Crocin and Kaempferol, powerful Skin antioxidants, in *Crocus sativus* L. flowers

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Introduction

The skin is the largest organ of the body. Aging of skin is an intricate biological process consisting of two types of events. While intrinsic or chronological aging is an inevitable process, photoaging involves the premature aging of skin due to cumulative exposure to ultraviolet radiations. These exposures induce the production of highly volatile molecules called free radicals, which go on to wreak havoc in the cellular environment of the skin. Herbal drugs have been intensively used since very ancient times in skin care, a complex of beautification techniques that should not be confused with medical therapies.

The saffron crocus (*Crocus sativus* L.) is an autumn-flowering geophyte extensively grown and cultivated in countries such as Italy, Spain, Greece, Turkey, Iran and India. Commercially used saffron is dried stigmas which have been used as a valuable spice and traditional medicine for centuries. Recently, attention has been paid to the identification of natural antioxidants from *Crocus sativus* flowers that are normally wasted [1]. The antioxidant activities are mainly attributed to carotenoid and flavonoid compounds, like crocin and kaempferol. These bioactive compounds have important biological activities, in fact as being antioxidants they are able to trap and neutralize free radicals. Crocin is a diester formed from the disaccharide gentiobiose and the dicarbolic acid crocetin. Crocin, with C.I. Number 75100, is known to inhibit nucleic acid synthesis and cell proliferation [2]. Kaempferol is a natural flavonoid. Numerous reports have shown that kaempferol and/or its glycosides can slow skin aging by contrasting enzymes that degrade the extracellular matrix, such as collagenases, elastases, and hyaluronidases [3].

Methodology

The antioxidants have been extracted from saffron flowers using methanol and ethanol in different concentrations. Isolation and purification have been done by flash column chromatography, and fractions have been analysed by Thin Layer Chromatography (TLC), and compared with authentic standards. Crocin and kaempferol have been further characterised by infrared (IR), mass spectroscopy (MS) and nuclear magnetic resonance (1H & 13C NMR) [1]. In this context, it has been used a new hydrogel, consisting of three polymers - Polyvinyl pyrrolidone (PVP), Agar and Polyethylene glycol (PEG) – and Kaempferol and/or Crocin. All these ingredients have been mixed, reticulated and at the same time sterilized by gamma irradiation at 25 kGy. For the in vitro experimental protocol, it has been used a primary culture of fibroblasts, taken from the subcutaneous tissue of a newborn mice, by seeding the cells on a little square (1cm² area) of both kinds of hydrogel. As the ISO protocol prescribes, the experiments have been repeated 3 times for each kind of hydrogel, stopping the culture at the 3rd, 7th and 14th day after the seeding [4]. For all the steps, three Petri dishes have been used as controls without biomaterials. At each fixed deadline, all the Petri dishes have been colored using the Wright method for cell counting and morphological evaluations.

Results and Conclusions

These preliminary results have shown that the new PVP-hydrogel is biocompatible with the cells and they grow very fast. The microscopic analysis has revealed the complete biocompatibility of the hydrogel at every step of the experiment, and the test has produced very interesting results. Saffron crocin and kaempferol as antioxidants scavenge free radicals and thereby possess the effective properties of preventing aging, fighting wrinkles, eliminating dark spot and acne when used in the formulation of health care and beauty care products, like this new kind of hydrogel [5].

The presence of these important groups of medicinal natural compounds in saffron flowers, up to this moment considered waste, now paves the way towards new biological effects. This study is currently in progress.

Key words

Antioxidant, Crocin, Kaempferol, PVP-hydrogel, Saffron, Skin Aging.
References: