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**Original Article**

# Comparison of Extraction and Measurement of Quercetin from Stigma, Style, Sepals, Petals and Stamen of *Crocus Sativus* by HPLC in Combination with Heat and Ultrasonic

**Kamkar Jaimand*, Mohammad Bagher Rezaee, Zahra Behrad and Akbar Najafy-Ashtiany**

*Department of Medicinal Plants, Research Institute of Forests and Rangelands, Tehran, Iran*

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## Abstract

Flavonoids, are a large class of polyphenols, with more than 4000 combinations. They have an antioxidant role in plant photosynthesis and in the human body they optimally act as an antioxidant, anti-inflammatory, anticancer and protector of the heart. Quercetin is in the flavonol group that is used to fight protection specifically against viruses and cancer cells. In this study, Comparison of extraction and measurement of quercetin in combination with heat and Ultrasonic from Stigma, style, sepals, petals and stamen, of *Crocus sativus* L. by HPLC method was carried out. Samples were collected in last October 2012 from experiment farms, in a research station in Hamand Abesard in the east of Tehran, Iran. Various methods of extraction with the heat and Ultrasonic were tested using different organs (viz. Stigma, style, sepals, petals and stamen). A total of eight samples were obtained for measurement of quercetin by high-performance liquid chromatography (HPLC). In stigma with heat were 0.0856%, and with Ultrasonic were 0.0356%, in style with heat Soxhlet were 0.001%, and with Ultrasonic were 0.009%, in sepals and petals with heat were 0.409%, and with Ultrasonic were 0.307%, and in stamen with heat were 0.009%, and with Ultrasonic were 0.005%.

**Key words**: *Crocus sativus* L., Quercetin, Ultrasonic, HPLC.

## Introduction

Saffron, the dried stigmas of *Crocus sativus* L. (Iridaceae), is a very expensive spice, and is used as a herbal medicine, for food coloring and as a flavoring agent in different parts of the world [1]. Saffron originally grew in Iran, India, Europe and other countries, and it has been successfully cultivated in different countries, including Iran. The most important Iranian production areas are Ghaen and Birjand (Iran). For saffron, the flowers are cultivated to produce the stigmas. After harvesting, the flowers are subjected to a delicate treatment which will give the saffron spice. The stigmas are picked by hand and dried. This procedure is performed the same day of harvest. One of the most traditional procedures is the separation of petals from stigmas. A large amount of petals is discarded for obtaining a small amount of stigmas. The production process involves a large amount of manual work and cannot be completely mechanized.

Saffron cultivation is in 70,043 hectares in Iran. Saffron production of over 200 tons of saffron. Sepals and petals production rate of about 3000 - 2500 tons of waste, which is mainly to be discarded [2]. In many cultures, saffron is used as a spice and for culinary purposes; however, it has many medicinal uses as well. Saffron, which has for decades been the world's most expensive spice by weight, is native to Southwest Asia. Saffron is characterized by a bitter taste and an iodoform- or hay-like fragrance; these are caused by the chemicals picrocrocin and safranal. It also contains a carotenoid dye, crocin that gives food a rich golden-yellow hue. These traits make saffron a much-sought ingredient in many foods worldwide. Saffron also has medicinal applications. Pharmacological studies have revealed that saffron extract has antitumor, radical scavenging properties [3,4], as well as antinociceptive, anti-

*Corresponding author: Department of Medicinal Plants, Research Institute of Forests and Rangelands, Tehran, Iran
E-mail Address: Jaimand@rifr-ac.ir

inflammatory [5], anticonvulsant [6], and antidepressant effects [7,8]. The main aroma factor in saffron is safranal, which comprises about 60% of the volatile components of the saffron [3]. The investigations demonstrate that saffron and its active constituents like safranal have antitumor, antioxidant and antigenotoxic effects [3,5]. Organic sunscreens are generally aromatic compounds conjugated with a carbonyl group [9]. In our previous study, because of the advantages of saffron besides having many aromatic and flavonoid compounds such as kaempherol and quercetin, the SPFs of the lotions containing ground saffron were evaluated and established [10]. Quercetin, a flavonol, is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It also may be used as an ingredient in supplements, beverages or foods Quercetin is a flavonoid widely distributed in nature. The name has been used since 1857, and is derived from quercetum (oak forest), after Quercus. [11,12] It is a naturally-occurring polar auxin transport inhibitor The American Cancer Society, says quercetin "has been promoted as being effective against a wide variety of diseases, including cancer [13]. Several laboratory studies show quercetin may have anti-inflammatory properties, [14,15] and it is being investigated for a wide range of potential health benefits[16, 17].

## Materials and Methods

Sample preparation

Sepals, stamens and dried stigma samples were collected in last October 2012 from experiment farms, in a research station in Hamand Abesard in the east of Tehran, Iran, (52° 5′ 35″ N and 35° 40′ 60″ E; alt. 1960 m). The samples (1g), from stigma, style, sepals, petals and stamen, of *Crocus sativus* L. were powdered and passed through a No.40 mesh sieve. Then samples were extracted with Soxhlete apparatus with 80 ml of methanol. After extraction, the samples were filtered to eliminate plant residues, and the filtrate evaporated to dryness under vacuum at room temperature. All samples kept at volume 30 ml. Ultrasonic Baths which are popularly known as Sonicator Baths or Sonicator, Sample (1g) was extracted with methanol, the extractions were performed in a ultrasonic bath (Branson the Heat System (USA) with a working frequency of 33 KHz. material extraction procedure was repeated three times. This solution was filtered by a 0.45 µm filter and 20 µL of it was injected into the HPLC system.

HPLC condition

Analysis for quercetin was carried out using a KNUAR liquid chromatograph with WellChrom 2000, with pump model Maxi-star K-1000 and spectrophotometer K-2500, spectra were recorded in the 290 nm range. Column used was Erospher 100 $C_{18}$ (250×4 mm, 5 µm). The mobile phase used for separation and determination of analytes was mixture of methanol, water and acetic acid (50:45:5) during a 20-min period; flow rate 1mL min-1.

Preparation of standards and calibration curve

Authentic standards of Quercetin dehydrate (with scientific name 3,3′,4′,5,6-Pentahydroxyflavonel) with formula of $C_{15}H_{10}O_7 . 2H_2O$, with mass 338.27 with 25 gr , were purchased from Fluka (St. Louis, USA), All solvents were of HPLC grade purity.

Quercetin combined with the preparation of the standard curve was determined as follows. Quercetin combination with different concentrations of the calibration curve for the seven standard concentration ppm 150, 300, 450, 750, 1050, 1500 and 2100 were prepared and then injected into the device.

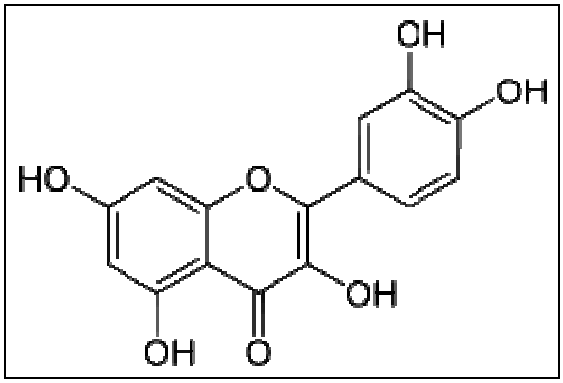


**Fig 1** Chemical structure of quercetin

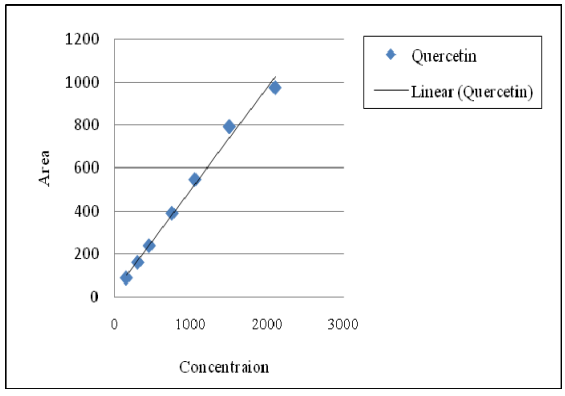


**Fig 2** Calibration curve of quercetin standard by microgram concentrations.

**Table 1** Measurement of quercetin in combination with heat and ultrasonic from *Crocus sativus* L. by HPLC method.

| Plant part | Quercetin concentration (%) | |
|---|---|---|
| | Soxhlete | Ultrasonic |
| Stigma | 0.086% | 0.036% |
| Style | 0.001% | 0.009% |
| Sepals and petals | 0.409% | 0.307% |
| Stamen | 0.009% | 0.005% |

## Results and Discussion

Saffron, the dried stigmas of *Crocus sativus* L. (Iridaceae), is a very expensive spice, and is used as a herbal medicine, for food coloring and as a flavoring agent in different parts of the world. Karimi, *et al*. 2010, had carried out studies on evaluation of *Crocus sativus* L. stigma phenolic and flavonoid compounds and its antioxidant activity, results show the free radical scavenging activity of methanolic saffron stigma extract was stronger than that of the boiling water extract, followed by the ethanol one[18]. Flavonoid compounds, which are widely found as secondary metabolites in plants, are important due to their ability to serve as antioxidants [19]. Many phenolic compounds have been reported to possess potent antioxidant activity and anti-cancer, anti-carcinogenic, anti-bacterial, antiviral or anti-inflammatory activities in a greater or lesser extent [20-23]. Flavonoids, which are found commonly in the flowering tissues and pollens are an important part of the diet because of their effects on human nutrition [24,25]. The most important function of flavonoids is their antioxidant activity, as they have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals [26,27].

The purpose of this paper is the analysis of flavonoid contents of *Crocus sativus* L. in different organs stigma style, sepals, petals and stamen extracts cultivated in Iran in order to characterize this commercial saffron from a quality point of view. In these areas, cultivation is effected under natural conditions and without the use of any chemical product in the drying and conservation phases.

Results on the flavonoid contents of *Crocus sativus* L. in different organs stigma style, sepals, petals and stamen extracts obtained are presented in Table 1. A total of eight samples were obtained for measurement of quercetin by high-performance liquid chromatography (HPLC). In stigma with heat were 0.0856%, and with Ultrasonic were 0.0356%, in style with heat Soxhlete apparatus were 0.001%, and with Ultrasonic were 0.009%, in sepals and petals with heat were 0.409%, and with Ultrasonic were 0.307%, and in stamen with heat were 0.009%, and with Ultrasonic were 0.005%.

Quercetin is available in supplement form in doses ranging from 50 to 500 mg. However, some people question how well quercetin is absorbed by the human body when taken orally. It is believed to have antioxidant, anti-inflammatory, and antiallergic properties. Quercetin appears to reduce production of inflammatory mediators, such as leukotrienes and histamine [28]. Quercetin is available in sepals and petals of *Crocus sativus* L. which extracted by Soxhlete apparatus with 80 ml of methanol were 0.409% that can be used in medicine.

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