Chemical composition of the essential oils of Centaurea tomentella Hand.-Mazz. and C. haussknechtii Boiss. (Asteraceae) collected wild in Turkey and their activity on microorganisms affecting historical art craft

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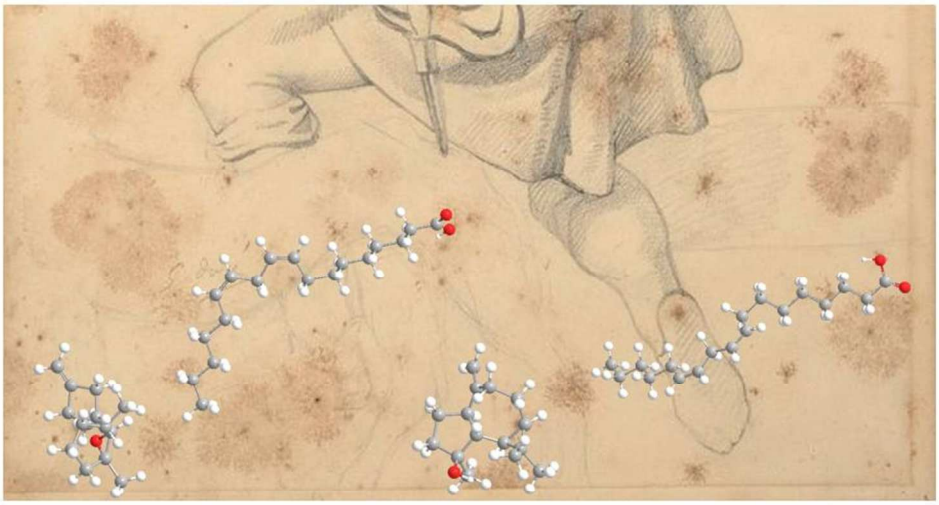

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RESEARCH ARTICLE

Chemical composition of the essential oils of *Centaurea tomentella* Hand.-Mazz. and *C. haussknechtii* Boiss. (Asteraceae) collected wild in Turkey and their activity on microorganisms affecting historical art craft

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Chemical composition of the essential oils of *Centaurea tomentella* Hand.-Mazz. and *C. haussknechtii* Boiss. (Asteraceae) collected wild in Turkey and their activity on microorganisms affecting historical art craft

In the present study the chemical composition of the essential oils from aerial parts of *Centaurea tomentella* Hand.-Mazz. and *C. haussknechtii* Boiss. collected in Turkey was evaluated by GC and GC-MS. The main components of *C. tomentella* L. were hexadecanoic acid (19.7%), caryophyllene oxide (6.6%) and spathulenol (4.8%) whereas *C. haussknechtii* was rich in hexadecanoic acid (26.2%), (*Z,Z*)-9,12-octadecadienoic acid (19.3%), heptacosane (5.3%) and nonacosane (5.1%). Antibacterial and antifungal activities against some microorganisms infesting historical art craft, were also determined.

Keywords: *Centaurea tomentella*; *Centaurea haussknechtii*; Volatile components; Hexadecanoic acid; (*Z,Z*)-9,12-octadecadienoic acid; Caryophyllene oxide; Antibacterial and Antifungal activity

1. Introduction

Centaurea tomentella Hand.-Mazz. (Asteraceae) belongs to subgenus *Cynaroides* Dostál. It is a perennial with erect, 25-80 cm high stem and firm, sparsely hairy or tomentose leaves; basal ones are oblong-cordate, petiolate while lower are lanceolate with winged petioledilated at the base; median leaves are lanceolate or broadly lanceolate, shortly decurrent, while upper ones are lanceolate and sessile. Capitula are arranged in a spike or raceme (peduncles up to 8 cm). Involucre is *c*. 30 high and 25-30 mm wide, ovoid to subglobose. Appendages are very firm, straw-coloured,totally concealing basal part of phyllaries, narrowly triangular, with 6-10 cilia on each side (5-6(-8) mm long) and endingin a 9-12(-17) mm spine. Flowers are purple. Pappus is *c*. 8 mm long. The species is Turkish endemic (Davis, 1975).

Centaurea haussknechtii Boiss. belongs also to subgenus *Cynaroides* Dostál. The species is morphologically similar to *C. tomentella,* but lower leaves are lyrate with oblong terminal segmentand 1-2 pairs of retrose lateral segments, while median ones are sessile or very shortly decurrent. Appendages are lanceolate with 7-9 cilia on each side (5-7 mm long) and a terminal spine 15-17 mm long. Flowers are rose-purple. The species is Turkish endemic (Davis, 1975).

Biodeterioration of archival and library materials is a worldwide problem, which is great damage to unique manuscripts and books (Zyska, 1997; Shamsian et al. 2006). The historical organic materials

such as paper, paintings, wood, papyri, incunabula, books, as well as textile and leather are mainly constituted of natural fibres that can be culture media of several heterotrophic microorganisms (bacteria and fungi). In the frame of search of "green" alternative to synthetic chemicals, several papers have been published on new natural biocides that can be used to prevent and reduce the dangerous effects of microorganisms on historical art craft (Rakotonirainy and Lavèdrine, 2005; Stupar et al. 2014; Casiglia et al. 2014a; 2014b; 2015a; 2015b; 2015c; 2016).

Bacillus, Chaetomium, Myrothecium, Memnoniella, Stachybotrys, Verticillium, Alternaria, Trichoderma, Fusarium, Penicillium and *Aspergillus* are often responsible of damages and deterioration of cellulosic materials, causing oxidation, depolymerisation and breakdown of molecular and supra-molecular structure, with consequent fibres weakening, involving loss of strength, stains and discoloration. The presence of glues and other substances of vegetable and animal origin can increase and facilitate the microbial growth. Generally, microorganisms can deteriorate the cellulosic support with extracellular enzymes, able to break long chain molecules, like cellulose, in smaller chemical units that can be absorbed into the cell through the cell membrane. Cellulases, a group of fibrolytic enzymes, can affect cellulosic fibres, cooperatively hydrolysing cell wall fibres of vegetables into glucose, cellobiose or oligosaccharides (Kamel et al. 2014). The degradation of wool, on the contrary, involves a keratinolysis process that starts with the fixation of disulfide bridges, the origin of the keratin resistance, followed by the hydrolysis of protein linear chains mediated by extracellular proteinases (Szostak-Kotowa 2004).

Since, at the best of our knowledge no investigations have been reported on this species, in the context of our on-going research on Mediterranean taxa of *Centaureae* (Senatore et al. 2006; Erdemgil et al. 2006; Formisano et al. 2008a; Formisano et al. 2011; Maggio et al. 2014; Riccobono et al. 2017), we were interested to investigate the chemical composition of the essential oils obtained from *Centaurea haussknechtii* and *C. tomentella*, as well as their biological properties against several microorganisms, including *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium oxysporum* and *Aspergillus niger*. These biological targets were chosen for their known ability to infest historical art craft materials (Basu and Ghose, 1962; Desai and Pandey, 1971; Agarwal and Puvathingal, 1969, McCarty and Greaves, 1988).

2. Results and discussion

Hydrodistillation of *Centaurea hausschnetchii* aerial parts (**CH**) gave a pale yellow oil. Overall, fifty-two compounds were identified, representing 91.3% of the total components. The components are listed in Table 1 according to their retention indices on a HP 5MS column and are classified on the basis of their chemical structures into 9 classes (Table 1).

The oil was particularly rich in fatty acid and esters (49.6%). Hexadecanoic acid (26.2%) and (*Z,Z*)-9,12-octadecadienoic acid (19.3%) were, by far, the main components of this class as well as of the oil. Monoterpenes were present in very low amount (1.4%). Oxygenated sesquiterpenes were quite abundant (10.3%) with spathulenol (2.9%) as the main component. Among sesquiterpene hydrocarbons (15 compounds, 8.6%) only germacrene D (2.5%) was present in significant quantity. Five hydrocarbons were recorded, forming 12.5% of the total, with heptacosane (5.3%) and nonacosane (5.1%) and as the most abundant ones. Among the phenolic compounds (4.5%) only 4-vinylguaiacol (3.5%) is worth of mention.

Hydrodistillation of *Centaurea tomentella* aerial parts (**CT**) gave a pale yellow oil. Overall, seventhy-three compounds were identified, representing 91.0% of the total components (Table 1). Also in this case the main class was represented by fatty acid and esters (29.5%) with hexadecanoic acid (19.7%) as the main component although the occurrence of unsaturated fatty acid was quite lower (3.9%) with respect to **CH**. Sesquiterpenes represented the 31.8% of the oil with caryophyllene oxide (6.6%), spathulenol (4.8%) and *β*-eudesmol (3.5%) as main compounds among the oxygenated sesquiterpenes (20.4%). Among carbonylic compounds (8.5%) is noteworthy the occurrence of hexahydrofarnesyl acetone (2.9%) and monoterpenes, more abundant than in the essential oil of *Centaurea hausschnetchii*, represented the 7.6% of the oil. The quite similar profile of the two oils (**CH** and **CT**) is in agreement with the fact that they have been enclosed into the same subgenus *Cynaroides* Dostál. Hexadecanoic acid, the main compound of *C. hausschnetchii* and *C. tomentella*, was shown to be the most abundant component also of essential oil of *C. aladagensis* (Kose et al. 2007), *C. luschaniana*, *C. tossiensis*, *C. wagenitzii* (Kose et al. 2008), *C. saligna* (Altintas et al. 2009), *C. paphlagonica* (Kose et al. 2009) and *C. hierapolitana*, *C. cadmea*, *C. calolepis*, *C. reuterana*, *C: depressa*, *C. urvillei* (Karamenderes et al. 2008) from Turkey and of *C. nicaeensis*, *C. parlatoris* and *C. solstitialis* ssp. *schouwii* (D.C), species growing wild in Sicily (Senatore et al. 2008). The occurrence of good amount of hydrocarbons (12.5% in **CH** and 8.7% in **CT**, respectively) was also detected in *C. sicana* and *C. giardinae*, both growing in Sicily (Formisano et al. 2008b).

The antimicrobial activity of the essential oils (**CH** and **CT**) is reported in Table 2. The oil of *C. hausschnetchii* showed significant properties both towards bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and toward moulds (*Fusarium oxysporum* and *Aspergillus niger*). These results can be related to the occurrence in the oil of high levels of fatty acids. In fact, palmitic acid (the main component of **CH**) has been shown to have an excellent antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Yff et al. 2002) and remarkable anti-fungal properties against *Fusarium oxysporum* and *Aspergillus niger* (Pohl et al. 2011). Furthermore (*Z,Z*)-9,12-octadecadienoic acid

(linoleic acid) has been proved to be very active against *Bacillus subtilis* and in lesser amount against *Staphylococcus aureus* (Dilika et al. 2000) and to have antifungal activity against *Fusarium oxysporum* (Pohl et al. 2011). On the other hands the good anti-microbial properties of the oil of *C. tomentella* against Gram+ bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) are probably due to the occurrence in the oil of some compounds with a well stated activity on these bacteria: palmitic acid (Yff et al. 2002), caryophyllene oxide (Ben Hsouna et al. 2013; Runyoro et al. 2010) and spathulenol (Runyoro et al. 2010).

3. Experimental

3.1. *Plant material*

Aerial parts of *Centaurea haussknechtii* Boiss. were collected in Turkey, Adıyaman; Kahta, between Kahta-Nemrut Mountain, 15 km, 800 m s/l, (37° 48 38' N, 41° 93 60'E), at the beginning of July 2016. Aerial parts of *Centaurea tomentella* Hand.-Mazz. were collected in Turkey, between Malatya-Darende, 57 km, 1700 m s/l, (42° 44 38 N, 37° 39 58' E), at the beginning of June 2016. Typical specimens, identified Prof. Dr. Sezgin Çelik, have been deposited in the Department Yıldız Technical University (voucher No. Celik 3850 and Celik 3838, respectively).

3.2. *Isolation of the essential oil*

The air-dried samples (200 g) were ground in a Waring blender and then subjected to hydrodistillation for 3 h, according to the standard procedure described in the European Pharmacopoeia (2004) using a Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulphate and stored under N2 at -20 ◦C in the dark until tested and analyzed. The samples yielded 0.08% (*C. haussknechtii*) and 0.09% (*C. tomentella*), respectively, of yellow oils (w/w), with a pleasant smell.

3.3. *Qualitative and quantitative analyses of essential oil*

The essential oil samples were analyzed to determine the chemical components by GC and GC-MS. The GC analyses were carried out with a Perkin-Elmer Sigma 115 gas chromatograph equipped with a flame ionization detector (FID) while Gas chromatography-Mass spectrometry (GC-MS) was recorded on an Agilent 6850 Ser. II apparatus coupled to an Agilent Mass Selective Detector MSD 5973 as previously described (Loizzo et al. 2013). Identification of constituents was made as elsewhere reported (Zito et al. 2013).

3.4. *Antimicrobial assay*

3.4.1. *Microbial strains*

The antimicrobial and antifungal activities of essential oil were tested against a panel which included eight bacteria species, selected as representative of the class of Gram positive and Gram negative, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853), two moulds, *Fusarium oxysporum* (ATCC 695) and *Aspergillus niger (*ATCC 16401). The strains were grown on Tryptone Soya Agar (Oxoid, Milan, Italy) for the bacteria, Saboureaud Dextrose Agar (SDA) with chloramphenicol for yeasts and SDA for moulds. For the antimicrobial tests, Tryptone Soya broth (Oxoid, Milan, Italy) for bacteria and Sabouraud dextrose broth (SDB) for yeasts and fungal strains were used.

3.4.2. *Antimicrobial screening*

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC), which includes minimum bactericidal (MBC) and minimum fungicidal concentrations (MFC), as previously described (Rigano et al. 2011), using the broth dilution method (Barry, 1976). Oil samples were tested in triplicate.

4. Conclusion

Essential oils of *C. haussknechtii* and *C. tomentella* showed a significant biocidal activity against microorganism like *Bacillus subtilis, Staphylococcus aureus, Fusarium oxysporum* and *Aspergillus niger,* affecting cellulosic materials stored in archives and libraries as well as historical textiles objects. The study of these plants can be crucial to find possible applications in the protection and disinfestation of museum objects.

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Table 1. Percent composition of the essential oils of *Centaurea hausschnetchii* Boiss and *Centaurea tomentella* Hand.-Mazz. aerial parts (subgenus *Cynaroides*).

RI[a]	RI[b]	Component	CH[c]	CT[d]	Ident.[e]
		Monterpene hydrocarbons	**0.8**	**7.2**	
1012	1157	δ^3-Carene		0.8	1, 2
1025	1278	*p*-Cymene	0.4	2.4	1, 2, 3
1030	1203	Limonene	0.3	2.3	1, 2, 3
1057	1256	*γ*-Terpinene	0.1	1.7	1, 2, 3
		Oxygenated monoterpenes	**0.6**	**0.4**	
1174	1567	*cis*-3-Pinanone	0.1		1, 2
1178	1673	Isopinocampheol	0.2		1, 2
1201	1613	Safranal	0.3	0.2	1, 2
1232	1772	Citronellol		0.2	1, 2
		Sesquiterpene hydrocarbons	**8.6**	**11.4**	
1363	1492	Cyclosativene	1.2	2.0	1, 2
1373	1493	*α*-Ylangene	0.4	1.2	1, 2
1377	1497	*α*-Copaene	0.3		1, 2
1393	1527	*iso*-Longifolene	0.7		1, 2
1403	1587	Longifolene	0.1		1, 2
1414	1612	*β*-Caryophyllene	0.6		1, 2, 3
1428	1654	Calarene (*β*-Gurjunene)		0.2	1, 2
1433	1620	Widdrene		0.1	1, 2
1465	1687	*γ*-Selinene	0.6		1, 2
1477	1726	Germacrene D	2.5	2.5	1, 2
1478	1704	*γ*-Muurolene	0.4		1, 2
1482	1741	*β*-Eudesmene	t[f]		1, 2
1487	1679	*α*-Amorphene	t		1, 2
1506	1760	(*E*,*E*)-*α*-Farnesene		0.4	1, 2
1513	2354	*δ*-Guaiene (*α*-Bulnesene)	0.1		1, 2
1535	2093	*α*-Cadinene		0.6	1, 2
1541	1918	*α*-Calacorene	1.0	1.3	1, 2
1554	1856	Germacrene B	0.2	1.4	1, 2
1568	1918	*β*-Calacorene	0.5		1, 2
1677	2256	Cadalene		1.7	1, 2
		Oxygenated sesquiterpenes	**10.3**	**20.4**	
1553	2076	*cis*-α-Copaen-8-ol		0.5	1, 2
1564	2056	Ledol		0.2	1, 2
1578	2150	Spathulenol	2.9	4.8	1, 2
1580	2008	Caryophyllene oxide		6.6	1, 2, 3
1596	2145	α-Cedrol		t	1, 2
1598	2107	Guaiol	0.3		1, 2
1599	2178	Widdrol	0.7	0.3	1, 2
1615	2324	Caryophylla-4(12),8(13)-dien-5*α*-ol	t	1.4	1, 2
1622	2182	*nor*-Copaanone		t	1, 2
1628	2185	Isospathulenol		0.5	1, 2
1648	2398	Aromadendrene oxide	2.1	1.1	1, 2
1648	2258	*β*-Eudesmol	1.7	3.5	1, 2
1662	2255	Vulgarone B	1.7		1, 2
1682	2356	Bisabolene oxide	0.1	1.5	1, 2
1689	2359	8-Cedren-13-ol		0.8	1, 2
1692	2342	(2*Z*,6*E*)-Farnesol	0.8		1, 2
1816	2567	Nootkatone		0.2	1, 2
		Carbonylic compounds	**2.5**	**8.5**	
963	1543	Benzaldehyde	0.2	t	1, 2, 3
967	1338	(*E*)-2-Heptenal		0.6	1, 2
1015	1507	(*E*,*E*)-2,4-Heptadienal		t	1, 2
1044	1663	Phenyl acetaldehyde	0.6	t	1, 2, 3
1104	1401	Nonanal		0.1	1, 2, 3
1206	1510	Decanal	t	0.4	1, 2, 3

1265	1595	(*E*)-2-Decenal		t	1, 2
1305	1779	(*E,Z*)-2,4-Decadienal		1.1	1, 2
1315	1827	(*E,E*)-2,4-Decadienal		0.3	1, 2
1335	1927	2,4,6-Trimethylbenzaldehyde		1.6	1, 2
1382	1838	(*E*)-*β*-Damascenone	1.7	0.9	1, 2
1385	1836	(*Z*)-*β*-Damascenone		0.3	1, 2
1436	1805	*α*-Ionone		t	1, 2
1484	1958	(*E*)-*β*-Ionone		0.3	1, 2
1845	2131	Hexahydrofarnesyl acetone		2.9	1, 2
		Fatty acid and esters	**49.6**	**29.5**	
1278	2190	Nonanoic acid		0.2	1, 2, 3
1424	2178	γ-Decalactone		t	1, 2
1364	2295	Decanoic acid		t	1, 2, 3
1467	2419	Undecanoic acid		0.1	1, 2, 3
1567	2503	Dodecanoic acid	1.8	1.1	1, 2, 3
1680	2396	*γ*-Dodecalactone		0.3	1, 2
1768	2723	Tetradecanoic acid	2.3	2.9	1, 2, 3
1870	2822	Pentadecanoic acid		0.5	1, 2, 3
1972	2931	Hexadecanoic acid	26.2	19.7	1, 2, 3
2104	3160	(*Z,Z*)-9,12-Octadecadienoic acid	19.3	2.4	1, 2, 3
2120	3095	(*Z*)-9-Octadecenoic acid	t	1.5	1, 2, 3
2172	3172	Octadecanoic acid	t	0.8	1, 2, 3
		Phenolic compounds	**4.5**	**2.5**	
1189	11789	Methyl salicylate	0.5	0.2	1, 2, 3
1239	1607	Thymol methyl ether		0.3	1, 2, 3
1293	2198	Thymol		1.2	1, 2, 3
1312	2180	4-Vinylguaiacol	3.5	0.8	1, 2
1353	2186	Eugenol	0.5		1, 2, 3
		Hydrocarbons	**12.5**	**8.7**	
1194	1197	Dodecene-1		0.1	1, 2
1353	1721	1,2-Dihydro-1,1,6-trimethylnaphthalene	0.8		1, 2
1373	1549	1,4,6-Trimethyl-1,2-dihydro-naphthalene	0.3		1, 2
2300	2300	Tricosane		1.7	1, 2, 3
2400	2400	Tetracosane		0.2	1, 2, 3
2500	2500	Pentacosane	1.0	1.4	1, 2, 3
2600	2600	Hexacosane		0.1	1, 2, 3
2700	2700	Heptacosane	5.3	2.8	1, 2, 3
2800	2800	Octacosane		0.2	1, 2, 3
2900	2900	Nonacosane	5.1	1.1	1, 2, 3
3000	3000	Triacontane		0.2	1, 2, 3
3100	3100	Hentriacontane		0.9	1, 2, 3
		Others	**1.9**	**2.4**	
1002	1243	2-Pentylfuran	0.2	0.5	1, 2
1235	1916	Benzothiazole		t	1, 2
1290	1471	Indole	0.1	t	1, 2
1487	2352	Dihydroctinidiolide	1.2		1, 2
1535	2296	Myristicine	0.4		1, 2
2821	3047	Squalene		1.9	1, 2
		TOTAL	**91.3**	**91.0**	

[a]: HP-5 MS column; [b]: HP Innowax column [c]: **CH** = *Centaurea hausschnetchii Boiss* aerial parts [d]: **CT** = *Centaurea tomentella* Hand.-Mazz.; [e]: 1: retention index, 2: mass spectrum, 3: co-injection with authentic compound; [f]: t: trace, <0.05.

Table 2. MIC (µg/mL) and MMC * (µg/mL) of essential oils of *Centaurea hausschnetchii* Boiss (**CH**) and *Centaurea tomentella* Hand.-Mazz. (**CT**) aerial parts.

Strain	**CH**	**CT**	**Chloramphenicol**	**Ketoconazole**
Bacillus subtilis ATCC 6633	12.5(25)	12.5	12.5	NT
Staphylococcus aureus ATCC 25923	25(50)	50	25	NT
Staphylococcus epidermidis ATCC 12228	50	50	3.12	NT
Streptococcus faecalis ATCC 29212	50(100)	50	25	NT
Proteus vulgaris ATCC 13315	100(100)	100	25	NT
Pseudomonas aeuriginosa ATCC 27853	100(>100)	100(>100)	100	NT
Fusarium oxysporum ATCC 695	6.25(12.5)	25	NT	3.12
Aspergillus niger ATCC 16401	6.25	25	NT	3.12

*MMC are reported in brackets when different from MIC; NT: not tested.