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**RESEARCH ARTICLE**

# Volatile compositions of three critically endangered and endemic species of the genus *Crocus* L. (Iridiaceae) and comparison with *C. sativus* L. (Saffron)

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**Abstract**

This study was carried out upon chemical researches of three species *Crocus* L. that distribute in Central Anatolia (Eskişehir), Turkey. The volatile compounds were obtained by microdistillation of the stylus, stigma-tepal parts of three critically endangered and endemic species of the genus *Crocus* L., viz. *C. chrysanthus* (Herb.) Herb., *C. antalyensis* B. Mathew and *C. ancyrensis* (Herb.) Maw, and the stylus, stigma parts of *C. sativus* L. and the volatiles were analyzed by GC-FID and GC-MS, simultaneously. Ethyl cinnamate (14.8%), heptanal (14%), and hexahydrofarnesyl acetone (12.8%) of *C. chrysanthus*. Hexanal (17.7%), nonanal (17.1%), and undecanal (14.7%) of *C. antalyensis*. *β*-Isophorone (14.4%), heptanal (11.5%), and heneicosane (8.5%) of *C. ancyrensis*. Safranal (77.9%), *α*-isophorone (13.5%), and *β*-isophorone (2.2%) were detected as main constituents in sample of *C. sativus.* In addition, chemical structures of *C. ancyrensis*, *C. chrysanthus* and *C. antalyensis* are given in this study for the first time.

**Keywords**: Iridaceae, *Crocus*, Saffron, Endemic, GC-FID, GC-MS, Microdistillation

## Introduction

Turkey is a rich country regarding the species occurrence of *Crocus* L. (Iridaceae). *Crocus* is distributed mainly in the Mediterranean region and includes 80 species worldwide (Mathew, 1984). There are 70 taxa (including subsp. and var.) of *Crocus* in Turkey (Güner et al 2000). Thirty-one of these are endemics for Turkey (Erol, 2011). Many species of the family Iridaceae are grown in parks and gardens as ornamental plants due to their beautiful flowers (Baytop, 1999).

The known chemical components of *C. sativus* can be listed as follows. Crosetins: crosin-1, crosin-2, crosin-3, crosin-4, crocetin, protocrosin, picrosin, safranal Flavonoids: camphorol, astragalin, helicrisoside, crosatoside A, camphorol 3-O-*β*-D-glucopyranosyl (1→2) *β*-D-glucopyranoside, camphorol-3-O-*β*-D-glucopyranosyl (1→2) *β*-D-6-acetylglucopyranoside, quercetin-3-O-*β*-D-glucopyranoside, isorhamnetin-3-O-*β*-D-glucopyranoside Pigments: carthamin, precaharthamine, safflor yellow A, B, *β*-carotene, zeaxanthin, lycopene Phenolic compounds: chlorogenic acid, caffeic acid, catechol, crosatoside B, 3,8-dihydroxy-1-methylantraquinone-2-carboxylic acid Triterpenes: ursolic acid, oleanolic acid, β-sitosterol, campesterol, stigmasterol Amino acids: 3,4-dihydroxyphenylalanine, proline, asparagine, arginine, glutamine, glutamic acid Organic acids: palmitic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid Other components: mangicrosine, nonacosane ( Bensky et al. 2004). Saffron's yellow-orange color crosin, sharp taste picrocrosin, aroma comes from safranal (Gruenwald et al. 2007).

Some *Crocus* species were used for medicaments making dye and perfume ((Abdullaev, 2003). The saffron (*Crocus sativus* L.) was the first to be cultivated and has been grown for economic purposes since ancient

times (Abdullaev, 2003) pointed out that the saffron could be useful in cancer chemoprevention in the immediate future. (Özdemir et al. 2006).

The *Crocus* is regarded as an ornamental plant as they grow like tulipa and flower in different colours. Because of these properties the *Crocus* species can sustain their life forms when they are cultivated in parks and gardens. It is estimated that the *Crocus* which affects people positively with their lovely flowers is going to be of an important economic value in the near future. Some studies reported that the *Crocus* species have antitumor, antimutagenic, cytotoxic activities and inhibits nucleic acid synthesis in human (Kravkaz et al. 2006; Fatehi et al. 2003).

The species known as endemic *C. ancyrensis* (Herb.) Maw "Ankara Çiğdemi", *C. chrysanthus* (Herb.) Herb., "Sarı Çiğdem", endemic *C. antalyensis* B. Mathew "Antalya Çiğdemi" and *C. sativus* L. "Safran, Çiğdem" in Anatolia (Guner et al. 2012).

The aim of this study to investigate chemical characteristics of *C. ancyrensis, C. chrysanthus, C. antalyensis* and *C. sativus*. In addition, volatile compositions of *C. ancyrensis*, *C. chrysanthus* and *C. antalyensis* are given in this study for the first time.

## Materials and Methods

### Plant material

Plant materials were collected from different localities in Eskişehir, Turkey and they were identified as herbarium materials (Table 1). Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE) in Eskişehir, Turkey.

Table 1. Information on the plant materials.

| Species | Collection date | Collection site | Voucher specimens no (ESSE) |
|---|---|---|---|
| *C. ancyrensis* | 10.03.2013 | B3:Eskişehir: Bozdağ, 39° 56' 32'' K - 030° 30' 54'' D, 1079 m | 14623 |
| *C. chrysanthus* | 10.03.2013 | B3:Eskişehir: Hekimdağ,39°54'34''K-030° 33'13''D, 1289 m | 14625 |
| *C. antalyensis* | 10.03.2013 | B3:Eskişehir: Bozdağ,39°56'32''K-030° 30'54''D, 1079 m | 14624 |
| *C. sativus* | 20.042013 | Eskişehir Geçit Kuşağı Tarımsal Araştırma Enstitüsü (TAGEM) | 15406 |

### Isolation of volatile components

#### *Microdistillation*

The volatiles were obtained after microdistillation of the plant material (650 mg) using an Eppendorf MicroDistiller® containing 10 mL of distilled water per sample vial. The sample vial was heated to 108 °C at a rate of 20 °C/min for 90 min followed by heating at 112 °C at the rate of 20 °C/min for 30 min. The sample was subjected to a final post-run for 2 min under the same conditions. The collecting vial, containing a solution of NaCl (2.5 g) and water (0.5 mL) *n*-hexane (350 µL) to trap volatile components, which were cooled to -5°C during distillation. Thereafter, the organic layer in the collection vial was separated and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) simultaneously.

***GC-FID and GC-MS analyses***

Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses processes were performed with reference to Demirci et al., (2017).

## Results and Discussion

In this study, the stylus-tepal parts of *C. chrysanthus*, *C. antalyensis*, *C. ancyrensis* and the stigma parts of *C. sativus* were distilled using Eppendorf Microdistiller® and analyzed by GC-FID and GC-MS. Ethyl cinnamate (14.8%), heptanal (14%), hexahydrofarnesyl acetone (12.8%) were detected as main constituents in sample of *C. chrysanthus*, hexanal (17.7%), nonanal (17.1%), undecanal (14.7%) of *C. antalyensis*, *β*-Isophorone (14.4%), heptanal (11.5%), heneicosane (8.5%) of *C. ancyrensis.* In the oil of *C. sativus* safranal (77.9%) *α*-isophorone (13.5%), and *β*-isophorone (2.2%) were detected as main constituents (Table 2).

There is a lot of works available in the literature related volatile and non-volatile components of the *Crocus sativus*. In previous work, volatile and colour compounds of *Crocus sativus* stigmas were obtained by microdistillation and extraction techniques, respectively. The samples were analyzed by GC-FID, GC-MS, HPLC systems (Başer et al. 2007). Previously, safranal, α-isophorone and β-isophorone were reported as the main components for *C. sativus* flowers by microdistillation method (Başer et al. 2007). Caballero-Ortega et al. have investigated and compared active compounds with the HPLC technique on 11 *C. sativus* samples obtained from different sites and sources (Caballero-Ortega et al. 2007). Zheng et al. have reported on chemical composition determination studies using the GC-MS method in their study of different parts of *C. sativus* such as stigma, stamen, and periant (Zheng et al. 2011). Maggi et al. conducted chemical composition studies on 418 *C. sativus* samples obtained from various sources around the world and found that saffron-specific aromatic properties originate from the safranal named compound in their research and comparison with the volatile compounds they obtained with the GC-MS technique (Maggi et al. 2009). Zhu et al. have comparatively examined the specimens in their study of the volatile oil composition they made on the *C. sativus* corm extract and stigma sections (Zhu et al.2008). Esmaeili et al. have identified phenolic compounds of *C. sativus* by GC-MS method (Esmaeili et al 2011). In a study by Campo et al. the content of picrocrosin on 345 *C. sativus* samples obtained from various sources was comparatively determined (Campo et al. 2010). Masuda et al. performed comparative volatile compound analysis on the corms in their study with *C. sativus* and *C. vernus* (Masuda et al.2012). Esmaeilian et al. have reported on chemical composition determination with GC-MS on *C. sativus* stigmas obtained at different harvest times. Esmaeili et al. conducted studies on antioxidant activity and identification of phenolic compounds on *C. sativus* (Esmaeilian et al. 2012). Goupy et al. have done studies to identify flavonol, anthocyanin, luteindiesters on *C. sativus* tepals (Goupy et al. 2013). Norbaek et al. were found in *C. chrysanthus* flowers in the anthocyanidins by HPLC method. Obtained anthocyanins are: 3-O- (6-O-malonyl-β-D-glucosyl) -7-O- (6-O-malonyl-O-malonyl- β-D-glucoside) -7-O- (6-O-malonyl-β-D-glucoside). Obtained camphorol, quercetin and myricetin flavonoids from *C. chrysanthus* periant segments and nine flavonol glycosides in their study on *C. antalyensis* flowers (Norbaek et al. 1998-1999). Norbaek et al. anthocyanins such as delfinidine, petudine and petunidine in the *C. antalyensis* periant segments (Norbaek et al. 1999).

To the best of our knowledge, this is the first report describing the volatile compositions of *C. ancyrensis, C. chrysanthus* and *C. antalyensis*. In this study these compounds are encountered in the samples and it is thought that the samples may be alternative sources to *C. sativus*.

Table 2. The volatile composition of the *Crocus* species.

| No | RRI | Compounds | *C.ancyrensis* % | *C. chrysanthus* % | *C. antalyensis* % | *C. sativus* % |
|---|---|---|---|---|---|---|
| 1 | 1093 | Hexanal | -- | -- | 17.7 | -- |
| 2 | 1194 | Heptanal | 11.5 | 14.0 | -- | -- |
| 3 | 1400 | Nonanal | 8.2 | -- | 17.1 | -- |
| 4 | 1400 | Tetradecane | -- | 5.6 | -- | -- |
| 5 | 1415 | β-Isophorone | 14.4 | 4.8 | -- | 2.2 |
| 6 | 1503 | 2,6,6-Trimethyl-1,4-Cyclohexadiene-1-Carboxaldehyde | -- | -- | -- | 1.9 |
| 7 | 1548 | (*E*)-2-Nonenal | 5.2 | -- | 7.1 | -- |
| 8 | 1600 | α-Isophorone | 5.8 | 4.1 | -- | 13.5 |
| 9 | 1617 | Undecanal | -- | -- | 14.7 | -- |
| 10 | 1661 | Safranal | 2.3 | 5.3 | -- | 77.9 |
| 11 | 1700 | Heptadecane | -- | 1.5 | -- | -- |
| 12 | 1714 | 6-Oxoisophorone | -- | 2.5 | -- | 1.2 |
| 13 | 1719 | Borneol | -- | tr | -- | -- |
| 14 | 1793 | Dihidro-4- Oxoisophorone | -- | -- | -- | 0.3 |
| 15 | 1800 | Octadecane | 2.4 | 2.8 | 1.8 | -- |
| 16 | 1900 | Nonadecane | 4.2 | 3.0 | 5.7 | -- |
| 17 | 1958 | (*E*)-β- Ionone | -- | -- | -- | 0.5 |
| 18 | 1960 | 4-(2,2,6-trimethylcyclohexan -1-yl)-3-buten-2-one | -- | -- | -- | 0.9 |
| 19 | 2000 | Eicosane | -- | -- | 9.2 | -- |
| 20 | 2100 | Heneicosane | 8.5 | 6.4 | -- | -- |
| 21 | 2131 | Hexahydrofarnesyl acetone | -- | 12.8 | 9.9 | -- |
| 22 | 2157 | (*E*)-Ethyl cinnamate | -- | 14.8 | -- | -- |
| 23 | 2174 | Fokienol | -- | 1.4 | -- | -- |
| 24 | 2300 | Tricosane | 3.4 | 3.1 | -- | -- |
| 25 | 2400 | Tetracosane | -- | 2.8 | -- | -- |
| 26 | 2500 | Pentacosane | -- | 4.2 | -- | -- |
| 27 | 2700 | Hexacosane | -- | 6.8 | -- | -- |
| | | Total | 65.9 | 95.9 | 83.2 | 98.4 |

RRI: Relative retention indices calculated against *n*-alkanes. % Calculated from FID data. Tr Trace (|<0.1 %).

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