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From Field to Lab: Exploring the Phytochemical Potential of Calabrian Saffron (*Crocus sativus* L.) Biowaste

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Abstract

Saffron (*Crocus sativus* L.) is widely cultivated for the spice obtained from the stigmas, while the remaining floral biomass is discarded as biowaste. Accessing the phytochemical composition of these residues could enable their valorization as a low-cost and sustainable resource for nutraceutical applications. In this context, a quantitative ^{1}H NMR-based metabolite profiling approach, complemented by HPLC-DAD and LC-MS, was employed to comprehensively characterize saffron biowaste. A total of 40 metabolites were identified and quantified by NMR, including amino acids (611.1 ± 36.5 mg/100 g FW), carbohydrates (2801.4 ± 33.7 mg/100 g FW), lipids (702.7 ± 28.2 mg/100 g FW), and saffron-specific compounds such as crocin (596.6 ± 21.5 mg/100 g FW), picrocrocin (1126.3 ± 18.9 mg/100 g FW), safranal (398.4 ± 14.8 mg/100 g FW), and crocetin (13.4 ± 0.4 mg/100 g FW). Targeted fractionation further allowed the identification of kaempferol 3-*O*-sophoroside (15.44 ± 0.61% *w*/*w* in dry ethanolic extract) and 3-hydroxy-γ-butyrolactone. Overall, the results highlight the rich metabolite composition of saffron production waste and support its potential reuse as a valuable source of functional ingredients within a circular economy framework.

Keywords: *Crocus sativus* L.; saffron; circular economy; ^{1}H NMR-based metabolite profiling approach

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1. Introduction

Saffron (*Crocus sativus* L.) is a perennial plant belonging to the Iridaceae family, widely cultivated from the Mediterranean to the Middle East [1]. Global saffron production is estimated at approximately 400–500 tons per year, with Iran accounting for more than

90% of the total production, followed by India, Greece, Spain, and Morocco [2]. In these regions, saffron represents one of the most valuable crops, known as 'Red gold' or 'Golden spice' due to the value of the produced spice [1]. Although saffron is also used in the pharmaceutical and cosmetic industries [3,4], a large portion of global saffron production is still employed in the food sector due to its distinctive organoleptic properties [4]. Crocin and crocetin are responsible for its color, picrocrocin for its bitterness, and safranal for its characteristic aroma [5]; according to the International Standardization Organization (ISO) and the National Nutrient Value of the United States Department of Agriculture (USDA), the concentration of these compounds is closely related to saffron's quality [6].

In addition, phenolic acids and flavonoids contribute to the well-documented antioxidant [7], cytotoxic [8], anti-inflammatory [9], antifungal, and antibacterial [10] activities of saffron extracts.

Saffron spice production involves manually separating the stigma from the rest of the flower. As a result, approximately 90% of the total biomass, including tepals, styles, and other floral tissues, is discarded as agricultural biowaste [11]. Notably, many bioactive compounds have also been reported in the non-edible parts of the plant. Phenolic compounds have been identified in the flowers and leaves, suggesting that these by-products may represent an underexploited source of valuable phytochemicals [12]. In recent years, growing interest in the sustainable reuse of agricultural biowaste has encouraged the development of strategies to recover valuable compounds for nutraceutical and pharmaceutical applications. However, most studies rely on targeted analytical approaches focused on selected classes of compounds, while comprehensive metabolomic characterization of the whole floral waste remains limited.

Cultivation strategies may further influence the phytochemical profile and quality traits of saffron and its residues. In Mediterranean regions, saffron is often grown under low-input or organic farming systems, considered more sustainable and environmentally friendly. Agronomic practices, including soil management, fertilization, irrigation, and harvesting conditions, may significantly influence plant metabolism and, consequently, the accumulation of bioactive compounds in both stigmas and floral by-products [13–15]. Therefore, understanding the biochemical composition of saffron waste materials should also consider the influence of the cultivation environment and management practices.

In Calabria (Southern Italy), saffron cultivation constitutes a niche but growing agricultural activity. According to regional agricultural reports [16], the crop currently covers only a few hectares and is managed by a limited number of small-scale farms operating under low-input or organic conditions, with production mainly directed toward local markets and short supply chains. Given that agronomic practices and environmental conditions may influence plant metabolism and phytochemical composition, the characterization of saffron by-products from this regional context is of particular interest.

Within this framework, the present study focuses on floral waste derived from a saffron cultivation site in Calabria and aims to provide a comprehensive phytochemical profiling of this biomass.

To achieve this goal, we adopted an integrated analytical approach combining quantitative NMR-based metabolite profiling with complementary chromatographic techniques (HPLC and LC-MS). NMR-based metabolite profiling was chosen for its high reproducibility, minimal sample preparation, and non-destructive nature, enabling the simultaneous identification and quantification of a broad range of metabolites in complex plant matrices. This strategy provides an in-depth evaluation of the biochemical potential of saffron biowaste as a sustainable source of functional ingredients.

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2. Materials and Methods

2.1. Sample Preparation According to the Bligh–Dyer Protocol

Fresh samples of saffron production residues, including tepals, styles, and parts of the floral tube, were obtained from a single cultivation site in Lamezia Terme (CZ), Italy (38°58′37.812″ N, 16°17′42.216″ E).

The cultivation was carried out in an open field under conventional management practices, using only organic fertilizer and no additional chemical treatments. Flower harvesting was performed between mid-October and early November, depending on microclimatic conditions that determine optimal humidity for flowering. Flowers were collected early in the morning while still closed in order to preserve product quality. Immediately after harvesting, stigmas were manually separated from the flowers and promptly subjected to drying and collected in November 2024 (Figure 1).

Figure 1. Fresh saffron flowers after manual separation of stigmas. Floral residues (**left**) and freshly collected stigmas (**right**) prior to drying.

For our study, the samples collected in November 2024 were homogenized in a mortar with liquid nitrogen. From the homogenized pool of saffron production residues, three aliquots of circa 0.3 g were weighed. For the analysis, samples were extracted following a modified Bligh–Dyer protocol [17]. Each aliquot was added to 2 mL of cold methanol, followed by 2 mL of cold chloroform and 1 mL of distilled water, with stirring after the addition of each solvent. Samples were then stored at 4 °C for 24 h. A centrifugation step was performed for 30 min at 4 °C with a rotation speed of 8600 g. The upper hydrophilic phase and lower lipophilic phase were then separated and dried under nitrogen flow. Prior to the analysis, the solvent-free residues obtained (dry extracts) were stored at −80 °C. The hydrophilic phase was resuspended in 0.7 mL of D_2O containing 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4, 2 mM), and the lipophilic phase was resuspended in 0.7 mL of $CDCl_3$ containing hexamethyldisiloxane (HMS, 2 mM), as internal and chemical shift reference standards; both samples were later analyzed by ^{1}H-NMR. Deuterium oxide (D_2O, 99.86% D), deuterated chloroform ($CDCl_3$, 99.8% D), 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt (TSP, 99% D), hexamethyldisiloxane (HMDSO), chloroform ($CHCl_3$), and methanol (CH_3OH) were purchased from Sigma (Sigma-Aldrich, Milano, Italy). Standard 5 mm NMR tubes and sealing caps were purchased from Hilgenberg (Hilgenberg GmbH, Malsfeld, Germany).

2.2. Targeted Extraction of Medium-Polarity Compounds

11 g of saffron waste was macerated in 100 mL of ethanol (BioUltra, ≥99.8%, Sigma–Aldrich, Milan, Italy):water (80:20 *v*/*v*) for 48 h in the dark at room temperature. After static maceration, the mixture was filtered and the resulting extract was concentrated under reduced pressure using a rotary evaporator. The concentrate was then frozen and lyophilized to yield a solvent-free residue (820 mg). Liquid–liquid partitioning was performed according to commonly adopted phytochemical fractionation procedures [18,19], with minor modifications. An aliquot (500 mg) of the total extract was dissolved in 100 mL of distilled water and fractionated by liquid–liquid partitioning using solvents of increasing polarity: dichloromethane (ACS reagent, Sigma-Aldrich, Milan, Italy), ethyl acetate (ACS reagent, Sigma-Aldrich, Milan, Italy), and *n*-butanol (ACS reagent, Sigma-Aldrich, Milan, Italy) (BuOH). The dichloromethane and ethyl acetate phases were dried over sodium sulphate, filtered and dried under reduced pressure giving, respectively,12.7 mg and 22.6 mg of waxy residues. The BuOH phase was concentrated under reduced pressure, and then frozen and lyophilized, yielding 106 mg of a violet solid. This was further fractionated by gravity column chromatography using 10 g of silica gel (70–230 mesh; Supelco®, Merck, Darmstadt, Germany) (ratio 1:100 *w*/*w*). The eluting system was a solution of *n*-butanol and distilled water (82:18 *v*/*v*). Four fractions were obtained: FR 8–12 (27 mg), FR 13–18 (9.4 mg), FR 22–36 (1.2 mg), and FR 37–46 (4.8 mg). Only FR 8–12, showing a single spot on TLC (silica gel 60 F_{254} TLC plates on aluminum backing, Supelco, Sigma-Aldrich, Milan, Italy) under UV lamp and after derivatization with H_2SO_4, was further analyzed by 1D and 2D NMR, HPLC-DAD and LC-MS. Kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12 was quantified by quantitative NMR (qNMR).

2.3. NMR Analysis

Qualitative ^{1}H NMR analysis of FR 8–12 was carried out on a Bruker Avance III 400 MHz instrument (Billerica, MA, USA) operating at 9.4 T at 298 K, with chemical shifts expressed in ppm. The chemical shifts were referenced to the internal solvent signal of CD_2HOD (quintet, δH 3.31 ppm). All other NMR experiments were carried out on a JNM-ECZ 600R spectrometer (JEOL Ltd., Tokyo, Japan) operating at a proton frequency of 600 MHz and equipped with a multinuclear z-gradient inverse probe head, working at 298 K. Spectral acquisition and processing were done using DELTA™ NMR Data Processing Software, version 6.1.0 (JEOL Ltd., Tokyo, Japan). For the hydrophilic extract, one-dimensional ^{1}H experiments employed a presaturation pulse sequence for water suppression (Single Pulse Experiment on DELTA), using a time length of 2 s, a spectral width of 9.03 KHz and 64k data points, corresponding to an acquisition time of 5.81 s. The pulse length of 90° flip angle was set to 8.3 µs; the recycle delay was set to 5.72 s. Final recycle delay was set to 7.723 s, 2 s for presaturation time and 5.72 s for measured total relaxation of TSP. The total relaxation delay time was set to 15 s. Similar parameters were employed for the lipophilic phase, without the presaturation sequence for water suppression, while the pulse length of 90° flip angle was set to 8.0 µs. Both aqueous and lipophilic spectra were acquired with 128 scans. Spectral window was set to 15 ppm, with the offset at 4.665 ppm. 0.2 Hz was chosen for Line Broadening Width. Phase correction for the aqueous fraction spectra was done with 0.59 [deg] for Φ_0 and 3.47 [deg] for Φ_1, while for lipophilic spectra it was 0.49 [deg] for Φ_0 and 3.60 [deg] for Φ_1. Akima fit in automatic mode was performed for baseline correction. Spectral assignment for both FR 8–12 and Bligh–Dryer samples was carried out based on chemical shift, multiplicity, *J* couplings, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC correlations, following the protocol reported by Sciubba et al. (2020) [20] with the bidimensional experiments following

spectral parameters in Spinelli et al. [21]. The putative assignments were corroborated by databases and literature compilations [22,23].

Quantification of metabolites was done by comparing integrals of non-overlapping and unambiguously assigned resonances (bold resonances reported in Table S1) with the integral of the respective internal standards (for TSP 9 protons in the aqueous fraction, for HMS 18 protons in the lipophilic fraction), according to the general formula:

$$C_m = \frac{A_m}{A_{IS}} \times \frac{H_{IS}}{H_m} \times C_{IS} \quad (1)$$

C_m stands for metabolite concentration, A_m is the area of the metabolite signal, A_{IS} is the area of the Internal Standard (IS) signal, H_{IS} is the number of protons generating the IS signal, H_m is the number of protons generating the metabolite signal, and C_{IS} is the concentration of the IS. Metabolite concentrations were normalized to the fresh weight (FW) of the homogenized saffron waste and expressed as mg per 100 g FW.

2.4. LC-MS and HPLC-DAD Analyses

LC–MS analyses were performed as previously described [24]. A Vanquish Core HPLC system equipped with an automatic sampler was used for confirmation of the molecular weight of KS. The analysis was performed on a Phenomenex Kinetex F5, 2.6 µm column, 2.1 × 100 mm. The mobile phases used were MeOH with 0.1% formic acid (LC–MS grade, Supelco®, Merck, Darmstadt, Germany) (A, organic phase) and H_2O with 0.1% formic acid (LC–MS grade, Supelco®, Merck, Darmstadt, Germany) (B, aqueous phase). The chromatographic gradient was as follows: initial condition of 30% B phase, then a linear increase to 55% in 3 min, then to 59% in 6 min and to 100% in 7 min. It was maintained at 100% for 2 min (9 min), then decreased from 100% to 30% at 10.5 min and remained at this condition until 12 min. The run took a total of 15 min. The flow rate was 0.25 mL/min, and the column temperature was kept constant at 35 °C. Mass spectrometry analysis was performed using an Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a heated electrospray ionization (HESI) source, operating in negative ionization. The source parameters were as follows: HESI temperature 300 °C, ion transfer tube temperature 280 °C, nebulization voltage of 3.2 kV (-), nebulizer gas 30 a.u., and auxiliary gas 10 a.u. The resolution was set at 120 K and the scan range at 100–1200 m/z.

The amount of KS in the ethanolic extract was assessed by HPLC-DAD performed with a Nexera XR (Shimadzu, Kyoto, Japan) instrument equipped with photo diode array detector (DAD) SPD-M40 model, an autosampler SIL-40C XR, a quaternary pump (LC-40D XR), a degassing unit (DGU-405), a control system CBM-40, a thermostatically controlled oven (CTO-40S) and a chromatographic column Shim-pack Velox C18 (150 mm × 3.00 mm, 2.7 µm). The mobile phases used were H_2O with 0.05% phosphoric acid (phase A) and ACN (LC–MS grade, Sigma-Aldrich, Milan, Italy) with 0.05% phosphoric acid (phase B). The chromatographic gradient was set as follows: the mobile phase was maintained at 5% solvent B for 10 min, then gradually increased to 100% B over 30 min, and held at 100% B for an additional 15 min. The flow rate was 0.5 mL/min, and the column oven temperature was set to 30 °C. The calibration curve of KS was built with its solutions in methanol ranging from 0.0048 to 0.957 mg/mL. 3 µL of each solution (prepared in triplicate) were injected. Peak area of KS at 348 nm (retention time = 16.3 min) was plotted vs. actual concentration. Valuation parameters are reported in Table S4. Three samples of ethanolic extract were dissolved in methanol (about 5 mg/mL) and analyzed by HPLC-DAD. KS amount is expressed as w/w% mean value ± standard deviation from three independently prepared extract replicates.

3. Results

Following the Bligh–Dyer protocol, a total of 40 metabolites were identified and quantitatively determined. The amounts of individual metabolites and their corresponding chemical classes are reported in Table 1. Representative ^{1}H NMR spectra of the hydrophilic and lipophilic phases are shown in Figures S1 and S2, while chemical shift, resonance assignment, and signal multiplicities are reported in Table S1. The identified metabolites were grouped into different chemical classes, including amino acids (alanine, arginine, asparagine, GABA, glutamine, isoleucine, leucine, phenylalanine, threonine, tryptophan, tyrosine, and valine), carbohydrates (glucose, fructose, myo-inositol, polygalacturonic acid, sucrose, and tagatose), lipids (polyunsaturated ω-3 fatty acids (ω-3 FA), polyunsaturated ω-6 fatty acids (ω-6 FA), monounsaturated ω-9 fatty acids (ω-9 FA), saturated fatty acids, and triacylglycerols), organic acids (formic acid, fumaric acid, gluconic acid, and malonic acid), and other metabolites (betaine, choline, crocetin, crocin, dimethylamine, ethanolamine, picrocrocin, safranal, uridine, uridine-XP, and trigonelline).

Table 1. Quantification of each metabolite from the hydrophilic and lipophilic phases by ^{1}H spectrum, reported in mg/100 g of FW of homogenized saffron waste.

Molecule	Amount (mg/100 g)	Molecule	Amount (mg/100 g)
	Crocus sativus L.		*Crocus sativus* L.
Total Amino Acids	611.1 ± 36.5	Total Lipids	702.7 ± 28.2
Alanine	60.8 ± 3.9	Polyunsaturated ω-3 fatty acids (ω-3 FA)	97.2 ± 4.2
Arginine	168.1 ± 8.6	Polyunsaturated ω-6 fatty acids (ω-6 FA)	38.5 ± 7.73
Asparagine	18.0 ± 2.1	Monounsaturated ω-9 fatty acids (ω-9 FA)	497.82 ± 20.13
GABA	29.1 ± 2.5	Total saturated fatty acids	46.33 ± 8.99
Glutamine	128.1 ± 10.5	Triacylglycerols	22.8 ± 1.4
Isoleucine	21.6 ± 0.8	Total Organic Acids	167.9 ± 20.1
Leucine	26.7 ± 0.2	Formic acid	0.5 ± 0.1
Phenylalanine	34.1 ± 2.9	Fumaric acid	5.6 ± 0.8
Threonine	54.6 ± 3.6	Gluconic acid	161.5 ± 20.3
Tryptophan	5.5 ± 0.5	Malonic acid	0.3 ± 0.1
Tyrosine	12.5 ± 2.1	Other Metabolites	2194.1 ± 30.0
Valine	52.1 ± 2.9	Betaine	11.1 ± 1.7
Total Carbohydrates and Polyols	2801.4 ± 33.7	Choline	26.0 ± 1.7
Glucose	1317.4 ± 62.6	Crocetin	13.4 ± 0.4
Fructose	585.1 ± 17.0	Crocin	596.6 ± 21.5
Myo-inositol	259.8 ± 10.6	Dimethylamine	10.5 ± 1.0
Polygalacturonic acid	183.3 ± 7.0	Ethanolamine	2.0 ± 0.0
Sucrose	99.2 ± 12.0	Picrocrocin	1126.3 ± 18.9
Tagatose	356.7 ± 76.7	Safranal	398.4 ± 14.8
		Uridine	4.3 ± 0.6
		Uridine-XP	5.4 ± 0.3
		Trigonelline	1.3 ± 0.1

To obtain a more comprehensive phytochemical profile, an extract of saffron waste was prepared by maceration and subsequently fractionated by liquid–liquid extraction, followed by gravity column chromatography of the BuOH phase. This approach allowed the isolation of a flavonoid, kaempferol 3-*O*-sophoroside (KS), whose structure was elucidated through NMR analysis (Figures S3–S6 for one- and two-dimensional spectra; Figures S7 and S8 for proposed structure and key HMBC correlations; Figures S9 and S10 for key spectral HMBC correlations; Table S2 for resonance assignment) and further confirmed by high-resolution mass spectrometry (HR-MS) which showed the following characteristic peaks: m/z 609 ($[M–H]^-$), consistent with a kaempferol derivative bearing two glucose moieties, m/z 429 ($[M–H–Glc–H_2O]^-$ and m/z 285 ($[M–H–2Glc]^-$.

In addition, 3-hydroxy-γ-butyrolactone was identified in fraction 8–12 in mixture with KS 1:2 (w/w) ratio (Table S3 for resonance assignment of 3-hydroxy-γ-butyrolactone). KS was quantified in the whole ethanolic extract (Figure 2) by HPLC analysis, and it was found to be present at a concentration of 15.44 ± 0.61 mg/100 mg of dry extract (Table S4 for calibration parameters, LOD, and LOQ of kaempfeol-3-*O*-sophoroside by HPLC-DAD analysis).

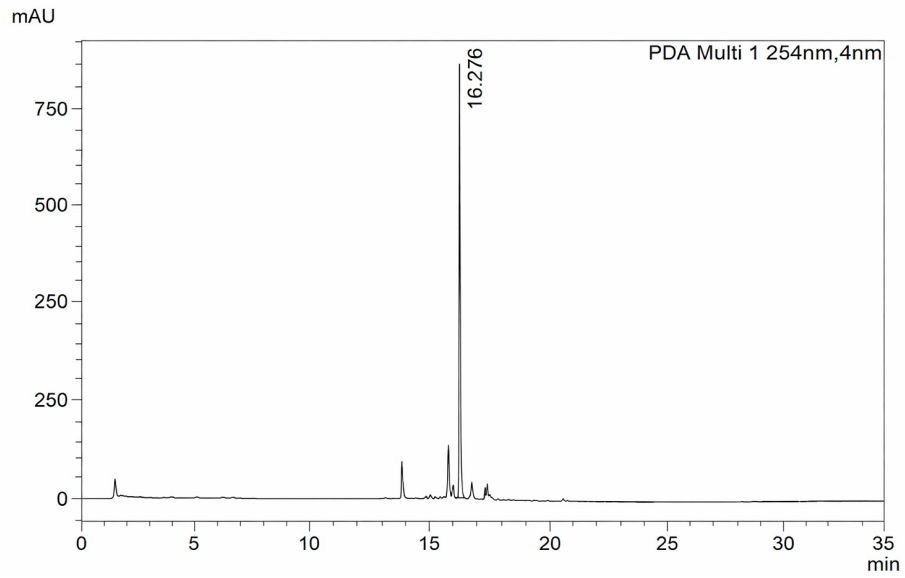

Figure 2. HPLC chromatogram of the ethanolic extract recorded at 254 nm. The peak with a retention time of 16.276 min corresponds to KS.

4. Discussion

In the context of circular economy, the processing and utilization of agricultural biowaste constitute one of its main challenges. Saffron cultivation represents a highly valuable crop in terms of market demand for its spice, but also a low-efficiency crop in terms of biomass utilization. In fact, it is estimated that between 158,000 and 300,000 flowers are needed to obtain 1 kg of spice, with tepals accounting for approximately 350 kg for each kilogram of spice [25]. Previous studies have mainly investigated saffron floral bioresidues, particularly tepals, as valuable sources of bioactive compounds such as phenolic compounds, including flavonoids, crocin, picrocrocin, and safranal [26,27]. Both reviews and experimental works consistently report significant radical-scavenging and antimicrobial activities of these extracts, supporting their potential exploitation as natural ingredients for food preservation, cosmetic formulations, and nutraceutical or pharmaceutical applications. In particular, antioxidant-rich tepal extracts have been proposed for functional foods and cosmeceuticals, while phenolic fractions from leaves and other by-products have shown additional bioactivities relevant to health-promoting applications [9,26,28].

With respect to the different parts of saffron waste, most investigations have adopted targeted approaches, focusing on specific classes of compounds in isolated matrices such as tepals rather than on the comprehensive characterization of the whole floral residue [26]. However, a complete phytochemical profile of the hydrophilic and lipophilic extracts of the whole waste is still lacking. Determining the total content of both known bioactive molecules and metabolites relevant for the development of nutraceuticals and functional ingredients, such as total amino acids, lipids, and carbohydrates, could provide a means for valorizing a biomass that would otherwise have no commercial value.

To date, quantitative data on free amino acids in saffron floral waste are not available. Therefore, only qualitative comparisons can be made with previous studies performed on saffron stigmas [29] in which alanine, proline, and aspartic acid were reported as the predominant free amino acids. This comparison revealed that several amino acids were common to both matrices, including tyrosine, phenylalanine, valine, and alanine. Conversely, serine, glutamic acid, aspartic acid, lysine, histidine, glycine, and proline were not detected in the present waste extracts under the adopted experimental conditions. Quantitative analysis of the waste matrix (Table 1) shows that the total free amino acid content accounts for approximately 9.4% (*w*/*w*) of the metabolites extracted from the whole saffron waste. Interestingly, 31.8% of the total amino acids are essential, including aliphatic ones such as isoleucine, leucine, threonine, and valine, as well as the aromatic amino acids phenylalanine and tryptophan, which are of particular interest for nutraceutical and nutritional applications [30].

Regarding carbohydrate composition, previous compositional studies on saffron floral by-products reported glucose as the predominant soluble sugar, followed by lower amounts of fructose and minor disaccharides and polyols [31]. However, more recent investigations [32] have highlighted that the relative abundance of glucose and fructose may vary among floral tissues and samples, with fructose occasionally exceeding glucose in certain waste fractions, suggesting a condition-dependent distribution of carbohydrates. In the present study the total carbohydrate and polyol content accounts for 43.3% of the whole extract. In particular, glucose represents almost half of the total carbohydrates and polyols, followed by fructose and tagatose (Table 1). The latter stands out as an uncommon carbohydrate, generally occurring in low amounts in nature [33]. It belongs to the class of carbohydrates with a high sweetness index and a low caloric contribution [33]. In addition to its nutritional value, it has also been reported to possess antioxidant activity and prebiotic effects [33]. Gluconic acid, a metabolite derived from the oxidation of the C1 position of glucose, is also present. In foods, it can act both as a flavoring agent, imparting a characteristic sour taste, and as a preservative [34].

Concerning the lipid profile, previous studies on saffron floral by-products mainly reported low total lipid contents [25,31,35] while the fatty acid profile has been described for anthers, which were shown to contain predominantly long-chain unsaturated fatty acids, largely represented by ω-3 and ω-6 species [36].

In the present study, lipids, which accounts for 10.8% of the extracted metabolites analyzed, are mainly composed of ω-9 monounsaturated fatty acids and ω-3 polyunsaturated fatty acids, followed by the saturated fraction and ω-6 polyunsaturated fatty acids. While ω-3 and ω-6 fatty acids are well known as essential fatty acids with established health benefits [33], several health benefits are also emerging for ω-9 fatty acids, particularly related to their anti-inflammatory activity [37].

Furthermore, ^{1}H NMR analysis of the extracts allowed the detection and quantification of the typical saffron metabolites, namely, in decreasing order of abundance, picrocrocin, crocin, safranal, and crocetin. Picrocrocin and crocin were among the most abundant metabolites detected in the analyzed extracts, accounting for 17.4% and 9.2% of the total

extracted metabolites, respectively, followed by safranal (6.2%) and crocetin (0.2%). Available literature data on these metabolites are largely focused on the dry saffron stigmas, employing different extraction methods and techniques. Reported crocin and picrocrocin content from the literature ranges from 6 to 16% and 1 to 13% respectively of saffron dry matter [38]. Studies on southern Tasmanian crops reported picrocrocin content ranges from 7.9% to 16.5%, depending on the harvest time, drying treatments and conditions [39]. Moroccan samples collected from different sites also showed a wide variability, with crocin content ranging from 18 to 37% and picrocrocin from 4.2 to 28.8% [40]. Saffron stigmas from Kashmir, India, have also been reported in the literature, showing 5.5–5.8% crocin, 2.2% crocetin, and 10.1 to 11.2% picrocrocin [41].

In addition, our analysis allowed the quantification of the aglycone moiety of picrocrocin, 4-hydroxysafranal, also known as hydroxy-β-cyclocitral (4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, HTCC), which represents another precursor of safranal. Studies on Greek saffron stigmas reported HTCC content in extracted essential oils ranging from 41.7 to 397 mg/ 100g, depending on the year of harvest and storage conditions [42]. Spanish samples from the Mancha region had higher amounts of HTCC compared to safranal (3.06 mg/g to 1.49 mg/g) [43]. The enzymatic conversion of HTCC to safranal has been reported to occur during storage periods at 4 °C [44].

From our results on the floral saffron residue from the Calabrian cultivar, we thus found comparable quantities, in respect to stigmas, of crocin and picrocrocin and lower content of crocetins.

Picrocrocin is responsible for the distinctive bitter taste and aroma of saffron spice [45]. Safranal, which is its hydrolysis product, is another key compound contributing to complex and characteristic aroma of saffron. Notably, the safranal-to-picrocrocin ratio in saffron depends on storage and drying conditions [46]. From the perspective of food supplement formulation and application, the presence of these metabolites could be valuable for imparting the unique sensory properties typically associated with the spice.

Crocetin and its glycosidic ester crocin are two carotenoids present in saffron with well-known pharmacological and bioactive properties [47]. Both are mainly used as food colorants in industry, particularly crocin, which is more water-soluble [48]. Given its antioxidant properties, crocin is also being investigated for use as a food preservative to inhibit oxidation processes [49].

Therefore, the detection of these characteristic metabolites in saffron floral waste highlights the potential of this by-product as a source of high-value functional and sensory ingredients, supporting its sustainable valorization beyond the spice fraction.

The NMR-based results were complemented by additional analytical investigations carried out on the ethanol extract of the whole matrix, using an approach developed from our previous experience in the field [50]. Maceration was chosen as it occurs at room temperature, thus preserving thermolabile compounds [51], while ethanol was selected for the extraction, since it is considered a green and environmentally friendly option [52].

The ethanolic extract, a highly complex mixture of polar and semi-polar compounds, was fractionated by liquid–liquid partitioning using a series of solvents of increasing polarity. This strategy, routinely employed in natural product isolation, facilitates the selective separation of constituent classes based on their differential solubilities [53].

Liquid–liquid partitioning was carried out with the aim of isolating medium-polarity compounds, such as flavonoids, which are typically not recovered using the Bligh–Dyer extraction method. Within this context, the ethanolic extract revealed kaempferol 3-*O*-sophoroside (KS), one of the main flavonoids present in saffron [54], which has been reported to be more abundant in saffron tepals than in other parts of the plant [49]. Several studies indicate that KS exhibits multiple biological activities, including antioxidant [49],

anti-inflammatory [54], neuroprotective and antidepressant-like effects [55]. These findings highlight the potential of saffron floral waste as a source of functional ingredients for nutraceutical and pharmaceutical applications, especially in the prevention or management of disorders related to oxidative stress, inflammation, and mood regulation.

Furthermore, 3-hydroxy-γ-butyrolactone (3-HBL) was detected in the saffron ethanolic extract. This molecule, previously reported in saffron waste [56], is widely employed in the pharmaceutical sector as a building block for the synthesis of various high-value compounds, including statins, antibiotics, antihyperlipidemic agents, and HIV inhibitors [57]. Recently, it was demonstrated that 3-HBL can be sustainably recovered from saffron floral waste using a green, scalable extraction method, suitable for both laboratory and industrial use [58]. Therefore, this represents a sustainable opportunity to repurpose otherwise wasted biomass into high-value chemical ingredients, aligning green chemistry and circular economy principles.

5. Conclusions

In this study, agricultural waste from *Crocus sativus* L. cultivated in Calabria (Southern Italy) was characterized for the first time. Using a combined untargeted and targeted NMR-based approach, a detailed phytochemical profile was established, enabling the quantification of 40 metabolites, with carbohydrates and polyols representing the most abundant fraction, followed by free amino acids and lipids. Notably, characteristic saffron metabolites such as crocin, picrocrocin and safranal, together with kaempferol-3-*O*-sophoroside and 3-hydroxy-γ-butyrolacton, were also detected in the waste biomass.

Overall, the complex metabolite composition identified highlights the potential of these regional by-products as a sustainable source of functional ingredients for food, nutraceutical, and pharmaceutical applications, supporting the valorization of saffron cultivation within a circular economy framework.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy16040485/s1. Table S1: Spectral assignation of each metabolite extracted from ^{1}H NMR spectrum of *Crocus sativus* L. The signals reported in bold are those chosen for quantification. Figure S1: ^{1}H-assigned spectrum of *Crocus sativus* L. hydrophilic extract. 1. Leucine, 2. Iso-leucine, 3. Valine, 4. 2,3- butanediol, 5. Threonine, 6. Alanine, 7. GABA, 8. Glutamine, 9. Picocrocin, 10. Dimethylamine, 11. Asparagine, 12. Malonic Acid, 13. Ethanolamine, 14. Choline, 15. Myo-inositol, 16. Betaine, 17. Tagatose, 18. Total Fructose, 19. Gluconic Acid, 20. Polygalacturonic Acid, 21. Glucose, 22. Sucrose, 23. Uridine, 24. Uridine-XP, 25. Crocin, 26. Fumaric Acid, 27. Tyrosine, 28. Phenylalanine, 29. Tryptophan, 30. Trigonelline, 31. Formic Acid. Figure S2: ^{1}H-assigned spectrum of *Crocus sativus* L. lipophilic extract. 1. Total Fatty Acids, 2. Omega 6, 3. Omega 3, 4. Safranal, 5. Triacylglycerols, 6. Total Unsaturated Fatty Acids and Omega 9, 7. Crocetin. Figure S3: ^{1}H spectrum of kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12. Figure S4: ^{1}H-^{1}H TOCSY spectrum of kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12. Figure S5: ^{1}H-^{13}C HSQC spectrum of kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12. Figure S6: ^{1}H-^{13}C HMBC spectrum of kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12. Figure S7. Molecule structure of kaempferol 3-*O*-sophoroside, observed in ^{1}H NMR spectrum of FR 8–12. Figure S8: Key HMBC correlation of kaempferol 3-*O*-sophoroside, observed in ^{1}H NMR spectrum of FR 8–12. Figure S9: ^{1}H-^{13}C HMBC spectrum of kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12. Spectral region 3.6–5.6 ppm, correlations G1-3 and G2-G1′. Figure S10: ^{1}H-^{13}C HMBC spectrum of kaempferol 3-*O*-sophoroside (KS) in the fraction FR 8–12. Spectral region 6.5–8.4 ppm, correlations 6′,2′- 2. Table S2: Resonance assignation of kaempferol 3-*O*-sophoroside Table S3: ^{1}H NMR (400 MHz, CD_3OD) of 3-hydroxy-γ-butyrolactone. Table S4: Calibration parameters, LOD, and LOQ of kaempfeol-3-*O*-sophoroside by HPLC-DAD analysis. Note: LOD and LOQ were calculated using the formulas

LOD = 3.3 × σ/S and LOQ = 10 × σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve.

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Abbreviations

The following abbreviations are used in this manuscript:

TSP-d_4	3-(Trimethylsilyl)-propionic-2,2,3,3-d_4 acid sodium salt
HMS	Hexamethyldisiloxane
BuOH	*n*-butanol
KS	Kaempferol 3-*O*-sophoroside
ACN	Acetonitrile
^{1}H-^{1}H TOCSY	^{1}H-^{1}H Total Correlation Spectroscopy
^{1}H-^{13}C HSQC	^{1}H-^{13}C Heteronuclear Single Quantum Correlation Spectroscopy
^{1}H-^{13}C HMBC	^{1}H-^{13}C Heteronuclear Multiple Bond Correlation Spectroscopy
ω-3 FA	Polyunsaturated ω-3 fatty acid
ω-6 FA	Polyunsaturated ω-6 fatty acid
ω-9 FA	Monounsaturated ω-9 fatty acid
3-HBL	3-Hydroxy-γ-butyrolactone

References

1. Ennami, M.; Khouya, K.; Taimourya, H.; Benbya, A.; Kaddi, M.; Khayi, S.; Diria, G.; Abdelwahd, R.; Gaboun, F.; Mentag, R. Genetic Diversity and Population Structure of Saffron (*Crocus sativus* L.) in Morocco Revealed by Sequence-Related Amplified Polymorphism Markers. *Horticulturae* **2025**, *11*, 174. [CrossRef]
2. Valenzuela, J.L.; Díaz, J.G.; Salas-Sanjuán, M.d.C. Improvement in Productivity and Quality of Soilless Saffron Crops by Implementing Fertigation. *AgriEngineering* **2024**, *6*, 1649–1659. [CrossRef]
3. Mir, T.U.G.; Wani, A.K.; Singh, J.; Shukla, S. Therapeutic Application and Toxicity Associated with *Crocus Sativus* (Saffron) and Its Phytochemicals. *Pharmacol. Res.—Mod. Chin. Med.* **2022**, *4*, 100136. [CrossRef]
4. Mzabri, I.; Addi, M.; Berrichi, A. Traditional and Modern Uses of Saffron (*Crocus sativus*). *Cosmetics* **2019**, *6*, 63. [CrossRef]
5. Semeniuc, C.A.; Mandrioli, M.; Urs, M.J.; Gallina Toschi, T. Quality and Authenticity of Saffron and Sensory Aspects. *Int. J. Gastron. Food Sci.* **2024**, *38*, 101067. [CrossRef]
6. Shamshiri, M.; Sánchez, C.; Rico, S.; Mokhtassi-Bidgoli, A.; Ayyari, M.; Rezadoost, H.; Shams-Bakhsh, M. Molecular, Metabolic, and Physiological Responses to Progressive Biotic Stress Caused by Cucumber Mosaic Virus and Turnip Mosaic Virus in Saffron. *Horticulturae* **2025**, *11*, 96. [CrossRef]
7. Maestre-Hernández, A.-B.; Vicente-López, J.-J.; Pérez-Llamas, F.; Candela-Castillo, M.-E.; García-Conesa, M.-T.; Frutos, M.-J.; Cano, A.; Hernández-Ruiz, J.; Arnao, M.B. Antioxidant Activity, Total Phenolic and Flavonoid Contents in Floral Saffron Bio-Residues. *Processes* **2023**, *11*, 1400. [CrossRef]
8. Vago, R.; Trevisani, F.; Vignolini, P.; Vita, C.; Fiorio, F.; Campo, M.; Ieri, F.; Di Marco, F.; Salonia, A.; Romani, A.; et al. Evaluation of Anti-Cancer Potential of Saffron Extracts against Kidney and Bladder Cancer Cells. *Food Biosci.* **2024**, *57*, 103501. [CrossRef]
9. Frusciante, L.; Geminiani, M.; Shabab, B.; Olmastroni, T.; Scavello, G.; Rossi, M.; Mastroeni, P.; Nyong'a, C.N.; Salvini, L.; Lamponi, S.; et al. Exploring the Antioxidant and Anti-Inflammatory Potential of Saffron (*Crocus sativus*) Tepals Extract within the Circular Bioeconomy. *Antioxidants* **2024**, *13*, 1082. [CrossRef]

10. Naim, N.; Fauconnier, M.-L.; Ennahli, N.; Tahiri, A.; Baala, M.; Madani, I.; Ennahli, S.; Lahlali, R. Chemical Composition Profiling and Antifungal Activity of Saffron Petal Extract. *Molecules* **2022**, *27*, 8742. [CrossRef]
11. Moratalla-López, N.; Bagur, M.J.; Lorenzo, C.; Martínez-Navarro, M.E.; Salinas, M.R.; Alonso, G.L. Bioactivity and Bioavailability of the Major Metabolites of *Crocus sativus* L. Flower. *Molecules* **2019**, *24*, 2827. [CrossRef]
12. Serrano-Díaz, J.; Sánchez, A.M.; Martínez-Tomé, M.; Winterhalter, P.; Alonso, G.L. Flavonoid Determination in the Quality Control of Floral Bioresidues from *Crocus sativus* L. *J. Agric. Food Chem.* **2014**, *62*, 3125–3133. [CrossRef]
13. Li, D.; Zuo, W.; Ma, S.; Li, R.; Ye, Z. Metabolomics Characterization of Two Saffron from Iran and China Using GC–MS and LC–MS Methods. *J. Anal. Sci. Technol.* **2024**, *15*, 7. [CrossRef]
14. Treccarichi, S.; Infurna, G.M.; Ciulla, A.; Rossitto, A.; Argento, S.; Fallahi, H.R.; Branca, F. Evaluation of innovative growing techniques for organic saffron production in the Mediterranean countries. *Acta Hortic.* **2022**, *1354*, 57–62. [CrossRef]
15. Salas, M.d.C.; Montero, J.L.; Diaz, J.G.; Berti, F.; Quintero, M.F.; Guzmán, M.; Orsini, F. Defining Optimal Strength of the Nutrient Solution for Soilless Cultivation of Saffron in the Mediterranean. *Agronomy* **2020**, *10*, 1311. [CrossRef]
16. ALSIA. The Production Reality of Officinal Plants in Calabria. Agrifoglio Online, 23 June 2021. Available online: https://www.alsia.it/opencms/opencms/agrifoglio/agrifoglio_online/dettaglio/articolo/La-realta-di-produzione-delle-piante-officinali-in-Calabria/ (accessed on 13 February 2026).
17. Patriarca, A.; Sciubba, F.; Tomassini, A.; Giampaoli, O.; De Rosa, M.; Marini, F.; Aureli, W.; Miccheli, A.; Brasili, E. Pisum Sativum L. 'Eso': Metabolic Profiling of Yellow Seeds to Define the Optimal Harvest Time. *Agriculture* **2024**, *14*, 855. [CrossRef]
18. Nakamura, M.; Ra, J.H.; Jee, Y.; Kim, J.S. Impact of different partitioned solvents on chemical composition and bioavailability of Sasa quelpaertensis Nakai leaf extract. *J. Food Drug Anal.* **2017**, *25*, 316–326. [CrossRef] [PubMed]
19. Otsuka, H. Purification by Solvent Extraction Using Partition Coefficient. In *Natural Products Isolation*; Sarker, S.D., Latif, Z., Gray, A.I., Eds.; Humana Press: Totowa, NJ, USA, 2006; Volume 20, pp. 269–273. [CrossRef]
20. Sciubba, F.; Tomassini, A.; Giorgi, G.; Brasili, E.; Pasqua, G.; Capuani, G.; Aureli, W.; Miccheli, A. NMR-based metabolomic study of purple carrot optimal harvest time for utilization as a source of bioactive compounds. *Appl. Sci.* **2020**, *10*, 8493. [CrossRef]
21. Spinelli, V.; Brasili, E.; Sciubba, F.; Ceci, A.; Giampaoli, O.; Miccheli, A.; Pasqua, G.; Persiani, A.M. Biostimulant Effects of Chaetomium Globosum and Minimedusa Polyspora Culture Filtrates on Cichorium Intybus Plant: Growth Performance and Metabolomic Traits. *Front. Plant Sci.* **2022**, *13*, 879076. [CrossRef]
22. Wishart, D.S.; Sajed, T.; Pin, M.; Poynton, E.F.; Goel, B.; Lee, B.L.; Guo, A.C.; Saha, S.; Sayeeda, Z.; Han, S.; et al. The Natural Products Magnetic Resonance Database (NP-MRD) for 2025. *Nucleic Acids Res.* **2025**, *53*, D700–D708. [CrossRef]
23. Hoch, J.C.; Baskaran, K.; Burr, H.; Chin, J.; Eghbalnia, H.R.; Fujiwara, T.; Gryk, M.R.; Iwata, T.; Kojima, C.; Kurisu, G.; et al. Biological Magnetic Resonance Data Bank. *Nucleic Acids Res.* **2023**, *51*, D368–D376. [CrossRef]
24. Bortolami, M.; La Montagna, D.; Toniolo, C.; Sciubba, F.; Patriarca, A.; Moretti, T.; Serafini, I.; Mura, F.; Cocco, E.; Maděra, P.; et al. Preliminary Insights into the Non-Volatile Constituents of *Commiphora ornifolia* (Balf.f.) J.B.Gillett Oleogum Resin from Socotra Island. *Plants* **2025**, *14*, 2999. [CrossRef] [PubMed]
25. Jadouali, S.M.; Atifi, H.; Mamouni, R.; Majourhat, K.; Bouzoubaa, Z.; Gharby, S. Composition of saffron by-products (*Crocus sativus*) in relation to utilization as animal feed. *Agric. Sci. Dig.-A Res. J.* **2022**, *42*, 475–481. [CrossRef]
26. Lahmass, I.; Lamkami, T.; Delporte, C.; Sikdar, S.; Van Antwerpen, P.; Saalaoui, E.; Megalizzi, V. The Waste of Saffron Crop, a Cheap Source of Bioactive Compounds. *J. Funct. Foods* **2017**, *35*, 341–351. [CrossRef]
27. Hosseini, S.R.; Ghavam, M. Saffron Petal Waste as a Source of Natural Antimicrobials: Comparative Analysis of Extraction Methods. *Discov. Appl. Sci.* **2025**, *7*, 418. [CrossRef]
28. Khadfy, Z.; Atifi, H.; Mamouni, R.; Jadouali, S.M.; Chartier, A.; Nehmé, R.; Karra, Y.; Tahiri, A. Nutraceutical and cosmetic applications of bioactive compounds of Saffron (*Crocus sativus* L.) stigmas and its by-products. *S. Afr. J. Bot.* **2023**, *163*, 250–261. [CrossRef]
29. Del Campo, C.P.; Garde-Cerdán, T.; Sánchez, A.M.; Maggi, L.; Carmona, M.; Alonso, G.L. Determination of free amino acids and ammonium ion in saffron (*Crocus sativus* L.) from different geographical origins. *Food Chem.* **2009**, *114*, 1542–1548. [CrossRef]
30. World Health Organization. *Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation*; World Health Organization: Geneva, Switzerland, 2005.
31. Serrano-Diaz, J.; Sánchez, A.M.; Martinez-Tome, M.; Winterhalter, P.; Alonso, G.L. A contribution to nutritional studies on Crocus sativus flowers and their value as food. *J. Food Compos. Anal.* **2013**, *31*, 101–108. [CrossRef]
32. Cerdá-Bernad, D.; Valero-Cases, E.; Pérez-Llamas, F.; Pastor, J.J.; Frutos, M.J. Underutilized *Crocus Sativus* L. Flowers: A Hidden Source of Sustainable High Value-Added Ingredients. *Plant Foods Hum. Nutr.* **2023**, *78*, 458–466. [CrossRef]
33. De Ortiz, A.C.; Fideles, S.O.M.; Reis, C.H.B.; Pagani, B.T.; Bueno, L.M.M.; Moscatel, M.B.M.; Buchaim, R.L.; Buchaim, D.V. D-Tagatose: A Rare Sugar with Functional Properties and Antimicrobial Potential against Oral Species. *Nutrients* **2024**, *16*, 1943. [CrossRef]
34. D'Angelo, S.; Motti, M.L.; Meccariello, R. ω-3 and ω-6 Polyunsaturated Fatty Acids, Obesity and Cancer. *Nutrients* **2020**, *12*, 2751. [CrossRef]

35. Jadouali, S.M.; Atifi, H.; Mamouni, R.; Majourhat, K.; Bouzoubaâ, Z.; Laknifli, A.; Faouzi, A. Chemical characterization and antioxidant compounds of flower parts of Moroccan crocus sativus L. *J. Saudi Soc. Agric. Sci.* **2019**, *18*, 476–480. [CrossRef]
36. Chichiriccò, G.; Ferrante, C.; Menghini, L.; Recinella, L.; Leone, S.; Chiavaroli, A.; Brunetti, L.; Di Simone, S.; Ronci, M.; Piccone, P.; et al. *Crocus sativus* By-Products as Sources of Bioactive Extracts: Pharmacological and Toxicological Focus on Anthers. *Food Chem. Toxicol.* **2019**, *126*, 7–14. [CrossRef]
37. Farag, M.A.; Gad, M.Z. Omega-9 Fatty Acids: Potential Roles in Inflammation and Cancer Management. *J. Genet. Eng. Biotechnol.* **2022**, *20*, 48. [CrossRef] [PubMed]
38. Samarghandian, S.; Borji, A. Anticarcinogenic Effect of Saffron (*Crocus sativus* L.) and Its Ingredients. *Pharmacogn. Res.* **2014**, *6*, 99–107. [CrossRef] [PubMed]
39. Gregory, M.J.; Menary, R.C.; Davies, N.W. Effect of Drying Temperature and Air Flow on the Production and Retention of Secondary Metabolites in Saffron. *J. Agric. Food Chem.* **2005**, *53*, 5969–5975. [CrossRef]
40. Lage, M.; Cantrell, C.L. Quantification of Saffron (*Crocus sativus* L.) Metabolites Crocins, Picrocrocin and Safranal for Quality Determination of the Spice Grown under Different Environmental Moroccan Conditions. *Sci. Hortic.* **2009**, *121*, 366–373. [CrossRef]
41. Sujata, V.; Ravishankar, G.A.; Venkataraman, L.V. Methods for the Analysis of the Saffron Metabolites Crocin, Crocetins, Picrocrocin and Safranal for the Determination of the Quality of the Spice Using Thin-Layer Chromatography, High-Performance Liquid Chromatography and Gas Chromatography. *J. Chromatogr. A* **1992**, *624*, 497–502. [CrossRef]
42. Kanakis, C.D.; Daferera, D.J.; Tarantilis, P.A.; Polissiou, M.G. Qualitative Determination of Volatile Compounds and Quantitative Evaluation of Safranal and 4-Hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC) in Greek Saffron. *J. Agric. Food Chem.* **2004**, *52*, 4515–4521. [CrossRef] [PubMed]
43. Lozano, P.; Delgado, D.; Gómez, D.; Rubio, M.; Iborra, J.L. A Non-Destructive Method to Determine the Safranal Content of Saffron (*Crocus sativus* L.) by Supercritical Carbon Dioxide Extraction Combined with High-Performance Liquid Chromatography and Gas Chromatography. *J. Biochem. Biophys. Methods* **2000**, *43*, 367–378. [CrossRef]
44. Raina, B.L.; Agarwal, S.G.; Bhatia, A.K.; Gaur, G.S. Changes in Pigments and Volatiles of Saffron (*Crocus sativus* L.) During Processing and Storage. *J. Sci. Food Agric.* **1996**, *71*, 27–32. [CrossRef]
45. Shahi, T.; Assadpour, E.; Jafari, S.M. Main Chemical Compounds and Pharmacological Activities of Stigmas and Tepals of 'Red Gold'; Saffron. *Trends Food Sci. Technol.* **2016**, *58*, 69–78. [CrossRef]
46. Criado-Navarro, I.; Ledesma-Escobar, C.A.; Pérez-Juan, P.; Priego-Capote, F. Distribution of Main Bioactive Compounds from Saffron Species as a Function of Infusion Temperature and Time in an Oil/Water System. *Molecules* **2024**, *29*, 3080. [CrossRef]
47. Zhao, C.; Kam, H.-T.; Chen, Y.; Gong, G.; Hoi, M.P.-M.; Skalicka-Woźniak, K.; Dias, A.C.P.; Lee, S.M.-Y. Crocetin and Its Glycoside Crocin, Two Bioactive Constituents From *Crocus sativus* L. (Saffron), Differentially Inhibit Angiogenesis by Inhibiting Endothelial Cytoskeleton Organization and Cell Migration Through VEGFR2/SRC/FAK and VEGFR2/MEK/ERK Signaling Pathways. *Front. Pharmacol.* **2021**, *12*, 675359. [CrossRef]
48. Ali, A.; Yu, L.; Kousar, S.; Khalid, W.; Maqbool, Z.; Aziz, A.; Arshad, M.S.; Aadil, R.M.; Trif, M.; Riaz, S.; et al. Crocin: Functional Characteristics, Extraction, Food Applications and Efficacy against Brain Related Disorders. *Front. Nutr.* **2022**, *9*, 1009807. [CrossRef]
49. Zhang, Y.; Gong, Y.; Hu, J.; Zhang, L.; Benito, M.J.; Usmanov, D.; Nishanbaev, S.Z.; Song, X.; Zou, L.; Wu, Y. Quercetin and Kaempferol from Saffron Petals Alleviated Hydrogen Peroxide-Induced Oxidative Damage in B16 Cells. *J. Sci. Food Agric.* **2025**, *105*, 967–973. [CrossRef]
50. Toniolo, C.; Patriarca, A.; De Vita, D.; Santi, L.; Sciubba, F. A Comparative Multianalytical Approach to the Characterization of Different Grades of Matcha Tea (*Camellia sinensis* (L.) Kuntze). *Plants* **2025**, *14*, 1631. [CrossRef] [PubMed]
51. Bitwell, C.; Indra, S.S.; Luke, C.; Kakoma, M.K. A Review of Modern and Conventional Extraction Techniques and Their Applications for Extracting Phytochemicals from Plants. *Sci. Afr.* **2023**, *19*, e01585. [CrossRef]
52. Matić, M.; Stupar, A.; Pezo, L.; Đerić Ilić, N.; Mišan, A.; Teslić, N.; Pojić, M.; Mandić, A. Eco-Friendly Extraction: A Green Approach to Maximizing Bioactive Extraction from Pumpkin (*Curcubita moschata* L.). *Food Chem. X* **2024**, *22*, 101290. [CrossRef] [PubMed]
53. Abubakar, A.R.; Haque, M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J. Pharm. Bioallied Sci.* **2020**, *12*, 1. [CrossRef]
54. Kim, T.H.; Ku, S.-K.; Lee, I.-C.; Bae, J.-S. Anti-Inflammatory Effects of Kaempferol-3-O-Sophoroside in Human Endothelial Cells. *Inflamm. Res.* **2012**, *61*, 217–224. [CrossRef]
55. Wang, R.; Hu, X.; Liu, S.; Wang, J.; Xiong, F.; Zhang, X.; Ye, W.; Wang, H. Kaempferol-3-O-Sophoroside (PCS-1) Contributes to Modulation of Depressive-like Behaviour in C57BL/6J Mice by Activating AMPK. *Br. J. Pharmacol.* **2024**, *181*, 1182–1202. [CrossRef]

56. Righi, V.; Parenti, F.; Tugnoli, V.; Schenetti, L.; Mucci, A. Crocus sativus Petals: Waste or Valuable Resource? The Answer of High-Resolution and High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance. *J. Agric. Food Chem.* **2015**, *63*, 8439–8444. [CrossRef] [PubMed]
57. Gao, W.; Li, Y.; Zhu, D. Phenolic Glucosides and a γ-Lactone Glucoside from the Sprouts of *Crocus sativus*. *Planta Medica* **1999**, *65*, 425–427. [CrossRef] [PubMed]
58. Corti, M.; Princiotto, S.; Zuccolo, M.; Beretta, G.; Dallavalle, S.; Pinto, A.; Annunziata, F.; Borgonovo, G. Valorization of Saffron Waste as a Natural Source of 3-Hydroxy-γ-Butyrolactone, a Valuable Chiral Building Block for Pharmaceutical Applications. *Tetrahedron Green Chem* **2025**, *5*, 100069. [CrossRef]