Petals of *Crocus sativus* L. as a Potential source of the antioxidants Crocin and Kaempferol

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Leicester – L’Aquila Research Programme on *Crocus Sativus* Flowers
Navelli plain
L’Aquila province
Abruzzo region
The domesticated saffron crocus, *Crocus sativus*, autumn-flowering perennial plant from a bulb.

Since ancient times, its stigmas have been used as a spice in cooking for its typical taste and flavouring.
Saffron harvest: The stamen are the valuable part of the flowers

.............daily, in the early morning, still closed and just blooming
Hand picking the stamens
separating stigmas from flowers
The stamens are carefully dried in a traditional way

......gathering all the fresh product (only few grams/each family)
The L’Aquila saffron "red gold", is defined by a high safranal and crocin content, a distinctive thread shape, an unusually pungent aroma, and an intense colour; it grows exclusively in Abruzzo region on eight hectares in the “Piana di Navelli”.

in this way stigmas are reduced to few milligrams .......... That’s the reason why the spice is very expensive
The petals are discarded, or at best used as compost.
Health benefits attributed to saffron
Effects of saffron (*Crocus sativus*) petal ethanolic extract on hematology, antibody response, and spleen histology in rats

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Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents

Yang Chen, Hao Zhang*, Xi Tian, Can Zhao, Le Cai, Ying Liu, Lin Jia, Hong-Xiang Yin, Chu Chen

HPLC quantification of major active components from 11 different saffron (*Crocus sativus* L.) sources

Heriberto Caballero-Ortega* a, Rogelio Pereda-Miranda b, Fikrat I. Abdullaev a,*
Some compounds from saffron:
• Crocins
• Kaempferol glycosides
• Saffranal

Are they present in the petals?
Hydrolysis of Kaempferol glycosides
Extraction and Purification
Hydrolysis of glycosides

A. HPLC profile before hydrolysis

B. HPLC profile after hydrolysis
After hydrolysis of the samples, we could detect Crocetin at 6 mg/g dried petals and Kaempferol aglycone at 126 mg/g dried petals.
The amount of kaempferol in the petals, **126 mg/g dry weight**, is very high. 

(Zeka et al. *Fitoterapia* 2015;107:128-34)

Cruciferous plants, e.g. broccoli, which are considered to be rich in kaempferol only contain in the order of 1 mg/g dry weight (Koh *et al.*, 2009).

Cancer

- Major cause of death in the UK: Lung Cancer (males)
  Breast Cancer (females)

- Over 200 different types of cancer reported

- Conventional treatments are often non-selective and have toxic side effects since healthy cells are destroyed as well as cancer cells (hair loss, nausea, vomiting, sterility etc.)

Cancer Therapy Goal:
To design and prepare drugs to selectively kill cancer cells whilst sparing normal cells
Cytochrome P450 CYP1-enzymes

- Cytochrome P450 are a family of enzymes that catalyse the oxidation of a xenobiotic compounds (e.g. drugs, pollutants)

- Human CYP1 family: CYP1A1(extra hepatic tissues), CYP1A2 (liver) & CYP1B1

- One sub-family CYP1-enzymes, CYP1B1, are over-expressed in a wide variety of human tumours but not detected in corresponding normal tissues.

This tumour-specific enzyme provides a target for activation within tumour of anticancer agents to toxic drugs that destroy cancer cells leaving normal cells unharmed.
CYP1B1 expression in human cancers and normal tissues

<table>
<thead>
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<th>TISSUE</th>
<th>NORMAL (NO. POSITIVE/NO. TESTED)</th>
<th>TUMOUR (NO. POSITIVE/NO. TESTED)</th>
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<tbody>
<tr>
<td>Bladder</td>
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<tr>
<td>Brain</td>
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</tr>
<tr>
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<tr>
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<td>11/11</td>
</tr>
<tr>
<td>Liver</td>
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<td>Not tested</td>
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<tr>
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<td>Skin</td>
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<tr>
<td>Small intestine</td>
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<tr>
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<tr>
<td>Total</td>
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</tr>
</tbody>
</table>

Potter et al demonstrated dietary phytoestrogen resveratrol (natural product) conversion to piceatannol, a natural plant metabolite with anticancer and antileukaemic properties, by CYP1B1.
Representation of action of tumour specific CYP1B1

Figure 2. Representation of the action of CYP1B1 enzyme in cancer cell

- Normal Cells (no CYP1B1)
- Prodrug
- Cancer Cell (with CYP1B1)
- CYP1B1
- Cytotoxicity
Cytotoxicity (*in-vitro*)

Human breast cell line model developed at De Montfort University: (Dr Paul Butler)

- MDA-MB-468, an aggressive breast cancer cells that constitutively expresses CYP1B1, and resistant to current chemotherapeutic agents like methotrexate, 5-flurouracil

- MCF10A, a non-tumour ‘normal’ breast cells with no CYP1-enzyme expression

- MCF7, breast cancer cells with inducible CYP1A1 expression by 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD)
- **Non-toxic prodrug**
- **Specific activation** by cytochrome P450 CYP1 enzymes over-expressed in cancer
- **Differential toxicity** between CYP1 expressing tumour cells versus non-expressing normal cells
Future studies

1. Further investigate our knowledge of the petal extract e.g. other useful compounds?

2. Investigate cheaper extraction method?
Bologna

Heraklion

Leicester

L’Aquila

3rd Annual International Conference on Pharmaceutical Sciences, 2-5 May 2016, Athens, Greece
De Montfort University, UK
Design & Isolation in-vitro screening & Analytical
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EPSRC National Mass Spectrometry Service Centre Swansea University
Leicester city
Multi-cultural, vibrant city
Collaborations?

Thank you