



Determination of Antioxidant Activities of Essential Oils of *Crataegus orientalis* var. *orientalis* in Tokat (Türkiye) Province

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ABSTRACT

Medicinal plants are quite popular in the medicine development process because they contain bioactive chemicals. Traditional medicine has made extensive use of *Crataegus orientalis* L., which also has significant biological effects. In this study, essential oils (EOs) of *C. orientalis* var. *orientalis* collected from Tokat-Türkiye were isolated by hydrodistillation. Chemical constituents were detected by GC-MS and benzaldehyde (%52.75), α -terpineol (%16.86), germacrene D (%7.03) and caryophyllene (%6.77) were determined as major components. Moreover, antioxidant analyses including DPPH•, ABTS+• and FRAP tests were carried out. EOs displayed good the DPPH activity (IC₅₀, 12.21, μ g/mL) in comparison to the standard BHT (IC₅₀, 10.23 μ g/mL). The same trend was observed for ABTS and FRAP assays.

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Introduction

Crataegus orientalis L. is a variety of hawthorn with orange, yellow and red colors belonging to the Rosaceae family. This plant, whose fruits are eaten, is known as medlar, bird berry among the people in Türkiye and is used in the treatment of hypertension, cardiovascular and arteriosclerosis diseases (Rigelski and Sweet, 2002; Nabavi et al., 2015). It has also been used as a traditional therapy method for a very long time in diseases related to gout, kidney stones, digestive system and depression. In addition, leaves, flowers and fruits are dried and prepared like tea, which is good for cough, sore throat and liver pain. Hawthorn fruit, flower and leaf contain flavonoids such as chlorogenic acid, triterpenoid acids, aromatic amines, phenolic acids, quercetin, luteolin, rutin and vitamin C (Zhang et al., 2001; Skerget et al., 2015).

Antioxidants are substances that prevent oxidative stress and cell damage caused by free radicals in our body. Endogenous enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), are constantly produced in the human body to eliminate the

harmful effects of free radicals. Antioxidants naturally found in plants make important contributions to the defence system rather than drugs (Pellegrini et al., 2009; Abed et al., 2020). The high number of flavonoids it contains are very powerful antioxidants and provide immunity in neurodegenerative and various cancer diseases (Verma et al., 2007; Poprac et al., 2017). In vitro and in vivo studies support that hawthorn berries have strong antioxidant activity. Anthocyanins are found in red fruits such as *Crataegus sinaica* and *Crataegus monogyna* (Froehlicher et al., 2009). Antinociceptive, anti-inflammatory and antioxidant effects were determined in the study conducted with ethanol extract using the leaves of *C. orientalis* fruit (Bor et al., 2012). The antioxidant potentials of the aqueous extracts of *C. aronia* berries and leaves were found to be significantly effective (Ljubuncic et al., 2006). It has been revealed that *Crataegus pinnatifida* leaf extract inhibits NO (Nitric Oxide) production and aldose reductase enzyme and increases the scavenging activity of O₂ radicals (Wang et al., 2011).

Essential oils (EOs) obtained from plants have been used in the pharmaceutical, food and cosmetic industries for many years. Especially in recent years, there has been a great increase in the demand for essential oils to be used in aromatherapy applications (Vasisht et al., 2016). Türkiye is among the leading countries in the trade of medicinal and aromatic plants with its geographical location, climate, rich plant diversity and agricultural potential (Samet and Cikili, 2015). For this reason, increasing the production of essential oils is important in supplying the needed drug substance. Compounds containing monoterpenoids, sesquiterpenoids, nortriterpenoids and triterpenes were determined in essential oils obtained from flowers of *C. jackii*, *C. flabellata* and *C. robesoniana* (Kovaleva et al., 2009). The leaf and flower volatile components of *C. orientalis* subsp. *orientalis* and *C. orientalis* subsp. *szovitsii*, which grows naturally in Muğla-Fethiye region, were determined (Ozderin et al., 2015).

Essential oils were reported to display the considerable biological activities such as antioxidant, antibacterial, anticancer and antifungal (Bakkali et al., 2008). They also keep plants against herbivores due to the compounds they contain in essential oils. Essential oils attract some of the insects that disperse the seeds and repel some of them (Turek and Stintzing, 2013). The chemical composition, antioxidant and anti-inflammatory activities of *C. orientalis* leaves and fruits extracts were investigated (Savikin et al., 2017).

In this study, the antioxidant activities of the essential oil of *Crataegus orientalis* var. *orientalis* grown in the province of Tokat was investigated at first.

Materials and Methods

Plant

C. orientalis var. *orientalis* was collected from Tokat region (40° 19' 11" N, 36° 28' 01" E) and identified by Dr. Bedrettin Selvi. A voucher specimen was deposited in the Herbarium of Tokat Gaziosmanpaşa University (No: 8250).

Essential Oil Isolation

The flowers and leaves were dried, then they (150 g) were subjected to hydrodistillation for 4 hours with the Clevenger apparatus to yield the essential oils (Karan et al., 2018).

GC-MS/MS Analysis

Chemical analyzes of essential oils were performed with GC/MS, Perkin Elmer Clarus 500 Series, in divided mode (50:1), equipped with a flame ionization detector (FID). 20 mg of essential oil was dissolved in 1.2 mL of acetone. BPX5 (30 m, 0.25 mm, 0.25 µm film thickness) column was used. The injection volume was set at 2.0 µL and the injection temperature was 250 °C. Helium was used with a carrier gas flow rate of 1 ml/min and a dispersion ratio of 50:1. The oven program was started at 50 °C and heated up to 100 °C by increasing 5 °C per minute and kept at this temperature for 2 minutes. Then, the temperature was increased to 220°C by increasing 3°C per minute and kept at this temperature for 2 minutes. Total program time was set to 60 minutes and ionization energy was set to 70 eV. Essential oil constituents were

determined by mass spectra and comparing with the standards in the database in the Adams and NIST library

Antioxidant Tests

For antioxidant analyses, 1,1-diphenyl-2-picrylhydrazil (DPPH[•]), [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] (ABTS^{•+}) scavenging activities and Ferric reducing antioxidant power assay (FRAP) were done according to literature (Erenler et al., 2018). Butylated hydroxy toluene (BHT), Butylated hydroxy anisole (BHA) and trolox were used as control group in the experiments.

Statistical Analysis

GraphPad Prism (8.0.1) with ANOVA was used for the statistical analysis. The multiple comparison test was executed by Tukey test. The results were indicated as mean ± SD (P<0.05).

Results and Discussion

The EOs were acquired from *C. orientalis* var. *orientalis* leaves and flowers by hydrodistillation. The chemical contents of the EOs were defined by the GC-MS/MS analysis. Thirteen components representing 100% of the EOs constituents were determined by comparing them with the standards in the database in the Adams and NIST library. The major components were benzaldehyde (%52.75), α-terpineol (%16.86), germacrene D (%7.03) and caryophyllene (%6.77) (Table 1).

2-hexenal (38.6%), capronaldehyde (6.8%) in *C. orientalis* subsp. *orientalis*, and propyl methyl ketone (26.6%), butyraldehyde (9.4%), 2-hexenal (6.6%) in *C. orientalis* subsp. *szovitsii* were the chief EOs constituents collected from Muğla-Fethiye in Türkiye (Ozderin, 2015). It is thought that the difference in chemical content in *Crataegus orientalis* essential oil is due to the collection of the plant from different regions and localizations (Sharma and Tripathi, 2008).

When other *Crataegus* sp. have also been investigated, tricosane, pentacosane, heptacosane and tetracosane major components were indicated from fruits of *C. pallasii* and *C. azarolus* EOs. This is compatible with the fact that EOs formation in plants is largely dependent on biotic and abiotic environmental factors, climatic conditions, genetic background, the distilled part of the plant and extraction methods (Agiel et al., 2019). In another study, the above-ground parts of the Algerian *C. azarolus* EOs were isolated and major constituents were 2,4-bis(1,1-dimethyl ethyl)-phenol, pentadecanoic acid 14-methyl-methyl ester, 8-octadecanoic acid methyl ester, tridecanoic acid 12-methyl-methyl ester isobutyl nonyl phthalate (Boudjada et al., 2018). Benzaldehyde (82.5%, 23.9%), 2-hexenal (21.7%, 2.5%), butyraldehyde (15.2%, 4.4%) from *C. azarolus* EOs collected from the Western Anatolia region of Türkiye, the main components were determined from the leaf and flower parts, respectively (Ozderin et al., 2016).

The antioxidant activities of EOs was examined using the DPPH[•], ABTS^{•+} and FRAP (Table 2). In the DPPH and ABTS tests, it was detected that EOs of *C. orientalis* var. *orientalis* (12.21±0.44 and 10.11±0.61 µg/mL, IC₅₀) have substantial activity. Regarding FRAP effect, the EOs activity was detected as 3.61 ± 0.04 µmol TE/mg extract.

Table 1. EOs of *C. orientalis* var. *orientalis*

No	RT	Compounds	Concentration, %
1	11.64	Benzaldehyde	52.75
2	12.35	β -Pinene	3.30
3	17.91	Linalool	2.24
4	18.55	Fenchol	1.40
5	21.50	4-Terpineol	1.67
6	22.11	α -Terpineol	16.86
7	32.09	Caryophyllene	6.77
8	33.45	Humulene	1.39
9	34.50	Germacrene D	7.03
10	36.64	α -Bisabolene	1.12
11	38.07	Caryophyllene oxide	1.70
12	40.30	α -Cadinol	2.31
13	50.82	Epimanol	1.46
Monoterpene hydrocarbons (Sr. No. 2)			3.30
Oxygenated monoterpenes (Sr. No. 3-6)			22.17
Sesquiterpenes hydrocarbons (Sr. No. 7-10)			16.31
Oxygenated sesquiterpenes (Sr. No. 11,12)			4.01
Ditrepene (Sr. No. 13)			1.46
Others (Sr. No. 1)			52.75
Total identified			1.46
Total			100

Retention times (min), % calculated from FID data.

Table 2. Antioxidant activities of *C. orientalis* var. *orientalis*

	DPPH	*ABTS**	**FRAP
<i>C. orientalis</i> var. <i>orientalis</i>	12.21 \pm 0.44 ^d	10.11 \pm 0.61 ^e	3.61 \pm 0.04 ^a
BHT	10.23 \pm 0.12 ^c	7.28 \pm 0.19 ^d	5.34 \pm 0.08 ^b
BHA	4.35 \pm 0.10 ^a	4.25 \pm 0.10 ^a	7.52 \pm 0.04 ^d
Trolox	4.54 \pm 0.14 ^a	5.52 \pm 0.22 ^b	-

*IC₅₀ (μ g/mL), ** μ mol TE/mg sample. The different letters indicate the significant differences of essential oils and standards (P<0.05).

Many pharmacological studies including antioxidant have been conducted on various extracts of *Crataegus orientalis*. When the phytochemical analyzes are evaluated, the hyperoside (quercetin-3-O-galactoside), flavonoids, oligomeric proanthocyanidins, phenolics and alkaloids contained in the leaves, flowers and fruits of *Crataegus orientalis* provide the plant with strong antioxidant properties (Horoz et al, 2008; Savikin et al., 2017). In the literature, there are essential oil studies of *Crataegus orientalis* collected from different regions and isolated from different parts, but there is almost no information about its biological activities such as antioxidant, and antibacterial. It has been reported that palmitic acid, α -farnesene, hexyl benzoate and linoleic acid are valuable antioxidant bioactive components in *Crataegus azarolus* (Lakache et al., 2014).

In present study, benzaldehyde, α -terpineol, germacrene D and caryophyllene were the major components identified by GC-MS in the EOs of the leaves and flowers of *C. orientalis* var. *orientalis*. It shows that our study is compatible with the literature, even if the essential oil components are in different amounts in other hawthorn species (Baghi et al., 2012). It has been previously determined that benzaldehyde is an insecticidal, antimicrobial and antioxidant component (Ullah et al., 2015). α -Terpineol is an aromatic compound widely used in cosmetics and food. It is an important monoterpene with antiproliferative, antinociceptive, anticonvulsant, antiulcer and antioxidant properties (Bicas et al., 2011;

Khaleel et al., 2018). It has been reported that germacrene D and caryophyllene, which are also present in *Crataegus azarolus* essential oil, have antioxidant, antimicrobial and anticarcinogenic properties (Tabaszewska et al., 2022).

Conclusion

Herein, EOs were generated from flowers and leaves of *C. orientalis* var. *orientalis* collected from Tokat province (Türkiye) at first. In the results, good antioxidant activity was determined in comparison with the standards. Biological activity studies of the essential oils of the plant, such as anticancer, antimicrobial and antiviral, are limited. In the future, larger studies should be conducted to emphasize its pharmacological effect.

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