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# Saffron byproducts as sources of bioactive extracts

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## Graphical Abstract

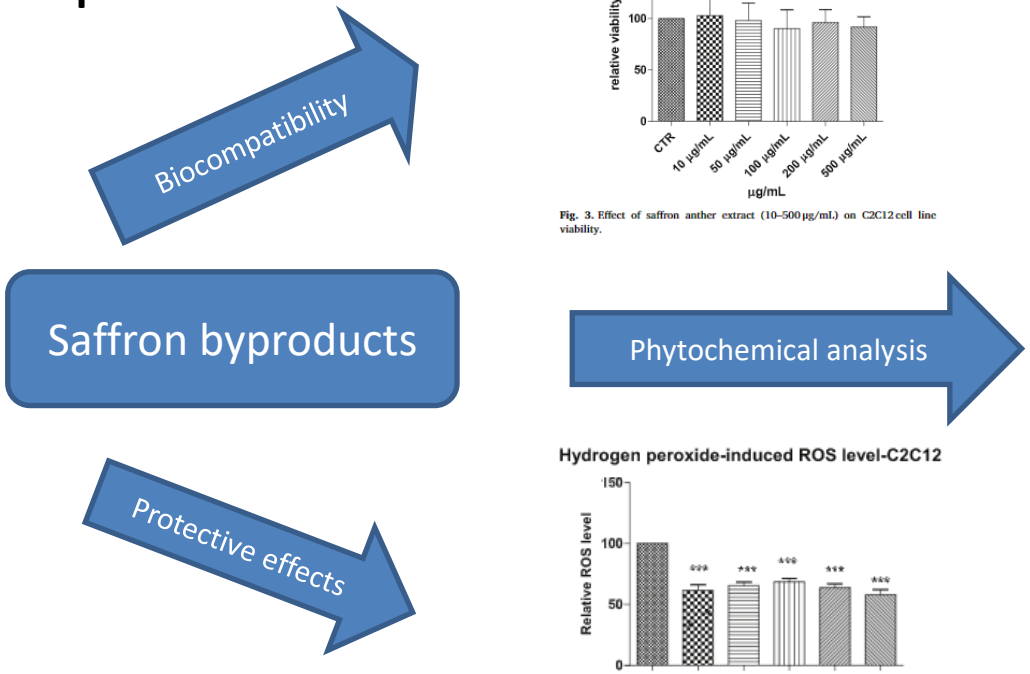





Fig. 3. Effect of saffron anther extract (10–500 µg/mL) on C2C12 cell line viability.




Fig. 7. Effect of saffron anther extract (10–500 µg/mL) on hydrogen peroxide-induced ROS production in C2C12 cell line. ANOVA, $p < 0.0001$, *post hoc* ***$p < 0.001$ *vs* hydrogen peroxide-treated group.

**Table 1**
Percentage fatty acid content in the pollen and anther fraction of saffron crocus.

| Fatty acids | % in pollen | s.d. | % in anther | s.d. | without pollen |
|---|---|---|---|---|---|
| Butyric | C4:0 | 0.44 | 0.31 | 0.63 | 0.01 |
| Capronic | C6:0 | 0.65 | 0.25 | 0.84 | 0.23 |
| Caprylic | C8:0 | 0.03 | 0.01 | 0.07 | 0.01 |
| Caprinic | C10:0 | 0.03 | 0.01 | 0.10 | 0.01 |
| Undecanoic | C11:0 | 0.00 | 0.00 | 0.02 | 0.00 |
| Lauric | C12:0 | 1.32 | 0.23 | 2.97 | 0.11 |
| Tridecanoic | C13:0 | 0.00 | 0.00 | 0.00 | 0.00 |
| Myristic | C14:0 | 0.00 | 0.00 | 0.00 | 0.00 |
| Myristoleic | C14:1 | 0.57 | 0.13 | 1.04 | 0.04 |
| Pentadecanoic | C15:0 | 0.09 | 0.01 | 0.12 | 0.04 |
| Palmitic | C16:0 | 15.09 | 0.69 | 15.13 | 0.33 |
| Palmitoleic ω7 | C16:1 | 0.05 | 0.00 | 0.30 | 0.03 |
| Heptadecanoic | C17:0 | 0.06 | 0.01 | 0.14 | 0.01 |
| Heptadecenoic | C17:1 | 0.03 | 0.01 | 0.26 | 0.30 |
| Stearic | C18:0 | 0.68 | 0.30 | 2.42 | 0.28 |
| Oleic ω9 | C18:1 | 4.66 | 0.13 | 4.26 | 0.15 |
| Vaccenic | C18:1 | 0.64 | 0.02 | 2.03 | 0.08 |
| Linoleic ω6 | C18:2 | 19.16 | 0.21 | 28.04 | 0.48 |
| Arachic | C20:0 | 0.27 | 0.01 | 0.25 | 0.00 |
| Linolenic ω3 | C18:3 | 50.34 | 0.97 | 28.85 | 0.49 |
| Eicosenoic ω9 | C20:1 | 0.92 | 0.13 | 1.54 | 0.59 |
| 11,14-Eicosadienoic | C20:2 | 0.15 | 0.00 | 0.35 | 0.00 |
| Behenic | C22:0 | 0.71 | 0.09 | 0.39 | 0.01 |
| Erucic | C22:1 | 2.73 | 0.06 | 6.09 | 0.22 |
| Lignoceric | C24:0 | 0.18 | 0.00 | 0.67 | 0.25 |
| Eicosapentaenoic | C20:5 | 0.72 | 0.01 | 1.07 | 0.41 |
| Docosapentaenoic | C22:5 | 0.43 | 0.01 | 1.96 | 0.20 |
| Docosahexaenoic | C22:6 | 0.11 | 0.04 | 0.51 | 0.23 |
| Total SFA | | 19.52 | | 23.73 | |
| Total MUFA | | 9.59 | | 15.50 | |
| Total PUFA | | 70.90 | | 60.78 | |
| ω6/ω3 | | 0.38 | | 0.97 | |

**Abstract:** Saffron (*Crocus sativus* L.) has been long described as a protective agent in experimental models of oxidative stress, inflammation and cancer. Multiple studies also revealed the potential application of high quality saffron byproducts as cheap sources of antioxidants. In this context and in collaboration with the Consortium of L'Aquila Saffron, the aim of the present work was to characterize the phytochemical amd antioxidant profile of water extracts from tepals and anthers, that are usually discarded during saffron harvesting. Tepal and anther extracts were equally tolerated by C2C12 cell line, as evidenced by biocompatibility MTT test. In order to further characterize their quality, we analyzed tepal and anther content of heavy metals, finding a more significant tendency to accumulate lead and cadmium in tepals compared to anthers. Considering this, we explored the pharmacological and toxicological potential of anthers, by evaluating genotoxic and protective effects in multiple cell lines and rat tissues challenged with LPS. Particularly, anther extract did not exert cytostatic, cytotoxic and genotoxic effects in Hs27 cells. Anther extract (10-500 µg/mL) was also able to reduce ROS production in MCF7 and C2C12 cell lines. In the same concentration range, anther extract was also effective on isolated rat peripheral and central tissues, challenged with LPS. In this context, LPS-induced levels of nitrites and malonildialdehyde were reduced by anther extract treatment.
Overall, the observed antioxidant effects suggest the valorization of saffron anthers as source of protective agents against the burden of oxidative stress occurring in inflammatory conditions.

**Keywords:** Saffron; Tepals; Anthers; Lipid peroxidation; Inflammation.

## Introduction

Saffron (*Crocus sativus* L.) is well known throughout the world for the expensive saffron spice that is obtained from dried stigmatic lobes of the pistil. In addition to its organoleptic properties, saffron spice is known for its therapeutic applications in many diseases and its potential arises from the antioxidant and anti-inflammatory properties of its components such carotenoid pigments and its derivatives. In the last decades the cultivation of saffron in Italy was subjected to renewed attention and the total cultivation is increasing. Given the high cost of the saffron spice there is growing interest for possible profitable uses of the floral parts such as the tepals and anthers, after the removal of the stigmatic lobes from the saffron flowers. In saffron crocus the anther consists of a wall of 4 monolayered tissues enclosing two techae each containing 2 pollen sacs where pollen develops, which is typically rich in pollenkitt.

A number of biological activities such as antioxidant and metal chelating and high quality byproducts have been reported for saffron flower wastes. Particularly, saffron tepals revealed to be very effective as antioxidant and antimicrobial agents, with promising industrial applications in Pacific white shrimp aquaculture. Additionally, Hosseinzadeh and Younesi (2002) described anti-inflammatory and antinociceptive effects related to saffron petals. Whereas, Moshiri et al (2006) observed clinical efficacy of saffron tepals in the treatment of mild-to-moderate depression. We recently showed the protective effects of high quality byproducts such as tepals and anthers in *in vitro* and *ex vivo* pharmacological models of inflammation and oxidative stress.

Multiple studies also revealed the potential application of high quality byproducts such as spaths, leaves, corms and floral-derived juices as cheap sources of bioactive compounds endowed with antioxidant activity.

Considering these findings, in the present study we aimed to further characterize saffron crocus byproducts from a phytochemical point of view. Particularly, we analyzed the total fatty acids of the anthers, distinguishing those of the pollen from those of the anther devoid of pollen, and the micro-macroelements content of tepals. Furthermore, we explored the pharmacological and toxicological potential of saffron anthers, by evaluating genotoxic and protective effects in multiple cell lines, brine shrimps and isolated rat tissues challenged with lipopolysaccharide (LPS).

## Results and discussion

Table 1 reports the total fatty acids of either the pollen, i.e. those present in the pollen cells plus those in the pollenkitt, or the anther devoid of pollen. The data show a variety of fatty acids in the pollen with high predominance of acids with long chain most of which are unsaturated (80.51%,) so that the ratio between unsaturated and saturated reaches a very high value (4.1). Among the unsaturated there are the omega acids (3,6,7,9) with a predominance of the linolenic acid. This acid well known for its health benefits for the man amounts to 50% in the pollen and this high value never reported so far for pollen enhances the quality of saffron pollen. From the comparison between the total fatty acids of the pollen reported in Table 1 and those that make up only the pollenkitt, it emerges that pollen cells contribute both to reduce the relative percentage of saturated acids and to increase that of polyunsaturated. The wealth of fatty acids can be related to the triploidy of saffron implying a surplus of chromosomes in the sporophyte and gametophyte cells .

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| Total PUFA | | 70.90 | | 60.78 | |
| ω6/ω3 | | 0.38 | | 0.97 | |

## Results and discussion

Table 2 shows the cytokinesis block proliferation index (CBPI), index of evaluation of cell proliferation and therefore of cytostatic and cytotoxic effects it respect to the control. Compared to the control, the CBPI increase for all experimental conditions, therefore there is no significant decrease in the rate of proliferation. In experiments conducted with cytochalasinB, the cytostasis/cytotoxicity can be quantified from the Replication Index (RI): we used the RI index determination to assess cell proliferation from at least 500 cells per culture (Fig 1) and to estimate cytotoxicity by comparing values in the Hs27 treated and control cultures. The RI showed a not significant increase in the presence of 10,100 and 500 µg of anther extracts. So we can assume that anther extract was not cytotoxic for Hs27cultures according to RI values. Fig 2 shows the numbers of Micronuclei (MN)/ 1000 binucleated cells (BNMN). As regards the induction of micronuclei, we observed an increase at the different concentrations of treatment. To this end, we can conclude that, despite an induction of the formation of Micronuclei in Hs27 cell, the extract we have tested did not provide statistically significant results, as regards genotoxic potential.

**Table 2**
Micronuclei at different concentrations of plant extracts. CBPI ((No. of mononuclear cells) + (2 × No. of binuclear cells) + (3 × No. of multinucleated cells)) ×500/(total number of cells). BNMN/1000: micronuclei/1000 cells. Positive control (Ctrl+) was evaluated with colchicine at the concentration of 5 µg/mL.

| | CBPI | BMNM/1000 |
|---|---|---|
| CTRL (-) | 1,11 | 4.33 |
| 10 µg/mL | 1,141 | 10.66 |
| 100 µg/mL | 1,141 | 18.33 |
| 500 µg/mL | 1,18 | 12.33 |
| CTRL (+) | 1,2 | |

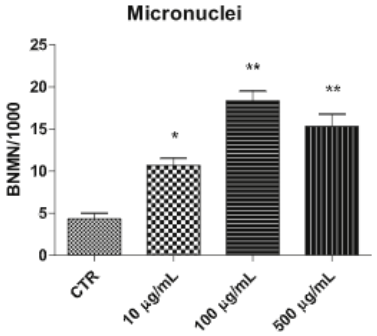


**Fig. 2.** Micronuclei induction in Hs27 at different concentrations of saffron anther extract (10–500 µg/mL). Micronuclei were evaluated *vs* negative control; BNMN/1000: micronuclei/1000 cells. Significance values were determined according to the t-Student: $^{*}p < 0.05$, $^{**}p < 0.005$ vs Control (CTR)-treated group.

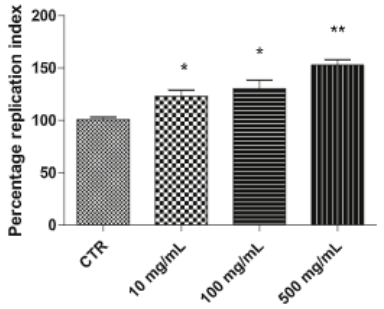


**Fig. 1.** Replication Index in Hs27. Induction of cytotoxicity according to RI in Hs27 cells in both the conditions (control and saffron anther extract at 10, 100 and 500 µg/ml exposure). Significance values were determined according to the t-Student: $^{*}p < 0.05$, $^{**}p < 0.005$ *vs* Control (CTR)-treated group.

**Results and discussion**

Anther extract also revealed to be well tolerated by MCF7 and C2C12 cell lines in the concentration range (10-500 µg/mL), as showed by the results of MTT test (Figures 3-4).

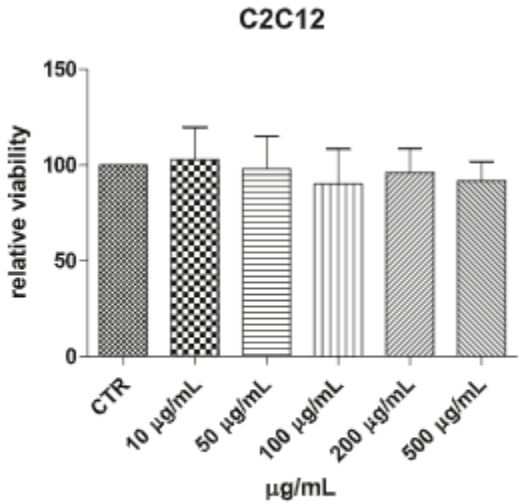


**Fig. 3.** Effect of saffron anther extract (10–500 µg/mL) on C2C12 cell line viability.

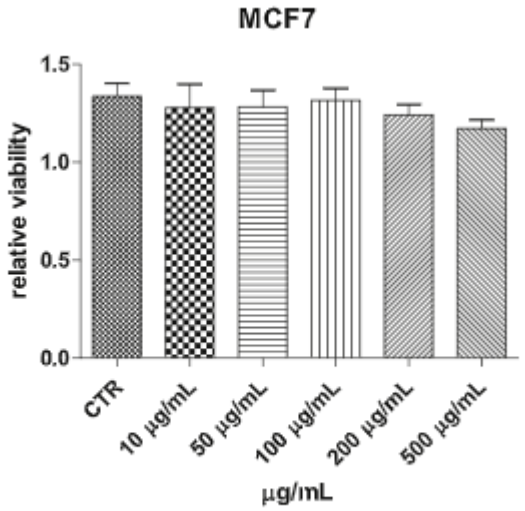


**Fig. 4.** Effect of saffron anther extract (10–500 µg/mL) on MCF7 cell line viability.

## Results and discussion

On the basis of viability test results, we further tested the effects of anther on basal and hydrogen peroxide-induced ROS production, in both C2C12 and MCF7 cell lines. We observed that C2C12 cell lines were more sensitive to the presence of the extract. Particularly, basal ROS production was increased only in C2C12 (Figures 5-6), which is a non-tumoral cell line, while hydrogen peroxide-induced ROS levels were blunted by the extracts in both cell lines (Figures 7-8), despite being the extracts more potent in C2C12 cells.

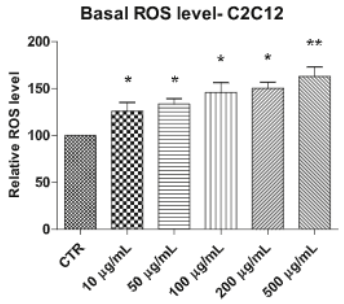


**Fig. 5.** Effect of saffron anther extract (10–500 µg/mL) on basal ROS production in C2C12 cell line. ANOVA, $p < 0.01$, *post hoc* $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* Control (CTR)-treated group.

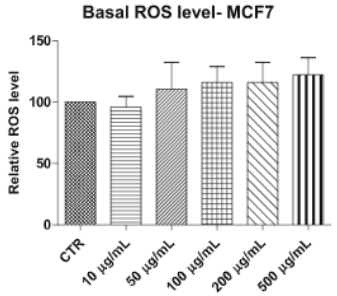


**Fig. 6.** Effect of saffron anther extract (10–500 µg/mL) on basal ROS production in MCF7 cell line.

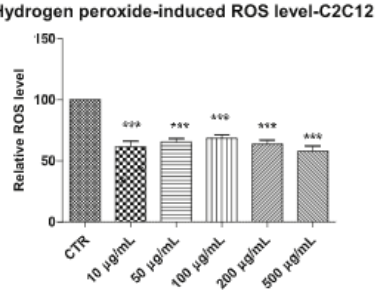


**Fig. 7.** Effect of saffron anther extract (10–500 µg/mL) on hydrogen peroxide-induced ROS production in C2C12 cell line. ANOVA, $p < 0.0001$, *post hoc* $^{***}p < 0.001$ *vs* hydrogen peroxide-treated group.

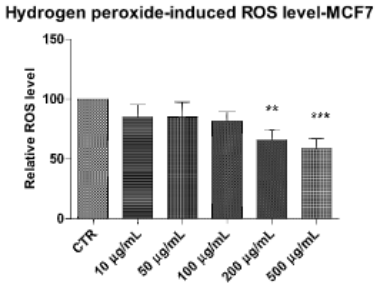


**Fig. 8.** Effect of saffron anther extract (10–500 µg/mL) on hydrogen peroxide-induced ROS production in MCF7 cell line. ANOVA, $p < 0.001$, *post hoc* $^{**}p < 0.001$, $^{***}p < 0.001$ *vs* hydrogen peroxide-treated group.

**Results and discussion**

We also tested anther extracts (125 µg/mL) on ex vivo rat peripheral and central tissues, such as bladder, kidney, stomach, esophagus, lung, prostate, cortex and hypothalamus challenged with LPS, a toxicity model for the evaluation of the efficacy of herbal extracts and drugs involved in inflammatory and oxidative stress modulation. The supplementation of tissue medium with anther extract revealed able to blunt the increased levels of biomarkers such as nitrites in all tested tissues (Figures 9).

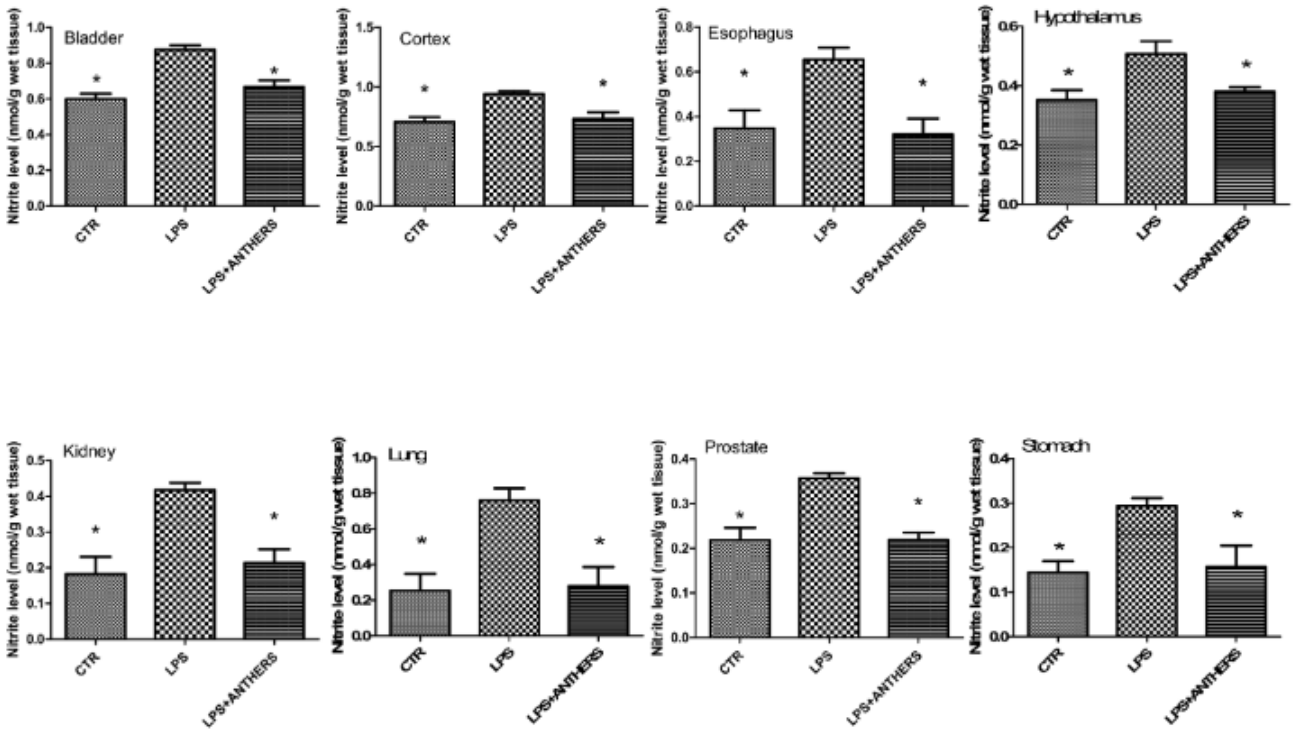


**Fig. 9.** Effect of saffron anther extract (125 µg/mL) on LPS-induced nitrite level (mmol./g wet tissue) in multiple rat tissue specimens (Panel A-H). ANOVA, $p < 0.001$; post-hoc, $^{*}p < 0.01$ vs. LPS.

# Conclusions

The present study emphasizes that saffron flower is a nutritional source with therapeutic potentiality useful for the human diet and human health.
Additionally, the promising results, deriving from the pharmacological and toxicological evaluations, support the valorization of saffron anthers, which are usually discarded, as potential protective agents. In agreement with the accepted principle of “Circular Economy”, our findings further support an intriguing approach to innovatively improve the high quality byproduct fraction with the final goal to optimize and develop of the productive chain of Abruzzo saffron.

## Acknowledgments

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