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Chemical composition of essential oils from *Crocus ancyrensis* (Herbert) Maw Spreading In Çorum (Türkiye) Region

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Abstract: *Crocus ancyrensis* is a yellow-flowered *Crocus* species and is in the same family and genus with saffron. Although various studies have been conducted on the antioxidant capacity and essential oil content characterization of saffron (*Crocus sativus*), there is no literature information about the *C. ancyrensis* plant, which is known as *Crocus* among the people. The members of *Crocus* family contain many valuable components including antioxidants, phenolic compounds and essential oils. The essential oils obtained from this family is a complex mixture of more than 30 components, which are primarily terpenes and their derivatives. These mixtures are used in paint, medicine, and food applications especially in the cosmetics sector. In the study, the essential oils of the *Crocus ancyrensis* plant were extracted with the clevenger system and characterized by GC-MS analyses. As a result, 23 volatile components were identified. 2-Hexenal, 1-ethylbutyl Hydroperoxide, 2-nitro-Hexane, β-Isophorone, α-Isophorone, 2-Caren-10-al and Eugenol are found as the main components of *Crocus ancyrensis* plant extract. Due to the antioxidant, antimicrobial, antifungal, anticancer and odorant properties of some of the identified components, *C. ancyrensis* can be used as a medicinal aromatic plant in various fields, especially in the cosmetics and perfume industry.

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1. INTRODUCTION

Essential oils found in parts of plants such as leaves, stems, roots, and flowers contain complex compounds with strong odor and are easy to crystallize (Bakkali *et al.*, 2008; Chavez-Gonzalez *et al.*, 2016). The chemical contents vary based on the type of plants, the geography, climate, production methods, and the area where the plant is grown (De Martino *et al.*, 2015). They are frequently used in cosmetics, food, and pharmaceutical (Asil, 2021) industries due to their unique different properties such as scenting, flavoring, and antibacterial activity (Buckle, 2015). Different methods such as supercritical CO2 extraction (Donelian *et al.*, 2016), ultrasonic extraction (Asil, 2018) and microwave enhanced hydrodistillation can be used for essential oil extraction from herbal plants (Hamidi, 2016; Jain *et al.*, 2022)

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Crocus belongs to the Iridaceae family containing approximately 2050 species around the world (Goldblatt *et al.*, 2008). The most well-known and researched one is *Crocus sativus* L., which has a high commercial value, and is called saffron. *Crocus* species include more than 80 species (about 30 are cultivated) and Türkiye is one of the richest countries in terms of these growing species. Especially, endemic *Crocus* taxa are found about 61% in Türkiye (Ozhatay, 2002). It is known that saffron is widely used as an aphrodisiac, antispasmodic, expectorant (Recio *et al.*, 1995; Asil & Göktürk, 2021), as well as various pharmacological effects such as antitumor, antioxidant, antidepressant, anti-inflammatory, memory and learning enhancement, treatment of hepatic disorders, and reducing insulin resistance (Sanchez-Vioque *et al.*, 2012).

For this reason, many studies have focused on *Crocus sativus*. (Srivastava *et al.*, 2010; Samarghandian & Borji, 2014; Mollazadeh *et al.*, 2015; Mzabri *et al.*, 2019; Cid-Perez *et al.*, 2021; He *et al.*, 2021; Abu-Izneid *et al.*, 2022; Butnariu *et al.*, 2022; El Midaoui *et al.*, 2022).

Sayarer *et al.* (2015) analyzed *Crocus ancyrensis* and found that safranal, α-isophorone and β-isophorone are the main components (Sayarer, 2015). In another study, Küçük *et al.* (2019) revealed that chemical composition of *Crocus ancyrensis* from Eskişehir region, Türkiye is compatible with the findings in Sayarer's study and contained β-Isophorone (14.4%), heptanal (11.5%) and heneicosane (8.5%) (Küçük *et al.*, 2019). For this reason, they have reported that *Crocus ancyrensis* could be an alternative source to *Crocus sativus L*., which contains safranal (77.9%), α-isophorone (13.5%), and β-isophorone (2.2%). Besides, it's found that alcohol and water extracts of *Crocus ancyrensis* showed an inhibitory effect on *Aeromonas hydrophila* bacterial fish pathogens (Turker *et al.*, 2009). On the other hand, Küçük *et al.* (2019) reported that local people used *Crocus ancyrensis* tea as a traditional medicine for abdominal pain and diuretic (Küçük *et al.*, 2019). However, there are limited number of studies on the *Crocus ancyrensis* extracts in the literature (Gunbatan *et al.*, 2016; Küçük *et al.*, 2019).

The aim of this study is to investigate the chemical composition of the essential oils obtained from the endemic *Crocus ancyrensis* plant known as "Ankara Çiğdemi" growing in Çorum region of Türkiye and to reveal its volatile components in detail for the first time.

2. MATERIAL and METHODS

2.1. Plant Materials

Crocus ancyrensis, which is shown in Figure 1, was collected from the region of Çorum location (40°40′26″ N, 34°48′15″ E) with the permission of the Republic of Türkiye, Ministry of Agriculture and Forestry. Collected herbs were identified by Dr. Bedrettin SELVİ from Tokat Gaziosmanpasa University, Faculty of Arts and Sciences, Department of Biology.

Figure 1. The picture of collected *Crocus ancryensis.*

2.2. Isolation of Essential Oils

Clevenger-type apparatus was used for the isolation of essential oils by hydro-distillation method. Air-dried aerial part (stigma, anthers, leaves, throat) of the plant material (30 g each) was added to beaker containing distilled water (200 mL). Distillation process was performed for 3 h and, enriched essential oils were dried over anhydrous sodium sulphate. The obtained essential oils were stored in the refrigerator at 4 °C for GC-MS analyses.

2.3. GC and GC-MS Analyses

GC MS analysis was carried out with Thermo Scientific- Trace GC Ultra system and TSQ Quantum XLS mass spectrometer. Additionally, TG-5MS apolar capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was used and 1.0 mL/minute high purity Helium (He) was selected as carrier gas. The injection temperature was adjusted to 250 °C. TG-5MS apolar capillary column was fixed from 50 to 120 °C at a rate of 3 °C/min, 120 to 220°C at a rate of 3 °C/min, held for 0.67 min, 220 to 250 at 5 °C/min, held for 5.0 min. TSQ Quantum XLS GC MS was set as the ionization energy at 70 eV, the ion source at 250 °C, and the transfer line temperature at 280 °C. Split/splitless (25:1 split) mode was used for the diluted samples (1/10 in acetone, v/v) of 1.0 µL. The results of Mass spectra of molecules were identified via library (WILEY and NIST) using the relative peak areas.

3. RESULTS

The analyses of the volatile components obtained through hydrodistillation method from the *Crocus* plant grown in Çorum region were accomplished by GC-MS and the results are given in Table 1. As a result of GC-MS analysis, 23 volatile components were determined in the plant extract (Figure 2). Among the substances obtained by GC-MS analysis, 2-Hexenal (22.27%), 1-ethylbutyl Hydroperoxide, (12.15%), 2-nitro-Hexane, (16.86%), β-Isophorone (7.76%), α-Isophorone (4.96%), 2-Caren-10-al (4.27%), and Eugenol (13.22%) were determined as the main components.

2-Hexenal, which was determined as the most dominant volatile component, is a simple long-chain unsaturated aldehyde naturally found in various vegetables and fruits. It is allowed to be used as a food additive by the US Food and Drug Administration (FDA), and has antimicrobial properties. It is colorless and sharp grass-smelling liquid and is mainly used in the perfume industry. However, its rapid evaporation limits its utilization in cosmetics (Lanciotti *et al.*, 2003; Joo *et al.*, 2012).

Eugenol, one of the components found in *Crocus ancryensis* extract, was detected as 13.27% by GC-MS analysis. Eugenol is a pale yellow volatile phenolic compound soluble in organic solvents and extracted from plants such as clove oil, nutmeg, cinnamon, basil and bay leaf (Marchese *et al.*, 2017; Nejad *et al.*, 2017). It has been reported to be effective in the treatment of skin infections, skin lesions, and inflammatory disorders (Kamatou *et al.*, 2012; Nejad *et al.*, 2017). In addition, there are studies showing that it can be used in the treatment of various diseases including leukemia, colon and melanoma cancers (Kim *et al.*, 2003; Ghosh *et al.*, 2005; Jaganathan *et al.*, 2011). Eugenol, the main component of clove oil, is widely used in dentistry due to its anesthetic and analgesic effects (Pramod *et al.*, 2010). Due to its antimicrobial and antioxidant properties, it is used in the pharmaceutical and cosmetic industry as well as its use as protective agent in food industry (Turker *et al.*, 2009; Woranuch & Yoksan, 2013). Although it has a wide use area, there are literature studies indicating that excessive use of eugenol has a toxic effect (Basch *et al.*, 2008; Kamatou *et al.*, 2012).

There are not enough studies in the literature on the biological activities of 2-nitro-Hexane, which was found to be 16.86% in essential oil obtained from *Crocus ancryensis*. Gafar *et al.* (2013) stated that this compound, which they detected in *Cucumis melo Linn* seed extracts, could be an industrial-based material (Gafar *et al.*, 2013). In addition, K J Abdulla *et al.* (2019) found 2-nitro-hexane as a degradation product in GC-MS analyzes in their study on the biodegradation of crude oil using local bacterial isolates (Abdulla *et al.*, 2019).

There is no literature on the biological activity of 1-ethylbutyl hydroperoxide, which was detected as 12.15% among the volatile components of the plant by GC-MS analysis (Meenakshi *et al.*, 2012; Padmashree *et al.*, 2018). In the meantime, Padmashree M., *et al.*, (2018) found as 5.85% 1-ethylbutyl hydroperoxide in ethanol extracts of Ipomoea staphylina leaves (Padmashree *et al.*, 2018).

While Ozcelik *et al.* (2020) detected as 7.39% and 14.93% hydroperoxide1-ethylbutyl, respectively, in the methanol extracts obtained from onion and garlic wastes (Ozcelik *et al.*, 2020), M., Al-Owaisi *et al*. (2014) found as 4.39% hydroperoxide1-ethylbutyl in the crude extracts of Moringa peregrina (Forssk.) Fiori leaves (Al-Owaisi *et al.*, 2014).

α-Isophorone and β-Isophorone, unsaturated cyclic ketones, were found to be 4.96% and 7.76%, respectively, in the plant extract as a result of GC-MS analysis. Isophorone is a colorless liquid with mint-like odor (Kataoka *et al.*, 2007; Ershova *et al.*, 2018) and derivatives are considered among the most important aromatic compounds of *Crocus sativus* (Panighel *et al.*, 2014; Condurso *et al.*, 2017). It has been reported to contribute to the aroma of honey, thyme, citrus, rosemary, and lavender (Graikou *et al.*, 2022). 1,8-cineole and camphor, which are structurally related to isophorones, show biological activity, anticancer, and antioxidant properties against some microorganisms (Vuuren & Viljoen, 2007; Kiran *et al.*, 2013; He *et al.*, 2021). Kuran İ *et al.* (2013) obtained two Isophorone derivatives from alternaria fungi and stated that these were effective against some microorganisms (He *et al.*, 2021). In addition, Isophorone has found application in various areas in industry such as paints, varnishes, printing inks, oils, waxes, pesticides, and solvent of natural and synthetic resins (Kataoka *et al.*, 2007; Panighel *et al.*, 2014).

It was determined that 2-Caren-10-al was found in the *Crocus* extract at a ratio of 4.27%. Abushama *et al*. (2013) reported that *Cuminum cyminum* L., which has a high ratio of 2-Caren-10-al among its volatile components, can be used as a potential antimicrobial and antitumor agent. However, they stated that more fractionation, isolation and chemical analysis should be done to identify the chemical compounds responsible for its bioactivity (Abushama *et al.*, 2013). Ghasemi *et al.* (2019) reported that 2 Caren-10-al, which is found in high amounts in the structure of *Cuminum cyminum* has an important contribution to the its antifungal activity (Ghasemi *et al.*, 2019).

Figure 2. GC-MS Chromatogram of Essential oil obtained from *Crocus* plant.

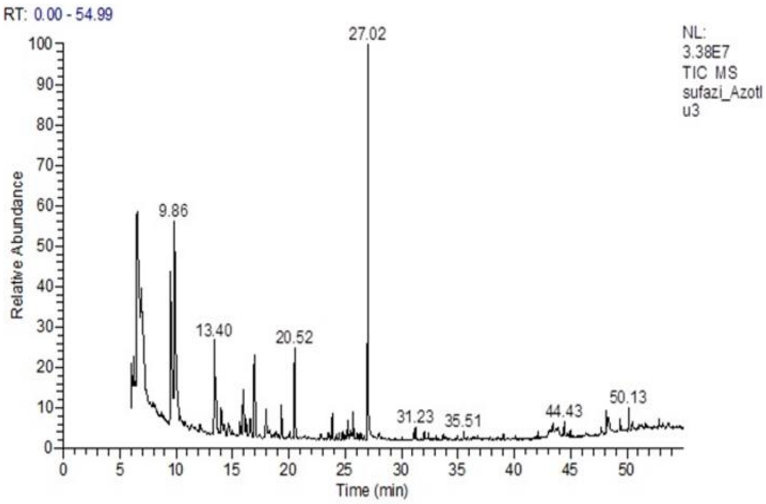

Table 1. Essential oil constituents of *Crocus ancyrensis*.

No	RT	Compound Name	Area %	RRI
1	6.56	2-Hexenal	22.27	845
2	9.53	1-ethylbutyl Hydroperoxide	12.15	921
3	9.89	2-nitro-Hexane	16.86	935
4	10.81	Nonanal	0.28	1081
5	13.41	β-Isophorone	7.76	1097
6	14.03	Tridecane	2.10	1117
7	14.64	1-ethyl-2-methyl-Benzene,	1.17	1124
8	15.67	3,3,5,5-Tetramethylcyclohexanol	0.78	1245
9	15.97	Tetradecane	3.18	1286
10	16.22	Undecane	0.88	1294
11	16.59	Isomenthol	1.11	1334
12	16.96	α-Isophorone	4.96	1534
13	17.98	2,6,6-Trimethyl-2-cyclohexene-1,4-dione (4-oxoisophorone)	1.96	1612
14	18.84	1,3,4-trimethyl-3-Cyclohexene-1-carboxaldehyde	0.62	1674
15	19.35	3-Caren-10-al	1.73	1697
16	20.08	α-Terpineol	0.51	1701
17	20.52	2-Caren-10-al	4.27	1724
18	24.76	2,6,10-trimethyl-Tetradecane	0.26	1784
19	25.72	Hexadecane	1.07	1811
20	27.02	Eugenol	13.22	1901
21	43.69	Heptacosane	0.60	2307
22	48.16	Tricosane	0.79	2181
23	48.33	9-Octadecenamide	1.47	2228

RRI: Relative retention indices. % Calculated from data (calculated according to *n*-alkanes).

4. DISCUSSION and CONCLUSION

In this study, essential oils of *Crocus ancyrensis* collected from Çorum region were extracted by hydrodistillation method and characterized by GC MS. As a result, 23 components were determined in the plant extract. Of these components, 2-Hexenal (22.27%), 1-ethylbutyl Hydroperoxide (12.15%), 2-nitro-Hexane, (16.86%), β-Isophorone (7.76%), α-Isophorone (4.96%), 2-Caren-10-al (4.27%) and Eugenol (13.27) can be considered as main components. It has been reported in the literature that some components of the essential oils showed different biological activities. By conducting more detailed research, the biological activities of the essential oils obtained from *Crocus ancyrensis* such as antimicrobial, antifungal, antioxidant and anticancer properties can be revealed and evaluated for industrial applications such as food, cosmetic, and pharmaceutical applications.

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Declaration of Conflicting Interests and Ethics

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors confirm that there were no ethical in preparing this manuscript. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. All authors consent to participating in this work. All authors consent to publishing this work. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Hacer Dogan: Analysis, Investigation, Resources, Writing -review & editing. **Omer Kayir:** Analysis, Investigation, Resources, Writing -review & editing. **Erol Alver:** Supervision, collecting, Methodology, Investigation, Resources, Writing -review & editing. **Ibrahim Bilici:** Supervision, collecting, Methodology, Investigation, Resources, Writing

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