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Antioxidant activity and cytotoxic effects of polar extracts from saffron (*Crocus sativus* L.) flowers

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ABSTRACT

Saffron is cultivated exclusively to obtain the stigma of its flowers, which represents less than 10% of the flower weight. In fact, to achieve a single kg of dried stigma, more than 60 kg of floral bio-residues are produced. These bio-residues were reported as having high bioactivity and important contents in bioactive compounds. Before considering the use of these bio-residues in different applications, it is important to assess its cytotoxicity. Herein, the antioxidant activity and cytotoxicity of saffron flower polar extracts were evaluated. All samples showed antioxidant activity, despite the higher effectiveness of the hydroalcoholic extract. Up to the concentrations corresponding to the EC_{50} values obtained in the antioxidant activity assays, none of the extracts showed high cytotoxicity against Caco-2 cell lines. Accordingly, saffron flowers might be used in different applications such as the development of food supplements or pharmaceutical related products.

1. INTRODUCTION

Saffron (*Crocus sativus* L.) flower is composed of six purple tepals, three yellow stamens and a white filiform style ending in a stigma with three threads, which represents less than 10% (w/w) of the flower weight. Nevertheless, saffron is cultivated for the stigma, which, after being dried, is the most valued spice [1]. For each kg of this spice, about 63 kg of floral bio-residues are produced, which so far are not exploited, being usually thrown away. The floral bio-residues were reported as having high phenolic content and bioactive properties, justifying the study of its cytotoxic effects [2]. Furthermore, the effectiveness of bioactive compounds extraction from plants, as well as their corresponding activity, is highly dependent on factors such as different types of solvent, solvent-to-solid ratios and specially the solvent polarity [3]. Accordingly, different polar extracts from saffron flower were prepared and their antioxidant activity and cytotoxicity were compared.

2. MATERIALS AND METHODS

Different polar extracts (ethanol, ethanol:water 1:1 v/v, and water) were prepared using a solid to solvent ratio of 1:30. The antioxidant activity was evaluated by: 1) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and calculated as a percentage of DPPH discoloration using the formula: $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution. 2) ferric reducing antioxidant power (FRAP), performed by mixing diluted extracts with the FRAP solution. Absorbance was measured at 595 nm. A calibration curve was prepared with ferrous sulfate and FRAP was expressed as mg of ferrous sulphate equivalents (FSE)/L of extract. 3) inhibition of β-carotene bleaching assay, evaluated through the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, measured as: β-carotene absorbance after 2h of assay/initial absorbance) × 100%. The cytotoxicity of extracts was evaluated in Caco-2 (ATCC® HTB-37™) cultures by using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) [4]. Caco-2 was cultured in DMEM medium supplemented with 10% heat-inactivated foetal bovine serum. Cells were incubated in a humidified atmosphere with 5% CO_2 at 37 ºC. To carry out the experiments, 10 000 cells were seeded in 96-well plates (flat bottom) and allowed to adhere overnight at 37 ºC. For cell treatment, these were incubated at 37 ºC for 1h and 24h in the presence of a variable number of extract concentrations in addition to a negative (medium without extracts) and positive control (Triton X-100, 1%). Absorbance was measured at 570 nm using a Cambrex ELX808 microplate reader (Biotek, Instruments, Inc; Winooski, VT, USA).

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

All samples proved to have antioxidant activity, despite the higher effectiveness of the hydroalcoholic extract. The antioxidant activity showed a concentration-dependent behavior, as it can be deduced from **Figures 1a-b**. The higher activity shown by hydroalcoholic extract is also reflected by the obtained EC_{50} values - DPPH scavenging activity, aqueous: 2.8±0.1 mg/mL; ethanolic: 2.4±0.1 mg/mL; hydroalcoholic: 1.5±0.1 mg/mL; β-carotene bleaching inhibition, aqueous: 2.7±0.1 mg/mL; ethanolic: 1.4±0.1 mg/mL; hydroalcoholic: 1.0±0.1 mg/mL. Likewise, the hydroalcoholic extract showed the highest FRAP: aqueous: 0.85±0.02 mM FSE/g dw; ethanolic: 1.02±0.03 mM FSE/g dw; hydroalcoholic: 1.32±0.02 mM FSE/g dw.

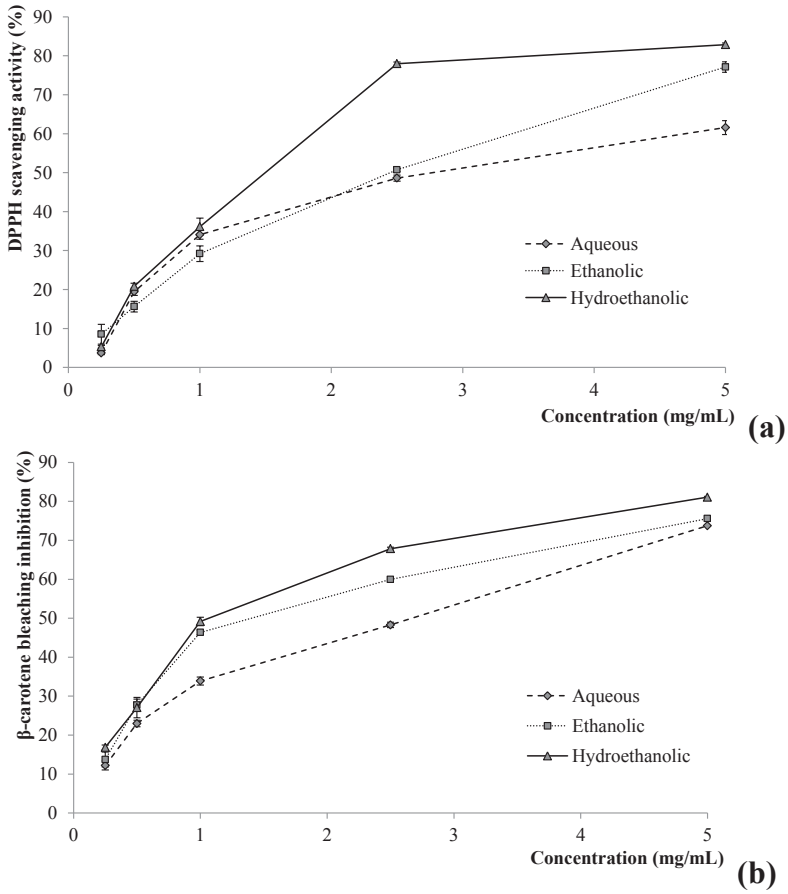
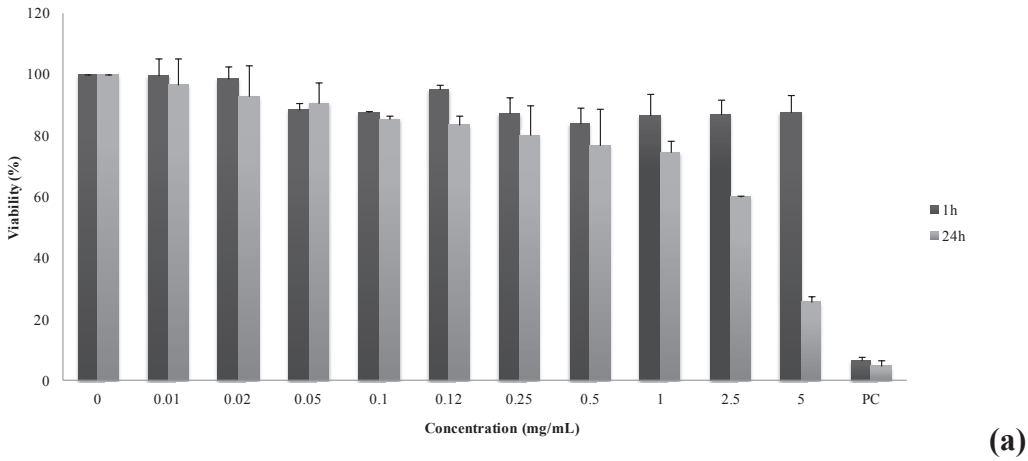


Figure 1. DPPH scavenging activity (a) and β-carotene bleaching inhibition (b).

3.2. Cytotoxicity

Results obtained from MTT assay are represented in **Figure 2a-c**. Saffron extracts induced dose-dependent decreases in viability percentages of Caco-2 cells, particularly pronounced at 24 h treatments (but also observable after 1h of exposure to hydroethanolic and ethanolic extracts). The comparison of the different tested extracts shows that the aqueous one is the less toxic to Caco-2 cells as a decrease in cell viability is observed only after 24h of treatment. The cytotoxicity observed herein is in agreement with previous studies [5], despite being lower when compared to the reported for different cell cultures [6].



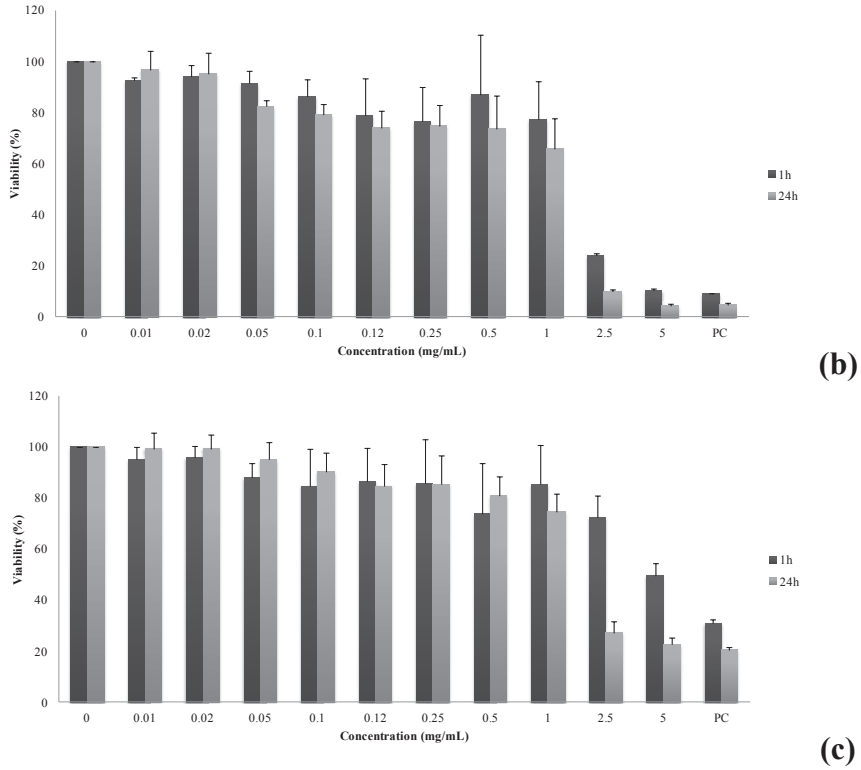


Figure 2. Viability of Caco-2 cells treated with saffron extracts. Results from MTT assay testing (a) aqueous extract, (b) hydroethanolic extract, and (c) ethanolic extract.

4. CONCLUSIONS

The main cytotoxic effects were only observed in the concentrations above the antioxidant activity EC_{50} values. The bioactivity and cytotoxicity were clearly influenced by the solvent polarity. Accordingly, saffron flowers might be used in different applications such as the development of food supplements or pharmaceutical related products.

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