Chapter 37
Crocus sativus L. Flower's Valorization as Sources of Bioactive Compounds

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Abstract The application of circular economy principles is of particular interest to the agricultural and agri-food sectors, given the large amount of waste matrix of some plant species. In recent decades, attention that has been given to the cultivation of saffron (*Crocus sativus* L.) has been rediscovered. The saffron produced from dried stigmas of *Crocus sativus* L. has been known since ancient times for its numerous therapeutic properties. The spice is obtained from the stigmas of the flowers, while petals and stamens are 90% waste material.

The recovery of the flowers, considering the considerable amount of polyphenols with high antioxidant activity present in this matrix (kaempferol and quercetin glycosides), allows its use for innovative purposes in different product sectors, such as foods, cosmetics, and biomedical applications. In this context, this work evaluated that the polyphenol content in flowers of *C. sativus* grown in Tuscanyto

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characterizes this product from a qualitative–quantitative point of view for various product sectors. The quali-quantitative analysis of the extracts was carried out by HPLC/DAD/MS (high performance liquid cromatography coupled with diode array and mass specrometer) analysis. Given the potential of this matrix, another aspect of the research consists of evaluating the possible tumour growth inhibition activity on kidney and bladder cancer cell lines by the extracts of petals.

Keywords Circular economy · HPLC/DAD/MS · Saffron · By products · Biological activity

37.1 Introduction

Saffron (*Crocus sativus* L.) is known throughout the world for the expensive spice obtained from dried stigmas. In addition to its organoleptic properties, saffron spice is known for its therapeutic applications in many diseases, and its potential derives from the antioxidant and anti-inflammatory properties of its components, such as carotenoid pigments and their derivatives (Shahi et al. 2016; Cardone et al. 2020). In recent decades, the cultivation of saffron in Italy has received renewed attention, and total cultivation is increasing. However, saffron is characterized by an enormous amount of manual work, which is the main factor influencing the final cost (Alonso et al. 2012). Given the high cost of saffron spice, interest in possible alternative uses of waste matrices, including flowers and petals, is growing. Compared to stigmas, saffron produces a large amount of biomass given by flower byproducts: based on agronomic measurements, flower byproducts represent approximately 90%. In addition to stigmas, saffron petals have also been shown to have some bioactive compounds (Zeka et al. 2015). Saffron flowers are rich in phenolic compounds, such as kaempferol derivatives, which show interesting antioxidant, antimicrobial, and anti-inflammatory activities. Kaempferol derivatives are flavonols present in different plant species (fruit and vegetables) with antioxidant and anti-inflammatory activity with beneficial effects in reducing the risk of chronic diseases, especially cancer (Chen and Chen 2013; Devi et al. 2015). The pharmacological properties of saffron petals include antibacterial, antispasmodic, immunomodulatory, antitussive, antidepressant, and antinociceptive activities (Shahi et al. 2016; Hosseini et al. 2018).

Considering these results, in this study, we aimed to further characterize saffron byproducts from a phytochemical point of view. In particular, analyses were carried out to evaluate the phenolic content, and given the potential of this matrix, a further aspect of the research was to evaluate the possible activity of tumour growth inhibition on kidney and bladder cancer cell lines by the extracts of the characterized flowers.

37.2 Material and Methods

The plant material was kindly provided by a local farmer from Montalcino (SI) (Azienda Pura Crocus) in 2019 and 2021. The collection of saffron flowers is handmade. The flowers are harvested in October in the early morning, and the stigmas are separated from the other parts of the flower. Saffron flowers were dried at 40 °C, and 300 mg of dried flowers was extracted with 15 mL of 70% ethanol (pH 3.2 for HCOOH) for one night and then filtered to eliminate plant residues.

These extracts were analysed by HPLC/DAD/MS for the determination of phenolic compounds. An authentic standard of safranal was purchased from Sigma-Aldrich (St. Louis, USA), and pOH benzoic acid, kaempferol 3 glucoside, quercetin 3-glucoside, and curcumin were purchased from Extrasynthèse S.A. (Lyon, France). All solvents were of HPLC grade purity (BDH Laboratory Supplies, United Kingdom).

37.2.1 Phytochemical Analysis

37.2.1.1 HPLC/DAD/MS Analysis

The analysis of polyphenols was carried out using an HP 1260 liquid chromatograph equipped with a DAD detector and an API (atmospheric pressure ionization)/electrospray interface (Agilent Technologies, Palo Alto, CA, USA), and polyphenols were separated by using a 250 ∗ 4.6 mm i.d. 5 μm Luna C18 column (Phenomenex) operating at 25 °C. UV/Vis spectra were recorded in the 190–600 nm range, and chromatograms were acquired at 280, 330, 350, and 440 nm. The mobile phase was a two-step linear solvent gradient system, starting from 90% H_2O (adjusted to pH 3.2 by HCOOH) up to 100% CH_3CN during a 40-min period, flow 0.8 mL min^{-1}.

The MS analyses were conducted with the following ESI parameters: nitrogen flow rate 10.5 L/min, drying gas temperature 350 °C; nebulizer pressure, 1811 Torr; and capillary voltage, 3500 V. The experiments were carried out in positive and negative ionization mode.

Quantification of individual compounds was directly performed by HPLC/DAD using a five-point regression curve ($r^2 = 0.998$) in the range 0–30 μg on the basis of authentic standards. In particular, kaempferol and quercetin derivatives were determined at 350 nm using kaempferol 3-glucoside and quercetin 3-glucoside as reference compounds, respectively, and crocetin derivatives were determined at 440 nm using curcumin as a reference compound. In all cases, actual concentrations of the derivatives were calculated after applying, where possible, corrections for differences in molecular weight. The identity of polyphenols was ascertained using data from HPLC/DAD/MS analyses by comparison and combination of their retention times, UV/Vis and MS spectra with those of authentic standards and previously reported data (Vignolini et al. 2008).

37.2.2 Pharmacological Studies

37.2.2.1 Cell Culture

RT4 and RT112 human bladder cancer cells were maintained in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine, and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) at 37 °C with 5% CO_2.

37.2.2.2 MTT Viability Assay

Cultured cell lines (5 × 103 cells/well) were seeded in 96-well plates and incubated for 72 h with serial logarithmic concentrations ranking from to µM of kaempferol or flower extract at 37 °C, 5%. Cell viability was then quantified by 3-(4,5-dimethy lthiazol-2-yl)-2,5-diphenyltetrazolium bromide staining (MTT) (working solution 0.5 µg/mL). After 1 h of incubation, the supernatants were removed, the formazan crystals were dissolved in dimethyl sulphoxide, and the absorbance at 570 nm was measured using a microtiter plate reader.

37.3 Results and Discussion

37.3.1 Saffron Byproduct Characterization

The application of circular economy principles is of particular interest to the agricultural and agri-food sectors, given the large amount of waste matrices. Among these, the rediscovery of saffron cultivation in recent decades has also led to the production of high quantities of waste matrix. This spice is considered the most expensive in the world, but it is obtained only from the stigmas of the plant, and flowers (petals and stamen) represent a percentage greater than 90%. To enhance the waste matrices of saffron, the first step is to analyse and characterize the tissues to evaluate the presence of bioactive compounds. The flower extracts harvested in 2019 and in 2021 were analysed. The characterization of secondary metabolites was performed by HPLC/DAD/MS analysis. This technique allows us to acquire chromatograms at different wavelengths, obtain information on the retention times and UV/Vis spectra of each component present, and compare them with those of similar substances and/or known standards for injection under the same analytical conditions. In Table 37.1, we report the qualitative and quantitative analysis of the extract analysed.

In particular, we confirmed the presence of flavonols (kaempferol and quercetin derivatives), which represented approximately 79%, and crocins (20%); the percentage was comparable in the 2 years, even if the total amount was higher in the 2021 sample. In particular, kaempferol derivatives are the main compounds present,

Table 37.1 Polyphenol content of saffron flowers, years 2019–2021. Data are expressed as mg/g dry sample. Data are the mean of three determinations (standard deviation <5%)

	2019	2021
	mg/g DW	mg/g DW
FLAVONOLS		
k 3 sophoroside 7 glucoside	7.89	8.06
k diglucoside	0.12	0.09
q diglucoside	1.31	1.46
q diglucoside	0.05	0.66
Methyl q diglucoisde	1.54	1.76
k 3 sophoroside	56.40	62.52
k sinapoyl glucoside	1.05	1.47
k glucoside	1.20	1.86
k cumaroylglucoside	0.17	0.28
q cumaroylglucoside	0.03	0.05
Quercetin	0.05	0.03
Kaempferol	1.54	1.66
Myricetin	0.80	0.13
q derivatives	0.98	0.80
k derivatives	1.50	5.37
CROCINS	19.45	23.56
TOTAL	94.08	109.75

q=quercetin, *k*=kaempferol

and kaempferol 3-sophoroside is the principal compound, as reported in a previous work (Vignolini et al. 2008), representing approximately 58% of total polyphenols. Due to the reported data on antioxidant and anti-inflammatory activity with beneficial effects in reducing the risk of chronic diseases, in particular cancer (Chen and Chen 2013; Devi et al. 2015), studies were performed to evaluate the possible tumour growth inhibition activity of flower extracts on the kidney and bladder cancer cell lines.

37.3.2 Effect of Crocus Sativus Flower Extract on Bladder Cancer Cell Viability

The following are the preliminary results of the studies conducted by the Urological Research Institute (URI) on the effect of *Crocus sativus* flower extract on bladder cancer cell viability. The RT4 and RT112 bladder cancer cell lines were used to determine the activity of *Crocus sativus* flower extract. Cells were incubated with scalar concentrations of kaempferol 3-glucoside as a standard and with the extract, and then the cell viability was measured (Fig. 37.1). Kaempferol 3-glucoside did not exert any toxic effect on cells at any of the tested concentrations. On the other hand, the extract was toxic to both cancer cell lines, although to different extents.

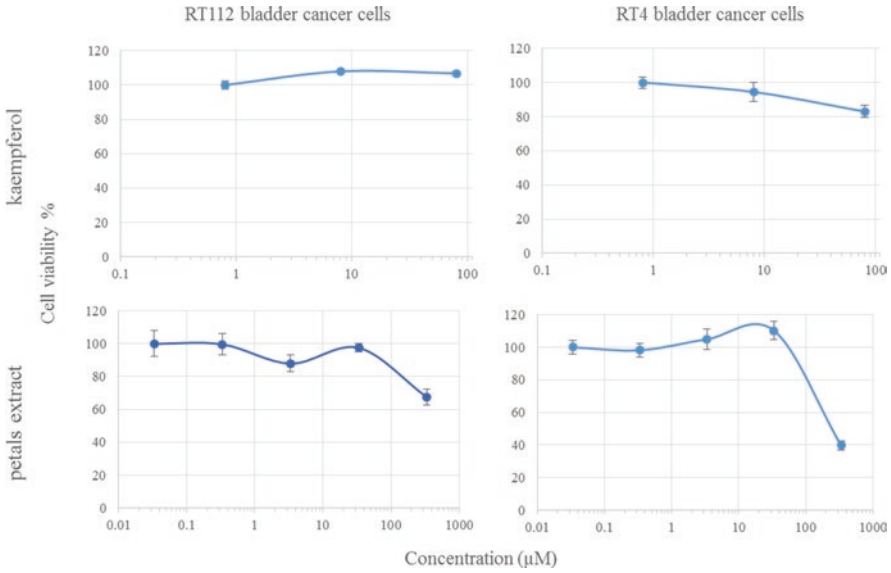

Fig. 37.1 Detection of cell viability of RT112 (left) and RT4 (right) bladder cancer cells incubated with increasing amounts of kaempferol 3-glucoside (top) or *Crocus sativus* petal extract (bottom) for 72 h. Reported values correspond to the mean of cell viability with standard deviation

This result suggests that the activity of the extract is unlikely due to kaempferol 3-glucoside at least alone but to the phytocomplex that contains it together with other flavonol derivatives, in particular kaempferol 3-sophoroside, which is the principal compound of the flower extract.

It is therefore clear that kaempferol derivatives, in particular kaempferol 3-sophoroside, are worthy of further studies to evaluate their activity.

37.4 Conclusions

The valorization of waste and secondary products has always been an important aspect of the agricultural sector, as well as a key element for the development of the circular economy in the agricultural context. Currently, the stigmas of saffron are used not only in the food sector but also in the cosmetic and phytotherapeutic sectors. Considering the tests and preliminary scientific tests, the evaluation of the use of the saffron flowers in the phytotherapeutic field could make it possible to use the entire flower for innovative production. The assessment of the therapeutic functionality of saffron waste tissues will allow an increase in the added value of saffron production itself.

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