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Petals of *Crocus sativus* L. as a potential source of the antioxidants crocin and kaempferol

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Abstract

Saffron from the province of L’Aquila, in the Abruzzo region of Italy, is highly prized and has been awarded a formal recognition by the European Union with EU Protected Designation of Origin (PDO) status. Despite this, the saffron regions are abandoned by the younger generations because the traditional cultivation of saffron (*Crocus sativus* L.) is labour intensive and yields only one crop of valuable saffron stamens per year. Petals of the saffron Crocus have had additional uses in traditional medicine and may add value to the crops for local farmers. This is especially important because the plant only flowers between October and November, and farmers will need to make best use of the flowers harvested in this period. Recently, the petals of *Crocus sativus* L., which are considered a waste material in the production of saffron spice, were identified as a potential source of natural antioxidants.

The antioxidants crocin and kaempferol were purified by flash column chromatography, and identified by thin layer chromatography (TLC), HPLC-DAD, infrared (IR), and nuclear magnetic resonance (¹H & ¹³C NMR) spectroscopy. The antioxidant activity was determined with the ABTS and DPPH test. The antioxidant activities are mainly attributed to carotenoid and flavonoid compounds, notably glycosides of crocin and kaempferol. We found in dried petals 0.6% (w/w) and 12.6 (w/w) of crocin and kaempferol, respectively. Petals of *Crocus sativus* L. have commercial potential as a source for kaempferol and crocetin glycosides, natural compounds with antioxidant activity that are considered to be the active ingredients in saffron-based herbal medicine.

**Key Words:** Antioxidant, Crocin, *Crocus sativus* L., Kaempferol, Petals, Waste.

**Chemical compounds studied in this article:**

Kaempferol (PubChem CID 5280863) Crocin (PubChem CID 5281233)
1. Introduction

The spice saffron consists of the stigmas of *Crocus sativus* L. (Iridaceae). This plant species is cultivated notably in Western Asia, with Iran as the world’s largest producer, but is also of significant economic importance to large parts of Mediterranean Europe. The temperate regions of the Northern hemisphere are characterized by cool to cold winters with autumn-winter-spring precipitation and warm summers with little rainfall [1]. It could be that these territorial and climatic conditions have affected the cultivation and the use of saffron. Nowadays, saffron is appreciated worldwide as a culinary spice. The unique bitter flavour of saffron spice is due to the monoterpene glycoside picrocrocin, whereas its aglycone safranal is the major volatile compound responsible for the aroma. The colour of saffron comes from crocetin and its glucosyl esters, the crocins. The quality of saffron spice is defined by these compounds [2]. In addition, saffron, its extracts and tinctures have been used in traditional and folk medicine. In Ayurvedic medicine, saffron is considered an aphrodisiac and as anti-stress agent [3]. In Iranian’s traditional medicine, ointments containing saffron are used to treat hot palpitation of heart [4]. Scientific studies have confirmed the validity of saffron extracts in the treatment of mild to moderate depression [5, 6], and agent against erectile dysfunction [7], and as anti-arrhythmic agent and restorer of cardiac functions [8, 9]. Other studies showed a role of saffron as a protector against age related macular degeneration [10, 11], in reducing cognitive decline in patients with Alzheimer Disease [12, 13], and as a source for potential anti-cancer agents [14, 15, 16].

In Italy, saffron is cultivated in several areas; this work focuses on the L’Aquila saffron which has been awarded Protected Designation of Origin (PDO) status. PDO covers agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognised know-how. The plain of Navelli, located in the Apennine mountain range at 740 m above sea level, in the province of L’Aquila, Abruzzo region of Italy (Fig. 1)
has traditionally produced highly prized saffron, using the same method since the 13th century, when a Dominican monk of the Santucci family brought bulbs from Spain.

Figure 1: The area of saffron production in the L’Aquila province, Abruzzo region, Italy.

The Navelli method of cultivation requires great expenditure of labour, which consequently leads to an increase of the final price of the product. To obtain 1 kg of the saffron stamens takes more than 500 hours work by a single farmer, and collection of approximately 200,000 flowers. The flower petals are thrown away after removal of the stigmas.

The cultivation cycle of "L'Aquila Saffron PDO" starts in August with plowing of the fields and fertilizing with about 300 kg/ha of matured cow manure, followed by and regular milling, refining, and levelling of the soil surface which serves to make it more porous. This way, series of beds are prepared with grooves of about 30 cm depth, where the bulbs are planted, spaced about 20 cm apart. The beds are separated by rows of about 40 cm width, necessary to allow the passage of farmers without causing damage to plants or bulbs.

In summer, when the plants are in vegetative stasis, C. sativus bulbs are collected from the previous year’s plots and transplanted onto the newly prepared beds. The planting density ranges from 500 to 600 thousand bulbs per hectare. Each bulb gives rise to 5-6 flowers.
maximum. The first rains in September trigger the vegetative cycle defined by the formation of a tuft of filiform leaves: the jets. This technique is used for the production of the "L’Aquila Saffron PDO", but also for the productions in Tuscany, Sardinia and Umbria.

The annual transplanting of the crops from one plot to another, with the use of the same plot only after ten years, seems to be a Navelli characteristic.

The flowering period usually starts in the first fifteen days of October, and lasts approximately six weeks, depending on the weather conditions. Flowers are hand-picked early in the morning, before sunrise when they are still closed, and are placed in traditional wicker baskets before moving to the stage of withering. Stigmas are still collected by a traditional rustic method: most of the time the work takes place in house and must be strictly manual due to the high sensitivity of the crop (Fig. 2). Brought into the home, the flowers are opened, the three stigmas collected, and the petals and other aerial parts of the plant discarded, or added to a compost heap to be used as fertilizer.

The stigmas are placed on a sieve floor put upside down (Fig. 3), hanging in the fireplace as a simple pot and placed in the Navelli’s traditional sieves that are made with horsehair, this

Figure 2: Cottage industry in L'Aquila: manually removing the stamens from the saffron flowers.
Figure 3: A traditional horsehair sieve with fresh stigmas, before roasting, in the house of an old farmer, Navelli, L'Aquila, Italy. Even the sieves are preserved according to the traditional production of the spice.

also seems to be a unique characteristic. The saffron of Navelli, or as the population call it ‘l’oro rosso di Navelli’ (the red gold of Navelli), its method of cultivation, and the historical importance of L’Aquila, the city Navelli village belongs to, are closely connected. Thanks to the method of production “the L’Aquila saffron PDO” is very high in safranal and crocin. The quality of saffron spice was highly appreciated from in medieval times onward and brought great prosperity to the city of L’Aquila which is now well-known for its beautiful Baroque and Renaissance buildings and churches. In 1458, King Ferrante I of Aragon signed the decree that granted the city the right to open its own university.

However, the harvest of saffron spice is limited to one season per year and though production has been optimised over the centuries for local farmers, the financial reward for saffron cultivation is modest. The traditional ways of saffron production may not be economically sustainable as the young population of the Navelli area prefer to work in industry rather than to cultivate saffron.

Petals, which form major part of saffron flowers by weight, are currently considered as waste material though their chemical profile is similar to that of the stamens. Phytochemical analysis has shown that saffron flowers are rich in antioxidant compounds like flavonols,
flavanones, crocins [17] and crocetin [18]. The antioxidants have been considered as the active ingredients responsible for a variety of health enhancing properties that were traditionally attributed to saffron [19].

This study focused mainly in two antioxidant compounds: Crocetin and Kaempferol (Fig. 4).

*Figure 4: Chemical structures of crocetin $R^1=R^2=H$ (1) and kaempferol $R^3=R^4=H$ (2). The petals contain glycosides, the most abundant ones in saffron petals are $R^1=R^2=$gentiobiose and $R^3=H, R^4=$sophorose. However, various other glycosides have been reported.*

Crocetin is considered a safer alternative to treat *all-trans* retinoic acids (ATRA) sensitive cancers in women of childbearing age [20]. Crocin, the gentiobiose diester of crocetin, was tested for treatment of adenocarcinoma. Treated cells exhibited a remarkable loss of cytoplasm and wide cytoplasmic vacuole-like areas. Long-term treatment with crocin selectively enhances survival in female rats with colon cancer without major toxic effects [21].

Crocins form the main pigment of *C. sativus* stigmas, but have also been identified in petals [22]. Since they seem to be the most abundant compounds in saffron stamens, in this study the main aim was to find whether they are also present in other parts of the flower.

Kaempferol (Fig. 2), a well-known flavonol, has been isolated from grapes, strawberries, Brussels sprouts, apples and other fruits and vegetables. Numerous reports have shown that
kaempferol and/or its glycosides induce cell death in a variety of cancer cell lines, derived from different tissues [23]. Plasma membrane potential and membrane fluidity were restored in kaempferol treated cells [24]. However, the introduction of kaempferol has been shown to reduce CDK1 levels in human breast cancer MDA-MB-453 cells [25]. Kaempferol has been shown to reduce the resistance of cancer cells to anti-cancer drugs such as vinblastine and paclitaxel [26]. Furthermore, several reports have shown that kaempferol and its glycosides can slow skin aging by inhibiting a variety of enzymes that degrade the extracellular matrix, such as collagenases, elastases and hyaluronidases [27]. The 7-O-glucopyranoside-3-O-sophoroside, and the 7-O-sophoroside glycosides of kaempferol are known to be present in saffron stigmas [28, 29]. More recently, kaempferol glycosides have also been identified in the petals of C. sativus [30, 31, 32]. Both crocins and kaempferol glycosides are natural plant secondary metabolites with antioxidant activity, and are part of the human diet [27]. They are important in protection of the body against damage caused by reactive oxygen species (ROS) and other free radicals, and are widely assumed to play a role in prevention of cancer and other degenerative diseases. The aim of this study was to see if the petals of C. sativus, like the spice, are also a good potential source of the antioxidants and chemo preventive compounds crocin and kaempferol.

2. Materials and Methods

2.1 Plant material

Whole flowers of saffron obtained from saffron spice production sites were collected on October 2012 from plain of Navelli, a small village near L’Aquila, in Abruzzo region, Italy. Floral petals were obtained after the stigmas were removed from flowers of saffron, according to traditional local procedures. Two hundred grams oven-dried flowers of saffron
were weighed on an analytical balance. Authentic standards of kaempferol and crocin were purchased from Sigma- Aldrich and used to confirm the identity of isolated compounds. DNA bar-coding of PCR-amplified internal transcribed spacer (ITS/ITS2) region was used to confirm the identity of the *Crocus sativus* L. petals.

2.2 Extraction and purification

For isolation and full identification of selected compounds from saffron petals, oven-dried flowers (200 g) with the stigmas removed were milled and left macerate in methanol (4 x 500 ml) for 48 hours at room temperature until complete discoloration of petals. The combined methanolic extracts were then concentrated *in-vacuo* at 30°C to a pale green crude extract (10 g). The crude extract was hydrolysed by reflux in HCl (2 M, 50 ml) at 90°C for 1 h under diminished light and in a nitrogen atmosphere. The reaction mixture was cooled with ice-water (40 ml) and subsequently extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were first washed with water (2 x 50 ml), and then with brine (saturated NaCl solution, 2 x 50 ml), and the organic fraction was dried over anhydrous magnesium sulphate. The methanol extract was split into two equal parts, one for kaempferol purification and one for crocetin purification.

Kaempferol was purified by flash chromatography [Column dimensions: length x ID= 40 x 4.1 cm; stationary phase: Fluka Silica 60; standard 30-45 µm fine grade 20-45 µm; mobile phase: chloroform: acetone: formic acid (75: 16.5: 8.5 v/v/v)]. Crocetin was purified by flash chromatography on a separate column [Column dimensions: length x ID= 40 x 4.1 cm; stationary phase: Fluka Silica 60; standard 30-45 µm fine grade 20-45 µm; mobile phase: ethylacetate: propan-2-ol: bi-distilled water (65: 25: 10 v/v/v)]. For both columns, fifty 10 mL fractions were collected, and analysed by thin layer chromatography (see section 2.3). Fractions with a similar TLC profile were pooled.
2.3 Thin layer chromatography

Thin layer chromatography (TLC) was performed on Merck flexible Aluminium Sheet- Silica Gel 60Å F254 coated plates. Several mobile phases were prepared to look for the ideal one. Reagents and solvents were used as received from Sigma-Aldrich Chemical Company (Dorset, UK) or Alfa Aesar (Lancashire, UK) and Fisher Scientific (Loughborough, UK). The mobile phase for crocin analysis was ethylacetate: propan-2-ol: bi-distilled water (65: 25: 10 v/v/v). The mobile phase for kaempferol analysis was chloroform: acetone: formic acid (75:16.5:8.5 v/v/v). The TLC plates visualised under Multiband UVGL-58 UV-254/366nm UV light and stained with 2, 4-dinitrophenylhydrazine, or iodine absorbed on silica, or phosphomolybdic acid reagent (PMA).

2.4 Spectroscopic analyses

Crocin and kaempferol were further characterised by mass spectroscopy (MS) and nuclear magnetic resonance (1H & 13C NMR). The 1H and 13C-NMR spectra were recorded on a 400MHz Bruker Avance Spectrometer at 30°C, operating at 1H frequency of 400.13 MHz and equipped with Bruker 5mm QNP probe and a 60-position sample changer. Deuterated methanol (99.8 atom % of deuterium) was obtained from Cambridge Isotope Laboratories, Inc (MA 01810 USA).

For each analysis, 256 transients were collected with a spectral width of 20ppm, using a 90°C pulse and inter-pulse delay of 3.95s. The acquisition was controlled by in-house Icon-NMR (Bruker Biospin, Karlsruhe, Germany). Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are reported in δ units relative to the TMS signal and coupling constants (J) expressed in Hertz (Hz). Infrared spectra (IR) were recorded on a Bruker-Alpha
(S/N 101965) 298 Spectrophotometer with absorption expressed in cm⁻¹. Mass spectra and Accurate mass were recorded on a Micromass Quattro II Low Resolution Triple Quadruple Mass Spectrometer (EPSRC National Mass Spectrometry Service Centre, Swansea UK). Mass spectra analysis has been exerted in order to compare the mass spectra of the fractions with the mass spectra of standard crocin and kaempferol. Ultraviolet spectra (UV) were recorded on Thermo Electron Corporation UV spectrophotometer. Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus in open glass capillary tubes.

2.5 Quantification of crocetin and kaempferol
Quantification of both crocetin and kaempferol in petal extracts was done by comparison of areas under the peak with those of accurately weighed authentic reference samples.

Extracts of petals were prepared by maceration of 100 mg of dried and milled petals in 10 mL of methanol for 16 h in the dark at ambient temperature. Following maceration, samples were either diluted by addition of 10 mL distilled water and left on ice, or hydrolysed by addition of 10 mL 10M hydrochloric acid followed by reflux at 90°C for 30 minutes, under diminished light and in a nitrogen atmosphere. Both hydrolysed and non-hydrolysed samples were extracted with ethylacetate (3 x 5 mL). Ethylacetate fractions were dried by addition of magnesium sulphate, and filtered through Whatman Grade 1 fluted filter paper. The filtered extracts were evaporated to dryness under reduced pressure. The dry residues were redissolved in 10 mL of methanol for HPLC analysis.

An Agilent 1100 Series HPLC, equipped with Diode Array Detection was used for the quantification of crocetin and kaempferol. The column used was Hichrom ACE Ultracore 5 Super C18 (150 x 4.6 mm, ID). Flow rate was 1 mL/min, sample size was 5 µL. Spectra were recorded from 190-400 nm.
For analysis of crocetin, the column was eluted isocratically with water: acetonitrile: acetic acid (55:44:1 v/v/v). Absorbance at 350 nm was used for quantification.

For analysis of kaempferol, the column was eluted isocratically with water: acetonitrile: water: acetic acid (71:28:1 v/v/v). Absorbance at 360 nm was used for quantification.

2.6 The DPPH (2, 2-diphenyl-1-picyrylhydrazyl) radical scavenging assay

A 1.0 mL of freshly made methanolic solution of DPPH radical (DPPH•) (100 μΜ) was mixed with tested compound solution at different concentrations. The contents were vigorously mixed, incubated at room temperature in the dark for 20 min and the absorbance was read at 517 nm. In each experiment, the tested compound alone in methanol was used as blank and DPPH• alone in methanol was used as control. The percentage of radical scavenging capacity (RSC) of the tested compounds was calculated according to the following equation [33]:

$$\text{RSC (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}\right] \times 100$$

Where $A_{\text{control}}$ and $A_{\text{sample}}$ are the absorbance values of the control and the test sample respectively. Moreover, IC$_{50}$ value showing the concentration that caused 50% scavenging of DPPH• was calculated from the graph plotted RSC percentage against extract concentration. All experiments were carried out in triplicate and at least on two separate occasions.

2.7 ABTS [2, 2’-azinobis (3-ethylbenzthiazoline-6-acid)] radical scavenging assay

The free radical-scavenging activity of the compounds was determined by ABTS radical cation (ABTS•+) decolorization assay as described by Cano [34] with some modifications. In brief, ABTS•+ was produced by mixing 2 mM ABTS with 30 μM H$_2$O$_2$ and 6 μM horseradish peroxidase (HRP) enzyme in 50 mM phosphate buffered saline (PBS-pH 7.5). Immediately after the addition of the HRP enzyme, the contents were vigorously mixed, incubated at room
temperature in the dark and the reaction was monitored at 730 nm until stable absorbance was obtained. Then, 10 μL of different extract concentrations were added in the reaction mixture and the decrease in absorbance at 730 nm was determined. In each experiment, the tested extract alone containing 1 mM ABTS and 30 μM H₂O₂ in 50 mM PBS-pH 7.5 was used as blank, while the formed ABTS⁺⁺ solution alone with 10 μL H₂O was used as control. The RSC percentage and the IC₅₀ values were determined as described above for the DPPH method. All experiments were carried out in triplicate and at least on two separate occasions.

3. Results

3.1 Extraction and purification

The DNA sequence of the PCR-amplified internal transcribed spacer (ITS/ITS2) region of dried petals was shown to be 97% identical to that of Crocus sativus L. in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/), confirming the identity of our sample. Identification of kaempferol and crocetin isolated from petals was confirmed by HPLC, TLC, NMR, MS, IR and UV spectroscopy. Extracted compounds gave results identical to the authentic standards (For MS, ¹H and ¹³C NMR data for both compounds, see electronic supplementary material). HPLC chromatograms of hydrolysed authentic crocins, and of hydrolysed petal samples showed one single crocin peak, which we assumed to be trans-crocetin [35]. The IR spectra of isolated kaempferol gave identical results compared with authentic standard sample; which confirm that our isolated compound is kaempferol. The isolated kaempferol was measured for melting point in a usual manner and revealed that it had a sharp melting point of 277-278°C compared to melting point 276 -278 °C for standard kaempferol.
3.2 Quantification of crocetin and kaempferol

Compounds extracted from *C. sativus* flowers were purified to a range of 96-98% as determined by HPLC-DAD. Whereas wavelengths of 350 nm and 360 nm were used for quantification of crocin and kaempferol respectively, UV-VIS spectra of samples were recorded over the range 190-400 nm (see section 2.5); purity assessment is based on monitoring the whole recorded range of the HPLC chromatogram. Little or no crocetin or free kaempferol could be detected in petal extracts before hydrolysis (Fig 5). After hydrolysis of the samples, we could detect crocetin at 6.4 mg/g dried petals and kaempferol aglycone at 126 mg/g dried petals. These results indicate that crocetin and kaempferol are present in the petals as glycosides.

The antioxidant activity of methanolic extract, as well as individual constituents isolated from saffron petals, were evaluated using the DPPH and ABTS radical scavenging methods. Both crocetin and kaempferol showed a clear and concentration dependent antioxidant effect (Fig 6).

4. Discussion

This study focused on the potential of *Crocus sativus* petals as a new source of antioxidants with characteristics similar to those found in the spice. The importance of saffron spice stigma for different kind of disease in folk medicine is well known [35]. At the same time, saffron flowers have been discarded or used as fertilizer only. Historically, petals of *C. sativus* were used by rich Roman families for their aromatic pillows, or in Greece for beautification of dresses [37]. However, we could not find reports on uses of petals in traditional medicine. Recent reports though have identified pharmacological activity in extracts of *C. sativus* petals [38, 39, 40, 41, 42, 43, 44]. The goal of our study was to extract and purify antioxidant compounds from saffron flowers, since these are often considered as
active ingredients with a wide pharmacological activity [45, 17]. The attention was concentrated on two compounds from different chemical families: the carotenoid crocins and the flavonol kaempferol. Both compounds are known to occur in *C. sativus* petals mainly as a variety of glycosides, e.g. seven different glycosides of kaempferol [46, 32] and at least six different glycosides of crocetin [30]. We measured the total amount of glycosides in the petal extracts, by quantifying the amounts of aglycone after hydrolysis. The amount of free
aglycone detectable before hydrolysis was negligible, and may even be an artefact caused by plant glycosidases that were activated during handling and cutting the crocus petals when the stamens were removed.

Notably the amount of kaempferol in the petals, 126 mg/g dry weight, is very high. Cruciferous plants, e.g. broccoli, which are considered to be rich in kaempferol only contain in the order of 1 mg/g dry weight [47].

Figure 6: ABTS and DPPH test to verify the antioxidant power of crocin (A) and kaempferol (B).
Another objective of this study was to compare the effectiveness antioxidant activity of the saffron petals. To be totally sure of the strong antioxidant activity of our target compounds, we used two different antioxidant tests: DPPH and ABTS assay. Jointly, the DPPH test and the ABTS assay showed that both compounds that were extracted from the petals have antioxidant activity, and have the ability to prevent the accumulation and/or reduce excessive cellular levels of reactive oxygen species (ROS). Thus, crocin, crocetin and kaempferol, may play a role in protection against diseases induced by oxidative stress [48]. Aqueous and ethanol extracts of saffron petals reduce the mean arterial blood pressure (MABP) in anaesthetised rats [38]. Our study shows that these extracts are rich in kaempferol glycosides.
and crocin. Therefore, we can speculate that it is these antioxidants that are responsible for the observed activity.

Crocin, the main pigment of *C. sativus* has been shown to exhibit antitumor activity against many human tumours, even though the exact mechanisms have yet to be elucidated [49, 50]. The antioxidant effects of crocin are more effective than those of alpha-tocopherol at the same concentration [51]. Crocin was poorly absorbed after oral administration to animals and healthy volunteers [52], and its aglycone crocetin has been demonstrated to be the main metabolite of orally administered crocin in vivo [53]. These results suggest that crocins are hydrolyzed to crocetin before or during absorption and then undergo the metabolic pathway of crocetin.

The planning of flower harvest is a very important moment because the flowering period is very short and flowers lose their properties if they remain exposed in bad weather conditions for a long period of time. Incorrect preservation of petals could lead to loss the active compounds or reduce their antioxidant power. Thus, extraction must be as soon as possible after the stigmas are removed from the flowers. Alternatively, petals can be rapidly dried and subsequently stored in a dark and dry environment. Rapid drying at temperatures of 70-90 °C has been shown to maintain the chemico-physical qualities of anthocyanins and flavonols [32].

Our results confirm that both crocin and kaempferol are predominantly present as glycosides. The glycosides are highly water soluble and may be extracted with water. However, the disadvantage of a water extract is it allows microbial growth, which will lead to a rapid deterioration of crocin and kaempferol glycosides. Methanol is considered an appropriate solvent for crocin (polar) and flavonol glycosides as well as free kaempferol (medium polarity) constituents.
5. Conclusion and future work

These results have shown that the saffron petals can be exploited to create a different conception of *Crocus sativus* L. The *Crocus sativus* petals are a good source of crocin, crocetin and kaempferol. It has been demonstrated that is possible to extract a good quantity of kaempferol from *C. sativus* petals, that is an impressive result considering that kaempferol is roughly 1000 times more expensive than the popular food supplement quercetin, but has better pharmacokinetic properties. These compounds are gaining increasing interest as for their antioxidant activity as a food supplement, in functional foods, beverages drinks, in pharmaceutical preparations and cosmetic formulations. Radical scavenging activity is strongly related to anti aging and correlated with prevention of different types of cancer.

Although numerous papers emphasize the link between antioxidant activity and pharmacological effects, it is recognised now that carotenoids and flavonols may affect the cell cycle through direct interference with cell signalling processes [54]. More investigations are necessary to fully understand the mechanism at the molecular level.

This can have a major impact on the local community of L’Aquila. The cultivation of saffron represents an opportunity to increase the economy, to develop the agricultural sector and to consider now the saffron not only for the spice but also for the rich powerful flowers. Local farmers and scientists may no longer view the *C. sativus* flowers as a waste, but as an important resource for health enhancing products.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.
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