@vizja.pl; miroslava.kacaniova@gmail.com

Abderrahim Lazraq
abderrahim.lazraq@usmba.ac.ma

[1] Euromed University, UEMF, Meknès Road, Bensouda Roundabout, 30 000 Fez, Morocco

[2] Biotechnology, Environmental Technology and Valorization of BioResources Team, Department of Biology, Laboratory of Research and Development in Engineering Sciences, Faculty of Sciences and Techniques, Abdelmalek Essaadi University, BP 34, Ajdir 32003, Al-Hoceima, Tetouan, Morocco

[3] Laboratory of Botany, National Agency for Medicinal and Aromatic Plants, P.O. Box 159, 34 025, 10 Taounate, Morocco

[4] Laboratory of Functional Ecology and Environmental Engineering, Department of Biology, Faculty of Sciences and Technologies, Sidi Mohamed Ben Abdellah University, Imouzzer Road, P.O. Box 2202, 30 000 Fez, Morocco

[5] Laboratory of Applied Sciences and Sciences of Education and Training, Higher School of Education and Training, Mohammed Premier University, PO Box: 1458, 62 000 Oujda, Morocco

[6] Laboratory of Chemistry and Biology of Membranes and Nano-Objects, Bordeaux University, 146 Rue Léo Saignat CS 61292, 33076 Bordeaux, France

[7] Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture, Trieda Andreja Hlinku 2, 94976 Nitra, Slovakia

[8] School of Medical & Health Sciences, VIZJA University, Okopowa 59, 01043 Warsaw, Poland

[9] Department of Chemistry and Technologies of Drug, Sapienza University, P. Le Aldo Moro, 5, 00185 Rome, Italy