

Volatile Oil Constituents of *Crataegus azarolus* L. and *Crataegus pallasii* Griseb.

Najat Agiel^{1,2}, Duygu Yiğit Hanoğlu³, Azmi Hanoğlu¹,

Kemal H. C. Başer¹ and Filiz Mericli^{1,4}

¹ Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Nicosia – Turkish Republic of Northern Cyprus

² The University of Tripoli, Faculty of Education, Department of Biology, Libya

³ Near East University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Nicosia – Turkish Republic of Northern Cyprus

⁴ Near East University, Faculty of Pharmacy, Department of Phytotherapy, Nicosia – Turkish Republic of Northern Cyprus

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Abstract: In this study the volatile oils constituents of the inflorescence and unripe fruits of *Crataegus azarolus* L. and *Crataegus pallasii* Griseb. were investigated. Fifty-four compounds were identified by GC and GC-MS analysis. The major outstanding constituents of the essential oil in all samples (dried and fresh *C. azarolus* and dried *C. pallasii*) were; tricosane (33.8%, 29.3%, 34.0 %), pentacosane (24.6%, 21.1%, 30.8%), heptacosane (12.5%, 10.2%, 11.7%), and tetracosane (6.0%, 5.6%, 5.7%), respectively. Besides these alkanes, ten compounds (nonanal, β -elemene, undecanal, β -caryophyllene, (*E*, *E*)- α -farnesene, eicosane, hexyl benzoate, (*Z*)-3-hexenyl benzoate, (*E*)-2-hexenyl benzoate, docasane) were determined in all samples. Carvacrol, carvacryl acetate, carvone, and thymol were determined for the first time from *C. pallasii* essential oil. (*E*)- β -damascenone was determined only in dried *C. azarolus* oil; sesquiterpene compounds valencene, α -selinene and β -selinene, δ -cadinene, germacrene D, selina-3,7(11)-diene, spathulenol, and δ -cadinol were determined only in fresh *C. azarolus* sample. On the other hand (2*E*,6*E*)-farnesol was determined in dried *C. azarolus* and *C. pallasii* samples.

Keywords: *Crataegus azarolus*; *Crataegus pallasii*; volatile constituents; GC-MS analysis. © 2019 ACG Publications. All rights reserved.

1. Introduction

Crataegus species (Rosaceae), more commonly known as “Aliç”, “Yemişen” or “Mosphilla” in Cyprus and as “Zaarour” in the Middle Eastern Countries, is a diverse genus of flowering, fruit bearing shrubs or small trees that grow mostly in temperate zones, including countries of North Africa, Europe and Mediterranean basin, Western Asia, India, China and North America [1,2]. *Crataegus* species (Hawthorn) have been used traditionally since ancient times and the first report of patients treated with *C. oxyacantha* that were suffering from various heart illnesses was in 1896 [3].

Researches documented that bioflavonoid-like complexes appeared to be primarily responsible for the cardiac actions of the plant which included oligomeric procyanidins (OPC) and

* Corresponding author: E-Mail: najataghil@yahoo.com; Phone: 090-533-8562632

flavonoids, hyperoside, quercetin, and vitexin. The action of these compounds on the cardiovascular system has led to the development of drugs from leaf and flower extracts, which are widely used in Europe [1,4].

Recent reports showed that numerous diverse chemical constituents were in the leaves, fruits, and flowers of *Crataegus* which include sugars and sugar alcohols, organic and phenolic acids, terpenes, essential oils (including mixtures of terpenoids and phenylpropanoids) [5]. While intensive work has been done on the major bioactive compounds in the chemical profile of *Crataegus* species little attention has been given to volatile constituents [6]. This could be due to the low concentrations of these metabolites but they could contribute to the synergic effect of the plant and explain some therapeutic effects such as sedative effect [4]. Therefore, we aimed to study the volatile constituents of the flowers and fruits of two *Crataegus* species. *C. azarolus* growing in Cyprus and *C. pallasii* growing in Libya.

2. Materials and Methods

2.1. Plant Material

Crataegus azarolus samples were collected from two regions in Northern Cyprus; Cengizköy / Lefke on March 1, 2018, and from Near East University Campus in Nicosia on March 26, 2018. Samples were deposited at the Herbarium of the Near East University with voucher numbers NEUN 6899 and NEUN 6900, respectively. *Crataegus pallasii* samples were collected from El-merj in Libya from Bata Region on March 23, 2018. Samples were authenticated by Dr. Mohammed Nuri Abuhadra and deposited in the Herbarium of the Faculty of Science, Botany Department, the University of Tripoli in Libya with voucher number: D6831131.

2.2. Isolation of Essential Oil

For the isolation of the essential oils, fresh and dry inflorescences and unripe fruits were separated from branches of *C. azarolus* and *C. pallasii*. 150 g of each fresh and dry *C. azarolus* and 85 g of dry *C. pallasii* inflorescences and unripe fruits were hydrodistilled in a Clevenger-type apparatus for 3h. The resulting oils were trapped with hexane and stored at 4 °C until used.

2.3. Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

2.3.1. GC-MS Analysis

The GC-MS analysis was carried out with an Agilent 5977B GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450. The sample was dissolved in 10% n-hexane and 1 µL was injected.

2.3.2. GC Analysis

The GC analysis was carried out using an Agilent 7890B GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a triplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.4. Identification of Compounds

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their linear retention index (LRI)

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to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, NIST Chemistry WebBook) [7,8] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data was used for the identification [9,10].

3. Results and Discussion

The essential oils isolated by hydrodistillation from the inflorescences and unripe fruits of *C. azarolus* and *C. pallasii* were analyzed simultaneously by GC-FID and GC-MS. The chemical profiles of the essential oils, the percentage contents and linear retention indices of the components are shown in table 1. The components identified from the distilled oil of fresh *C. azarolus* were thirty-three forming 91.1% of the total oil composition and twenty-seven were identified from dried *C. azarolus* and thirty-one were determined from *C. pallasii* comprising 85.5% and 97.6% of the total oil composition, respectively. Oil yield of all samples was measured less than 0.01% on dry weight basis. The moisture content of *C. azarolus* was calculated as 65.6%.

The major outstanding constituents and common to all samples of the essential oils of *C. azarolus* (fresh and dried) and *C. pallasii* were the alkanes namely; tricosane (33.8%, 29.3% and 34.0%), pentacosane (24.6%, 21.1% and 30.8%), heptacosane (12.5%, 10.2% and 11.7%) and tetracosane (6.0%, 5.6%, and 5.7%), respectively. Docosane (1.7%, 2.0%, and 1.3%), was also present in all three samples. Carvacrol (6.8%), carvacryl acetate (0.3%), carvone (t) and thymol (0.1%), were determined for the first time from *C. pallasii* essential oil. (*E*)- β -damascenone was determined only in dried *C. azarolus*; sesquiterpene compounds valencene, α -selinene and β -selinene, δ -cadinene, germacrene D, selina-3,7(11)-diene, spathulenol, and δ -cadinol were determined only in fresh *C. azarolus* sample. On the other hand (*2E,6E*)-farnesol was characterized in both dried *C. azarolus* (6.3%) and *C. pallasii* (0.4%) samples.

Analysis of our readings documented an apparent difference between both samples of *C. azarolus*, most predominate the presence of hexacosane (1.3%) in fresh *C. azarolus* and (*2E,6E*)-farnesol (63%) in dry *C. azarolus* samples. Eighteen compounds were common to both, this variation in chemical composition is suspected to be due to, mainly two reasons. Firstly, samples have been collected from different localities in Cyprus and secondly, the difference in sample conditions (fresh and dried). Reports on the essential oils of *C. azarolus* in different regions of the world also showed considerable qualitative and quantitative variations. This is in accordance with the fact that essential oil formation in the plants is highly dependent on climatic conditions, genetic background, biotic, and abiotic environmental factors, part of the plant distilled, stage of plant development as well as the extraction methods [11].

Comparison of our investigations with the published data regarding the essential oil of *C. azarolus*; Lakache and his colleagues reported sixty-one compounds from the essential oil of leaves and flowers of *C. azarolus* growing in Algeria by two extraction methods; hydrodistillation assisted by microwave heating (HD-MW) and hydrodistillation (HD). The main groups of detected volatiles were acids and esters (12 compounds) 27.5% for HD-MW, 50.7% for HD; alkanes and alkenes (17 components) comprised 40.8 % for HD-MW, 29.5% for HD. It is obvious that the latter group of compounds obtained by using HD-MW method is quite similar to our results [12]. The Algerian *C. azarolus* aerial parts were also investigated by Boudjada *et al.*, five volatile compounds were identified; 2,4-bis (1,1-dimethyl ethyl)-phenol, tridecanoic acid 12-methyl-methyl ester, pentadecanoic acid 14-methyl-methyl ester, 8-octadecanoic acid methyl ester, and isobutyl nonyl phthalate [13]. The water, methanolic and ethanolic extract of the fresh leaves of the Lebanese *C. azarolus* were examined by gas chromatography coupled with mass spectrometry. In total 11 compounds were determined in the water extract with the major compound pluchidiol (33.6%). The methanolic extract revealed 8 compounds with the major compound α -tocopherol-beta-d-mannoside (21.9%) and in the ethanolic extract, 7 compounds were determined with the major compounds γ -tocopheryl methyl (43.7%) and phytol isomer (20.5%) [14].

Table 1. Volatile oil composition of inflorescences and immature fruits of *C. azarolus* and *C. pallasii*

LRI _{EXP}	LRI _{LIT} [*]	Compound Name	A%	B%	C%
1020	1025 ^b	α -pinene	0.9	0.3	-
1190	1177 ^b	α -terpinene	-	-	0.1
1213	1212 ^c	Limonene	1.9	0.5	-
1259	1245 ^b	γ -terpinene	-	-	0.7
1288	1282 ^c	<i>p</i> -cymene	0.4	-	0.3
1408	1391 ^b	Nonanal	0.2	0.2	0.1
1514	1491 ^b	α -copaene	0.2	-	-
1555	1543 ^b	Linalool	-	-	0.4
1613	1591 ^b	β -elemene	0.1	0.2	0.2
1622	1617 ^a	Undecanal	0.1	0.3	0.2
1620	1617 ^a	Terpinen-4-ol	-	-	0.3
1624	1623 ^a	β -caryophyllene	0.3	0.2	0.2
1728	1719 ^a	Borneol	-	-	0.2
1744	1726 ^a	Germacrene D	0.2	-	-
1748	1741 ^a	β -bisabolene	-	-	0.3
1758	1742 ^d	β -selinene	0.2	-	-
1761	1744 ^a	α -selinene	0.1	-	-
1763	1760 ^a	(<i>E, E</i>)- α -farnesene	0.3	0.4	0.4
1770	1740 ^a	Valencene	0.5	-	-
1770	1751 ^a	Carvone	-	-	t
1786	1755 ^b	δ -cadinene	0.2	-	-
1817	1797 ^a	Selina-3,7(11)-diene	0.2	-	-
1835	1830 ^a	Tridecanal	-	0.2	0.1
1854	1838 ^a	(<i>E</i>)- β -damascenone	-	0.1	-
1875	1854 ^b	(<i>Z</i>)-geranyl acetone	-	0.1	-
1893	1880 ^e	2,2,4-trimethyl-3-carboxyisopropyl pentanoic acid isobutyl ester	-	0.1	-
1901	1890 ^a	Carvacryl acetate	-	-	0.3
1941	1931 ^a	Phenylethyl alcohol	-	-	0.1
1973	1954 ^a	(<i>E</i>)- β -Ionone	-	-	0.1
2033	2008 ^a	Caryophyllene oxide	0.5	-	-
2052	2053 ^a	(<i>E</i>)-nerolidol	0.8	1.0	-
2075	2055 ^a	Anisaldehyde	-	-	0.1
2100	2100 ^a	Eicosane	1.9	3.8	1.6
2110	2095 ^a	Hexyl benzoate	0.1	0.3	0.1
2138	2118 ^a	Hexahydrofarnesyl acetone	0.4	0.2	-
2159	2144 ^a	Spathulenol	0.2	-	-
2163	2148 ^a	(<i>Z</i>)-3-hexenyl benzoate	0.5	0.6	0.2
2182	2170 ^f	(<i>E</i>)-2-hexenyl benzoate	0.1	0.2	0.1
2197	2191 ^a	3,4-dimethyl-5-pentylidene-2(5H)-furanone	-	t	-
2200	2200 ^d	Docosane	1.7	2.0	1.3
2210	2198 ^a	Thymol	-	-	0.1
2228	2223 ^a	Methyl hexadecanoate	-	0.3	-
2230	2233 ^a	δ -cadinol	0.2	-	-
2242	2239 ^a	Carvacrol	-	-	6.8
2265	2278 ^d	Torilenol	0.3	-	-
2300	2300 ^d	Tricosane	33.8	29.3	34.0
2332	2351 ^a	Eudesma-4(15),7-dien-1- β -ol	0.2	-	-
2370	2369 ^a	(2 <i>E</i> ,6 <i>E</i>)-farnesol	-	6.3	0.4
2402	2400 ^d	Tetracosane	6.0	5.6	5.7
2416	2376 ^a	Manoyl oxide	0.2	0.7	-
2500	2500 ^d	Pentacosane	24.6	21.1	30.8
2602	2600 ^d	Hexacosane	1.3	-	0.7
2623	2613 ^a	Phytol	-	1.6	-
2700	2700 ^d	Heptacosane	12.5	10.2	11.7
Total			91.1	85.8	97.6

LRI_{EXP}: Linear retention indices calculated against n-alkanes by using FID data. LRI_{LIT}^{*}: LRI from literatures: [8]^a, [19]^b, [20]^c, [21]^d, [22]^e. t: trace (<0.1 percent).

A: *Crataegus azarolus* fresh inflorescences and immature fruits, NEU campus, TRNC; **B:** *Crataegus azarolus* dried inflorescences and immature fruits, Cengizköy / Lefke, TRNC; **C:** *Crataegus pallasii* dried inflorescences and immature fruits, Bata region, Libya.

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Using solid phase microextraction (SPME) method, analysis of the volatile components of the leaf and flower of 7 *Crataegus* taxa collected from the Western Anatolia part of Turkey was conducted by Özderin *et al.* Forty volatile components from two samples of *C. azarolus* var. *aronia* essential oil were reported with the major components; benzaldehyde (82.5%, 23.9%), 2-hexenal (21.7%, 2.5%), butyraldehyde (15.2%, 4.4%) [15]. Due to the high popularity of the fruit jam of *C. azarolus* in Cyprus, Hadjimitsi and Zabetakis performed a study that involved the identification and quantification of volatile compounds from the commercial jam of the species purchased from a local producer in Cyprus. In that study, simultaneous distillation–extraction method was used and 44 components were identified by GC-MS. The major constituent identified was 2-furaldehyde (21.4%) [16]. A study to evaluate the chemical profile of *C. azarolus* fruits in Northern Italy attempted an innovative approach, namely, a specific fingerprint, coupled to the multivariate data analysis (PCA), which was used to show the single bioactive class contribution to the total fruit phytocomplex. Five monoterpenes; phellandrene, sabinene, γ -terpinene, terpinolene and limonene constituting a total of (15.4%) were characterized [17]. Hydrosol beverage of *C. azarolus* leaf and fruits that are used for the treatment of cardiovascular diseases in Persian nutrition culture and folk medicine was investigated by Azadeh *et al.*, and analyzed by GC-MS, to reveal the presence of hexadecanoic acid (7.7%), *p*-xylene (20.1%), thymol (28.7%) and thymolethanoate (2.3%) [18].

Other *Crataegus* species have also been investigated for their essential oil constituents. *C. monogyna* inflorescence essential oil was evaluated by Kowalski *et al.*, and 65 compounds were identified, the major compounds were; tricosane (12% - 17%), heneicosane (11% - 16%), linalool (6% -11%), n-hexadecanoic acid (1%-11%), nonadecane (3%-7%), (*E,E*)- α -farnesene (1%-5%), caryophyllene oxide (1%-4%) and methyl eugenol (6%) [23]. Whereas in early 1993, Robertson *et al.*, also identified the major volatile compounds of *C. monogyna* flowers as alcohols, ketones and aldehydes; 3-methyl-1-butanol (23.2%), benzaldehyde (16.1%), 2-butanone (10.9%), 3-methyl butanal (9.6%), 4-methoxybenzaldehyde (9.2%) 4-methoxy benzoic acid (9.6%) and 3-pyridinecarboxaldehyde (8.3%) [24]. The essential oil of the flowers of *C. jackii*, *C. robesoniana*, and *C. flabellata* was reported by Kovaleva *et al.*, 46 compounds were identified. The major compounds were alkanes, mainly; tricosane (11.1%, 19.2%, 17.9%) which is in agreement with our results [25]. Özderin *et al.*, identified fifty-three volatile components, from *C. orientalis* subsp. *orientalis*, collected from Muğla-Fethiye in Turkey. Major components were aldehydes; 2-hexenal (38.6%), capronaldehyde (6.8%) and from *C. orientalis* subsp. *szovitsii* major components were propyl methyl ketone (26.6%), butyraldehyde (9.4%) and 2-hexenal (6.6%) [26]. Horvat and Chapman also investigated volatile oils from fruits of *C. opaca*, *C. aestivalis* and *C. rufula* from South Georgia. Twenty-four compounds were identified comprising mainly esters and aldehydes, constituting 70.4% of the volatiles [27]. The chemical characterization of *C. oxyacantha* essential oil from Algeria was determined by Chouitah and Meddah. Twenty-five compounds were detected, representing 97% of the total essential oil, eugenol (24.3%), longifolenaldehyde (17.5%), β -selinene (15.6 %) were the main components [28]. In a study by Nojima *et al.*, to identify volatile compounds from hawthorn fruit (*Crataegus* spp.) that act as behavioral attractants for hawthorn-infesting *Rhagoletis pomonella* flies. Six volatiles were mentioned: ethyl acetate (94.3%), 3-methylbutan-1-ol (4%), isoamyl acetate (1.5%), 4,8-dimethyl-1,3(*E*),7-nonatriene (0.1%), butyl hexanoate (0.01%), and dihydro- β -ionone (0.1%) [29].

4. Conclusion

To the best of our knowledge, this is the first report regarding the volatile oil analysis of inflorescences and unripe fruits of two *Crataegus* species, *Crataegus azarolus* growing in Cyprus and *Crataegus pallasii* growing in Libya. Fifty-four compounds were identified by GC and GC-MS analysis. Scientific studies document that alkanes are considered important substances in practical and clinical uses with huge potential in the nutraceutical and pharmaceutical industries [30] and in pest management programs [31]. Therefore, the alkanes concentration of *C. azarolus* and *C. pallasii* can be evaluated for these purposes.

Moreover, the presence of monoterpenes, sesquiterpenes, aldehydes, esters, ketones and the important constituent (*E*)- β -damascenone which is regarded as a useful marker for characterisation of

the quality of rose oil and wine [32,33] that was isolated for the first time in minute amounts from Bulgarian rose oil [34] also suggests the use of *C. azarolus* and *C. pallasii* in food, cosmetics, and pharmaceutical industries to improve flavor and taste [35,36,37]. On the other hand, current literature documents the importance of flavonoids and oligomeric-proanthocyanidins in the treatment of cardiovascular disease by *Crataegus* species, it is noteworthy to investigate whether the volatile oil of *Crataegus* sp. would be responsible for other pharmacological properties of this genus *e.g.*: anxiolytic, antiviral, antimicrobial, antioxidant, etc. [1,38]. Thus, further research is recommended to investigate the biological and economic importance of the essential oil of *C. azarolus* and *C. pallasii*.

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Conflict of interest statement

The authors have no conflict of interest to declare.

Supporting Information

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ORCID

Najat Agiel: [0000-0003-4620-0019](https://orcid.org/0000-0003-4620-0019)

Duygu Y. Hanoglu: [0000-0003-1345-4768](https://orcid.org/0000-0003-1345-4768)

Azmi Hanoglu: [0000-000207586-9080](https://orcid.org/0000-000207586-9080)

Kemal H. C. Baser : [0000-0003-2710-0231](https://orcid.org/0000-0003-2710-0231)

Filiz Mericli : [0000-0002-4172-5417](https://orcid.org/0000-0002-4172-5417)

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