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Anthemis cotula L. from Jordan: Essential oil composition, LC-ESI-MS/MS profiling of phenolic acids - flavonoids and *in vitro* antioxidant activity



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Abstract Essential oils of the leaves and flowers of *Anthemis cotula* L. (family Asteraceae) grown in Jordan were extracted by hydro-distillation and then analyzed by GC–MS. Sesquiterpenes hydrocarbons (SH) were the dominant components in the oils extracted from leaves and flowers of *A. cotula*. γ -Murolene and aromadendrene, were the major compounds that were obtained from the flowers oil, while γ -murolene and *trans*-cadinene ether were detected as major ingredients in the leaves extract. LC-MS analysis was carried out to identify the significant compounds from each extract. Additionally, butanol (B), aqueous methanol (M) and water (W) extracts prepared from the flowers and the leaves of *A. cotula* were analysed by LC-MS/MS. Apigenin and chlorogenic acid were the main constituents detected in the flowers' alcoholic extracts and leaves' aqueous extract. Moreover, the essential oils and all prepared extracts were assayed for their total antioxidant activity using the DPPH, ABTS, and ferrous ion chelating effect (FIC) assay methods. All investigated

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oils and extracts showed interesting activity as compared to the positive controls employed (α -tocopherol and ascorbic acid).

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1. Introduction

The genus *Anthemis* belonging to the Asteraceae (Compositae) family, is nowadays defined as the second largest genus of the Anthemideae tribe, comprising approximately 210 species widely distributed throughout Europe and the Mediterranean basin, spanning to the south of the Arabian Peninsula and some tropical regions (Fernandes 1976; Bremer 1994; Maggio et al. 2014). It represents a heterogeneous combination of annual and perennial herbs and dwarf shrubs of attractive and beautiful heads (Maggio et al. 2014). In Jordan, sixteen *Anthemis* species were reported to grow wild all over the country (Al-Eisawi 1982; Oran and Al-Eisawi, 1998). Folk herbal medicine uses *Anthemis* species mainly for the treatment of gastrointestinal disorders, hemorrhoids, dysmenorrhea, and stomach ache. Also, members of this genus have been found to possess antibacterial, antispasmodic, anti-inflammatory, hepatoprotective, anticholinesterase, antibiofilm and antioxidant activities (Fernandes 1976; Bremer 1994; Saroglou et al. 2006; Ghafoor 2010; Riccobono et al. 2017; Chemsal et al. 2018). Phytochemical investigation of several *Anthemis* plants revealed the presence of sesquiterpene lactones, polyacetylenes, flavonoids, and essential oils (Bohlmann et al. 1963; Bohlmann et al. 1965a; Bohlmann et al. 1965b; Bohlmann and Kleine 1966; Bulatovic et al. 1997; Williams et al. 2001; Van et al. 2003; Pavlovic et al. 2006; Staneva et al. 2008; Saroglou et al. 2010; Gonenc et al. 2012; Tuba et al. 2014; Doğan et al. 2015; Chemsal et al. 2018)).

A. cotula L. is an annual glandular plant, characterized by its harsh taste and intensive acrid smell with 12 to 24 in. in height (Zohary 1966; Oran and Al-Eisawi, 1998). *A. cotula* is well-known in folk herbal medicine for the treatment of psoriasis, fever, gastrointestinal problems, dysentery, and gouty arthritis (Quarengi et al. 2000).

Previous investigations on the aerial parts of *A. cotula* revealed the presence of 7-methoxy-6-acetyl-2,2-dimethylchromene, quercetagenin, quercetagenin-7-glucoside, quercetin, quercetin-7-glucoside, patuletin,

patuletin-7-glucoside, kaempferol, kaempferol-7-glucoside, kaempferol-3-rutinoside, apigenin and hispidulin in its extract (Quarengi et al. 2000). From the aerial parts of *A. cotula*, six linear sesquiterpene lactones were isolated (Van et al. 2003). In addition, investigation of the extracts obtained from the roots of *A. cotula* afforded four acetylenes, three prenylated acetophenones, and polyacetylenes (Bohlmann et al. 1965a; Bohlmann et al. 1965b; Van et al. 2003; Vucković et al. 2006). A previous work by Rezaei and Jaymand showed variable qualitative and quantitative composition of the essential oils obtained from the flowers and the leaves (Rezaei and Jaymand 2007). The major components of the flower oil were *n*-nonadecane, cedrane, and (E, E)- α -farnesene, while the leaves oil contained 1-eicosane, benzyl salicylate, and aromadendrene (Rezaei and Jaymand 2007). It is worth mentioning that *A. cotula* from Jordan has neither been investigated for its essential oil, phenolic acids and flavonoids content nor evaluated for its antioxidant activity.

In the current study, essential oils extracted by hydrodistillation (HD) from the flowers and leaves were investigated for their chemical composition by GC/MS. Additionally, these two organs were also subjected separately to extraction with methanol and water. The obtained extracts were then analysed for their phenolic and flavonoid compounds by LC-MS/MS. Moreover, the hydrodistilled essential oils, butanol (B), aqueous methanol (M) and water (W) extracts were screened for their *in vitro* antioxidant activities using the DPPH, ABTS and FIC assay methods.

2. Results and discussion

2.1. Essential oil composition

Hydrodistillation (HD) of from the *A. cotula* flowers and and leaves afforded afforded yellow-colored oils (yields: 0.05 %

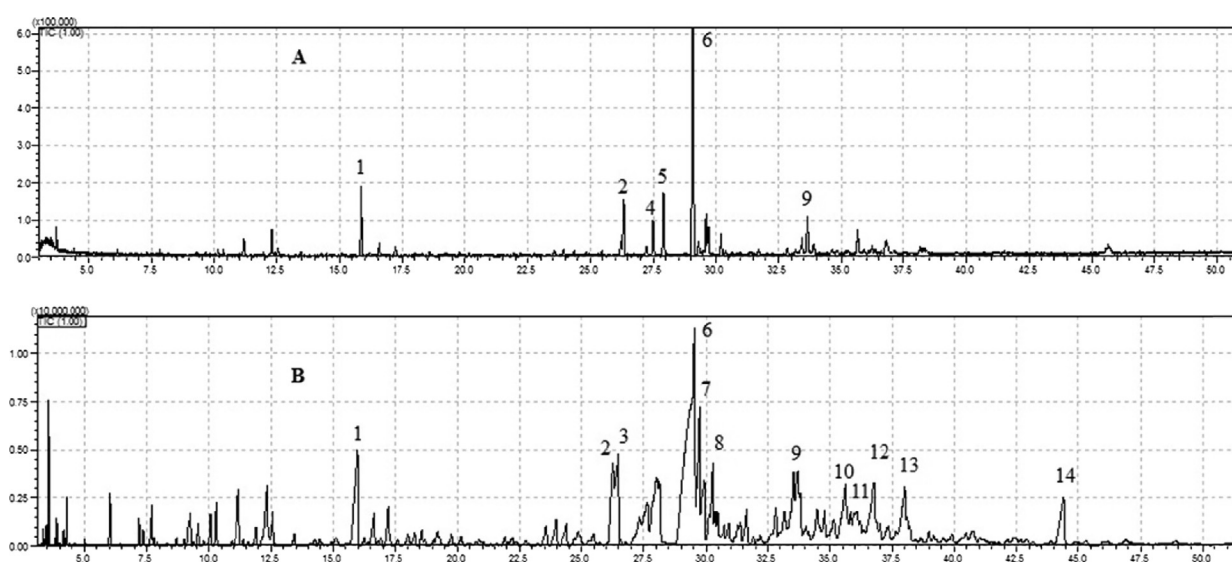


Fig. 1 GC chromatogram of essential oils from the flowers (A) and leaves (B) of *A. cotula* L. (1: α -terpineol; 2: Z-caryophyllene; 3: β -cedrene; 4: neryl acetate; 5: aromadendrene; 6: γ -muurolene; 7: Germacrene D; 8: δ -Selinene; 9: *trans*-cadinene ether; 10: ledol; 11: β -oplophenone; 12: lepi-cubenol; 13: β -eudesmol; 14: *n*-heptadecanol.

and 0.08 % (w/w), respectively). The obtained HD-essential oils were then analyzed using GC/MS and GC/FID techniques (Fig. 1). The main components, their Kovats retention indices (KIs) and relative concentration values are summarized in Table 1. The compounds are arranged in order of their elution from the DP-5 column (Table 1).

A total of 73 compounds were identified in the leaves hydrodistilled essential oils that amounted to 100 % of the total oil content. On the other hand, 30 compounds (accounting for 99.05 % of the total content) were identified in the flowers' hydrodistilled-essential oil. The components were classified based on their chemical structures into 5 classes (Fig. 2): monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and others (aliphatic alkane, alkene, alcohols, acids, and esters). The main class of compounds detected in the leaves and flowers oils was sesquiterpenes hydrocarbons (leaves HD-oil: 54.29 %; flowers HD-oil: 65.39 %). Oxygenated monoterpenes accounted for 14.31 %, 17.97 % of the total content of HD-oils of the leaves and flowers oils respectively.

The major constituents in leaves oil were γ -muurolene (19.03 %), *trans*-cadinene ether (6.49 %), germacrene D (6.02 %), 1-*epi*-cubenol (4.57 %), and aromadendrene (3.93 %). For the flowers oil, γ -muurolene (34.20 %), aromadendrene (7.18 %) β -cedrene (6.81 %), α -terpineol (6.80 %), and δ -selinene (5.94 %).

Results of the current investigation indicated noticeable qualitative and quantitative differences between the essential oils obtained from leaves and flowers. Many components were characteristic in one organ. *n*-Heptadecanol (2.41 %), selina-3,7(11)-diene (1.38 %), *epi*-cedrene (1.12 %), α -pinene (0.56 %) and linalool (0.44 %), were detected in the leaves, while davanol D2 (1.13 %), *n*-hexadecanol (0.78 %), methyl undecanoate (0.77 %) and *epi*-cedrol (0.70 %) were detected in the flowers oil. Of all 77 compounds detected in the oils of both organs, only 26 compounds were common.

Previous investigations related to essential oil composition of *Anthemis* species revealed the richness of oxygenated monoterpenes, such as α - and β -thujone, yomogi alcohol, borneol, terpinen-4-ol, and α -terpineol, and by terpene esters, especially *cis*-chrisanthemyl acetate and other chrysanthemyl esters (Pavlović et al. 2006; Saroglou et al. 2006; Gonenc et al. 2012; Bardaweel et al. 2014; Doğan et al. 2015; Tawaha et al., 2015). The findings of the current study were quite different. The essential oils obtained from the flowers and leaves of *A. cotula* from Gilan province included each of *n*-nonadecane and cedrane (flowers oil), and 1-eicosane and benzyl salicylate (leaves oil) (Rezaei and Jaymand 2007). The chemical variability between the oils reported in these studies could be due to the environmental factors (Barra 2009).

2.2. LC-MS/MS profiling of phenolic acids and flavonoids alcoholic and water extracts

The leaves and flowers of *A. cotula* from Jordan were subjected separately to the extraction procedure described in the experimental section to prepare the butanol (B), aqueous methanol (M) and water (W) fractions. Then these fractions were subjected to analysis using LC-ESI-MS/MS technique to determine their chemical profiles. Twenty constituents numbered

1–20 were detected and tentatively identified belonging to both phenolic acid and flavonoid. The molecular formulas of all identified compounds and their relative concentrations are shown in Table 2. By comparing the extract's contents with standard references, the phenolic components were identified. The results showed that the B extracts of leaves and flowers of *A. cotula* contained appreciable amounts of phenolic acids (5.25%) and flavonoids (26.32%) (Table 2). The M extracts of leaves contained slightly lower contents of phenolic acids (3.93 %) but much greater amounts of flavonoids (67.82 %). Flowers M extract contained the highest content of flavonoids (77.53 %) when compared to all other investigated extracts. On the other hand, the W extract of the leaves contained the highest content of chlorogenic acid (77.25 %), as compared to all other extracts. The leaves M extract contained cinnamic acid, vitexin, luteolin, and apigenin as major components. While B extract was characterized by the presence of apigenin, quercetin, and luteolin.

The B extract of the flowers was characterized by the presence of chlorogenic acid, apigenin, Luteolin, and 7-hydroxy coumarin, The M extract of flowers revealed the presence of apigenin, vitexin, cinnamic acid, and luteolin. In addition, in the W extract of flowers cinnamic acid, apigenin, vitexin, and ellagic acid were detected.

Due to the high content of phenolic acids and flavonoids in the extracts of *A. cotula*, the plant could be considered as a promising source for natural antioxidant agents. In the light of the current results, the oils and extracts were assayed for their antioxidant activities using the DPPH, ABTS and FIC assay methods.

2.3. Antioxidant activity

Several methods are used to evaluate the antioxidant and the free radical scavenging activity of plant extracts (Al-Jaber et al. 2014; Al-Qudah et al. 2014; Al-Qudah 2016; Al-Jaber et al. 2018; Al-Qudah et al. 2018a; Al-Qudah et al. 2018b; Al-Humaidia et al. 2019; Abu-Orabi et al. 2020). In the present study, the antioxidant and radical scavenging capacity of the hydrodistilled essential oils and all prepared extracts including the M, B, and W extracts of the leaves and flowers of *A. cotula* were evaluated. Three different methods were used including the DPPH, ABTS radical scavenging, and the ferrous ion chelating activity (FIC) assay methods. The measurements and results are given in Fig. 3. The IC₅₀ values were compared with those of α -tocopherol and ascorbic acid as positive controls (Table 3). Higher radical scavenging activities are associated with lower IC₅₀ values.

The DPPH radical scavenging activities of the essential oil and crude extracts of leaves and flowers of *A. cotula* quantified in terms of inhibition percentage (%) are presented graphically in Fig. 3. The results show that the DPPH radical scavenging activities are concentration dependent and had the following order: M > B > essential oil > W. The DPPH radical scavenging activity of the flowers' different extracts had the following order: B > W > M > essential oil (Table 3).

The ABTS^{•+} radical scavenging of the leaves and flowers extracts, as well as the standards increased with increasing concentrations (Fig. 3). The order of ABTS^{•+} radical scavenging activity of the different extracts obtained from the leaves had

Table 1 The chemical composition of the essential oils obtained from leaves and flowers of *A. cotula* L.

No.	¹ KI _{exp}	Name of compounds	Relative percentage amounts (%)		² Identification
			Leaves	Flowers	
1	806	4E-Octene	0.13	0.89	a, b
2	813	2Z-Octene	0.14	0.68	a, b
3	818	2E-Octene	0.9	—	a, b
4	830	Perenyl acetate	0.05	0.98	a, b
5	835	isoValeric acid	0.18	—	a, b
6	849	3E-Hexenol	0.12	—	a, b
7	856	2EHexenal	0.34	—	a, b
8	930	α - Pinene	0.56	—	a, b, c
9	970	Verbenene	0.31	—	a, b, c
10	975	Sabinene	0.18	—	a, b, c
11	987	β -Pinene	0.57	—	a, b, c
12	990	<i>cis</i> -meta-Menta-2,8-diene	0.1	—	a, b
13	1015	α - Terpinene	0.09	—	a, b, c
14	1023	p-Cymene	0.08	—	a, b, c
15	1028	1-p-Menthene	0.48	—	a, b
16	1029	o-Cymene	0.4	—	a, b
17	1037	3E-Octene-2-one	0.32	—	a, b
18	1050	E- β - Ocimene	0.49	—	a, b, c
19	1055	γ - Terpinene	0.63	—	a, b, c
20	1079	<i>cis</i> - Vertocitral C	1.38	1.89	a, b
21	1083	Terpinolene	0.09	—	a, b
22	1097	Linalool	0.44	—	a, b, c
23	1107	α -Thujone	1.71	2.18	a, b, c
24	1108	2E,4E-Octadienol	0.16	—	a, b
25	1112	<i>trans</i> -Thujone	0.68	1.34	a, b
26	1131	α -Campholenol	0.17	—	a, b
27	1188	α -Terpineol	3.76	6.8	a, b
28	1203	γ - Terpeneol	0.82	1.35	a, b
29	1215	<i>trans</i> -Pulegol	1.05	0.67	a, b
30	1232	Nerol	0.21	—	a, b, c
31	1238	E-Ocimenone	0.18	—	a, b
32	1244	Hexyl-2E-butanoate	0.39	—	a, b
33	1247	2E-Hexenyl isovalerate	0.12	—	a, b
34	1257	Thymoquinone	0.25	—	a, b, c
35	1260	Benzyl propanoate	0.08	0.1	a, b
36	1270	<i>n</i> -Decanol	0.14	—	a, b, c
37	1315	<i>cis</i> -Dihydro- α -terpinylacetate	0.11	—	a, b
38	1350	Citronellyl acetate	0.39	—	a, b
39	1359	<i>neiso</i> -Dihydrocarveol acetate	0.75	—	a, b
40	1368	Cyclosativene	0.75	—	a, b
41	1379	α -Copaene	0.21	—	a, b
42	1408	Z-Caryophyllene	3.18	1.65	a, b, c
43	1413	β - Cedrene	3.23	6.81	a, b, c
44	1415	β -Duprezianene	0.08	—	a, b
45	1429	Methylundecanoate	—	0.77	a, b
46	1431	Neryl acetate	2.41	3.74	a, b
47	1446	Aromadendrene	3.93	7.18	a, b
48	1448	<i>epi</i> -Cedrene	1.12	—	a, b
49	1478	γ -Muurolene	19.03	34.2	a, b, c
50	1486	Germacrene D	6.02	1.66	a, b
51	1494	δ - Selinene	2.59	5.94	a, b
52	1496	γ - Amorphene	1.4	2.26	a, b
53	1504	α -Muurolene	0.53	1.65	a, b
54	1508	E,E- α -Farnesene	0.68	—	a, b
55	1517	Z- α -Bisabolene	1.1	—	a, b
56	1523	δ - Cadinene	0.97	—	a, b
57	1547	Selina-3,7(11)-diene	1.38	—	a, b
58	1553	<i>cis</i> -Cadinene ether	1.6	1.77	a, b
59	1565	<i>trans</i> -Cadinene ether	6.49	3.93	a, b
60	1572	α -Cedrene epoxide	0.87	1.17	a, b
61	1581	<i>n</i> -Hexylbenzoate	1.59	—	a, b

Table 1 (continued)

No.	¹ KI _{exp}	Name of compounds	Relative percentage amounts (%)		² Identification
			Leaves	Flowers	
62	1587	Caryophellene oxide	0.87	–	a, b, c
63	1595	Globulol	1.21	–	a, b
64	1604	Ledol	3.41	2.56	a, b, c
65	1613	β-Oplopenone	3.08	1.44	a, b
66	1619	epi-Cedrol	–	0.7	a, b
67	1626	1-epi-Cubenol	4.57	2.07	a, b
68	1627	Davanol D2	–	1.13	a, b
69	1636	γ- Eudesmol	0.81	–	a, b
70	1651	β- Eudesmol	3.64	0.76	a, b
71	1669	E-Bisabol-11-ol	0.29	–	a, b
72	1673	Spathulenol	0.25	–	a, b
73	1687	Davanone	0.52	–	a, b
74	1698	Acorenone B	0.27	–	a, b
75	1703	n-Heptadecane	0.56	–	a, b, c
76	1773	n-Heptadecanol	2.41	–	a, b
77	1869	n-Hexadecanol	–	0.78	a, b
		Total			

¹ Kovats retention indices on a DP-5 column, ²Identification method: a = linear retention index, b = identification based on comparison of mass spectra, c = co-injection with standard compounds.

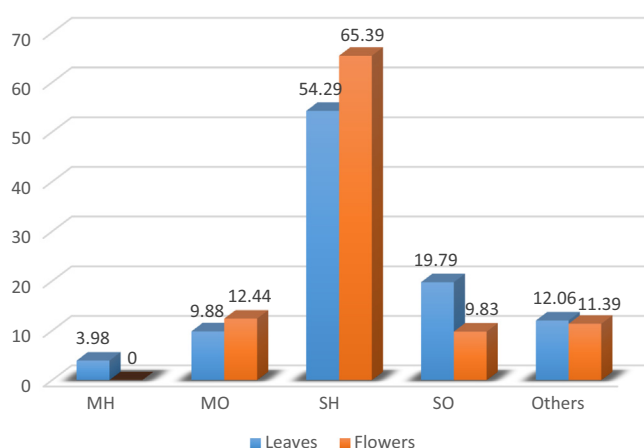


Fig. 2 Variation of essential oils composition between leaves and flowers oil from *A. cotula* L. Monoterpenes hydrocarbons (MH), Oxygenated monoterpenes (OM), Sesquiterpenes hydrocarbons (SH), Oxygenated sesquiterpenes (OS).

the following order: **M** > **B** > essential oil > **W**. For the flowers extracts the order was **B** > **W** > **M** > essential oil (Table 3).

The essential oil and crude extracts from leaves and flowers of *A. cotula* were evaluated for their ferrous ion chelating activity, which was found to increase with increasing concentrations, as shown in Fig. 3. The values of IC₅₀ of FIC of the essential oils and extract fractions of *A. cotula* L. on Fe²⁺ and ferrozine complex formation are shown in Table 3. The results showed that the **M** extracts obtained from the leaves had the best chelating activity for ferrous ions (IC₅₀ = 29.00 ± 0.50 × 10⁻² mg/L) as compared with the other crude extracts. Yet, the flowers **B** extracts had the highest chelating activity for ferrous ions (IC₅₀ = 7.60 ± 0.50 × 10⁻² mg/L).

The antioxidant efficiency of different extracts obtained from the leaves and flowers of *A. cotula* has been attributed mainly to their high phenolic and flavonoids contents, being especially rich in chlorogenic acid, cinnamic acid, vitexin, apigenin, quercetin, and luteolin (Romanova et al. 2001; Babaei et al. 2020). For the essential oils, the observed activity could be related to their high sesquiterpene hydrocarbon contents that are recognized as strong antioxidants (Victoria et al. 2012).

3. Materials and methods

3.1. Instrumentation

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using Agilent 6890 series II – 5973 mass spectrometers interfaced with HP chemstation. The UV-spectra were recorded on wave light II UV-visible spectrophotometer. A Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Daltonik Elute UHPLC system (Bremen, Germany) was used for screening flavonoids and phenolics compounds.

3.2. Materials and chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-Azino-bis(3-ethyl benzthiazoline-6-sulphonic acid) diammonium salt (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-P,P'-disulfonic (Ferrozin), Iron(II) chloride, Potassium persulfate, anhydrous sodium carbonate, Aluminum chloride, Sodium hydroxide, Methanol, *n*-Butyl Alcohol, Petroleum ether and Hexane were used as purchased without further purification.

3.3. Plant material

Plant material was collected from Irbid (AL Koura region (N 32.458559; E 35.676982), Jordan in 2019. Botanical authenti-

Table 2 LC-MS/MS data for standard phenolic and flavonoid compounds detected in the crude extracts *A. cotula* L. from Jordan.

No.	Rt (min)	Compound	Molecular Formula	M.wt	Relative peak area percentage (%)					
					Leaves			Flowers		
					B*	M*	W*	B*	M*	W*
1	1.9	Gallic acid	C ₇ H ₆ O ₅	170.12	–	–	–	2.31	–	–
2	3.2	Syringic acid	C ₉ H ₁₀ O ₅	198.17	–	–	–	1.37	–	–
3	3.5	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.31	0.57	–	77.25	22.55	1.43	5.38
4	3.9	Aesculetin	C ₉ H ₆ O ₄	178.14	1.32	–	2.77	3.39	–	–
5	4.0	4-Hydroxy coumarin	C ₉ H ₆ O ₃	162.14	–	–	–	11.43	–	–
6	4.0	Catechin	C ₁₅ H ₁₄ O ₆	290.27	0.74	–	–	0.39	–	–
7	4.5	Ellagic acid	C ₁₄ H ₆ O ₈	302.19	–	–	–	–	0.67	6.83
8	4.6	Vitexin	C ₂₁ H ₂₀ O ₁₀	432.38	7.42	20.39	5.88	2.16	20.85	7.79
9	4.6	Isoorientin	C ₂₁ H ₂₀ O ₁₁	448.38	0.88	3.95	2.92	0.73	4.53	–
10	4.7	Gallic acid ethyl	C ₉ H ₁₀ O ₅	198.17	–	–	–	0.58	–	–
11	4.7	Cinnamic acid	C ₉ H ₈ O ₂	148.16	1.61	26.53	–	–	16.77	48.69
12	4.9	7-Hydroxy coumarin	C ₉ H ₆ O ₃	162.14	0.87	1.71	–	11.42	3.56	–
13	5.0	Naringin	C ₂₇ H ₃₂ O ₁₄	580.54	0.90	–	4.45	0.88	3.00	8.00
14	5.2	Hyperoside	C ₂₁ H ₂₀ O ₁₂	464.40	4.68	3.93	–	0.09	–	2.46
15	5.2	Hesperidin	C ₂₈ H ₃₄ O ₁₅	610.57	1.19	2.82	3.05	2.14	3.20	–
16	5.3	Rutin	C ₂₇ H ₃₀ O ₁₆	610.52	0.77	1.59	–	2.10	0.63	–
17	6.5	Quercetin	C ₁₅ H ₁₀ O ₇	302.24	27.30	2.52	3.67	2.82	1.69	–
18	6.8	Luteolin	C ₁₅ H ₁₀ O ₆	286.24	14.87	15.29	–	16.95	8.46	–
19	7.6	Apigenin	C ₁₅ H ₁₀ O ₅	270.24	35.44	12.99	–	8.97	32.54	28.83
20	7.6	Kaempferol	C ₁₅ H ₁₀ O ₆	286.24	1.41	8.27	–	0.70	2.63	–

* B: butanal extract, M: Aq. Methanol extract, W: water extract.

cation of the plant species was confirmed by the botanist Prof. Jamil N. Lahham (Yarmouk University, Department of Biological Sciences). The aerial parts were dried at room temperature (in the shade for about a month). A reference specimen (AC/C/2019–1) was stored in Prof. Mahmoud A. Al-Qudah Laboratory, Yarmouk University, Irbid, Jordan.

3.4. Extraction and fractionation

The fresh aerial parts of the plant material were dried at room temperature in a shady place for a month. The air-dried powdered plant samples were successively extracted in Soxhlet extractor with petroleum ether to remove fatty acids and followed by methanol. The plant material was dried before the following extraction process with the next solvent system. The extracts were concentrated by a rotary vacuum evaporator and then dried. The alcohol residue was partitioned between CHCl₃ and H₂O (1:1). After the separation of CHCl₃ and H₂O phases, the dried CHCl₃ fraction was partitioned between 10 % aqueous methanol and hexane (H) yielding the aqueous methanol (M) and hexane (H) fractions. The polar organic compounds were extracted from water by *n*-butanol affording Water (W) and butanol (B) fractions. The extracts obtained were used directly for the estimation of the assessment of antioxidant potential through various chemical assays.

3.5. Essential oil extraction

Fresh leaves or flowers (200 g) of *A. cotula* were finely chopped and subjected to the classical hydrodistillation method using a Clevenger-type apparatus for 4 h. Subsequently, oils were dried over anhydrous sodium sulfate and immediately stored

in GC-grade *n*-hexane at 4 °C by gas chromatography/mass spectrometry (GC/MS).

3.6. GC–FID analysis

The oils were analyzed by an Agilent (Palo Alto, USA) 6890 N gas chromatograph fitted with a 5 % phenyl–95 % methyl silicone (HP5, 30 m × 0.25 mm × 0.25 μm) fused silica capillary column. The oven temperature was programmed from 60 °C to 240 °C at 3 °C/minute and hydrogen was used as a carrier gas (1.4 mL/minute), and 1.0 μL of a 1 % solution of the oils in hexane was injected in split mode (1:30). The injector was kept at 250 °C and the flame ionization detector (FID) at 280 °C. Concentrations (% contents) of oil ingredients for *A. cotula* L. species were determined using their relative area percentages obtained from GC chromatogram, assuming a unity response by all components.

3.7. GC–MS analysis

Chemical analysis of the essential oils was carried out using gas chromatography-mass spectrometry (GC–MS). The chromatographic conditions were as follows: column oven program, 60 °C (1 min, isothermal) to 246 °C (3 min, isothermal) at 3 °C/min, the injector and detector temperatures were 250 °C and 300 °C, respectively. Helium was the carrier gas (flow rate of 0.90 mL/min) and the ionization voltage was maintained at 70 eV. An HP-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. Retention indices (RIs) were calculated by the injection of a series of *n*-alkanes (C₈–C₂₀) in the same column under the same conditions specified above for gas chromatography analysis. Identification of

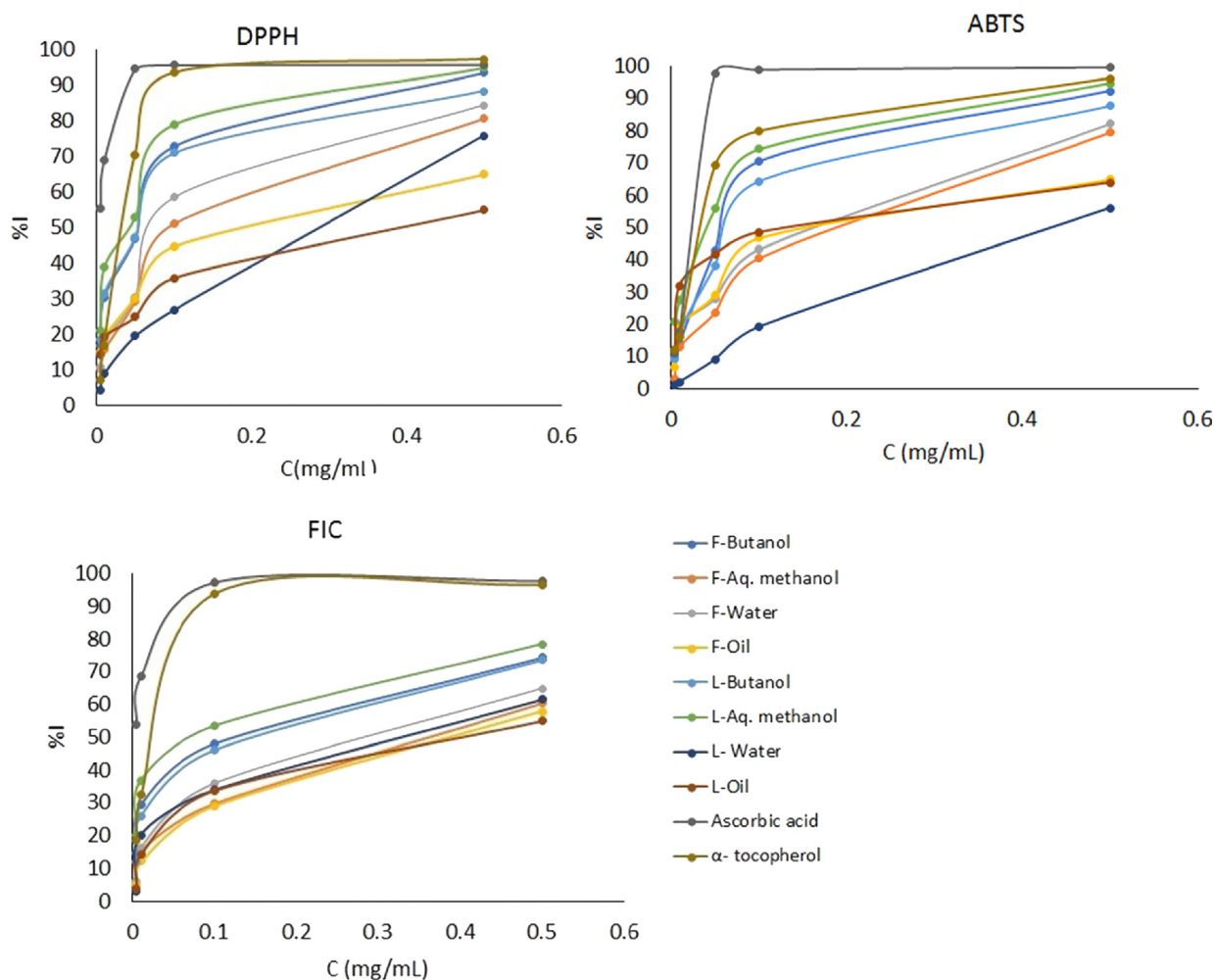


Fig. 3 Antioxidant activities of essential oils and crude extracts of the leaves and flowers from *A. cotula* and standards by using DPPH, ABTS and FIC methods.

Table 3 IC_{50} (mg/L) values of the essential oils and crude extracts from flowers and leaves of *A. Cotula* L. and standards by using DPPH, ABTS⁺ and FIC methods. Results are expressed as mean $\pm$ SD (n = 3).

Parts of plant	Crude	IC_{50} (mg/L)		
		DPPH	ABTS	FIC
Leaves	Aq. Methanol	$(23.00 \pm 0.20) * 10^{-2}$	$(23.00 \pm 0.60) * 10^{-2}$	$(29.00 \pm 0.50) * 10^{-2}$
	Butanol	$(24.00 \pm 0.10) * 10^{-2}$	$(25.00 \pm 0.30) * 10^{-2}$	$(31.00 \pm 0.40) * 10^{-2}$
	Water	$(62.80 \pm 5.40) * 10^{-2}$	$(75.20 \pm 6.30) * 10^{-2}$	$(41.20 \pm 0.30) * 10^{-2}$
	Oil	$(54.80 \pm 3.46) * 10^{-2}$	$(14.60 \pm 1.18) * 10^{-2}$	$(28.40 \pm 1.80) * 10^{-2}$
Flowers	Aq. Methanol	$(9.40 \pm 0.30) * 10^{-2}$	$(13.34 \pm 1.18) * 10^{-2}$	$(18.40 \pm 3.18) * 10^{-2}$
	Butanol	$(3.68 \pm 0.8) * 10^{-2}$	$(5.17 \pm 0.25) * 10^{-2}$	$(7.60 \pm 0.50) * 10^{-2}$
	Water	$(26.00 \pm 0.2) * 10^{-2}$	$(28.00 \pm 0.2) * 10^{-2}$	$(20.90 \pm 0.50) * 10^{-2}$
	Oil	$(17.00 \pm 2.80) * 10^{-2}$	$(21.08 \pm 1.60) * 10^{-2}$	$(36.70 \pm 5.90) * 10^{-2}$
Ascorbic acid		$(1.80 \pm 0.06) * 10^{-3}$	$(1.90 \pm 0.06) * 10^{-3}$	$(1.90 \pm 0.02) * 10^{-3}$
α -tocopherol		$(2.30 \pm 0.04) * 10^{-3}$	$(1.80 \pm 0.01) * 10^{-3}$	$(2.90 \pm 0.02) * 10^{-3}$

oil components was based on computer search using the library of mass spectral data and comparing the calculated retention indices (RIs) with those of the available authentic standards and literature data. Standard solutions of α -pinene, β -pinene, α -terpineol, β -cedrene, and γ -muurolene were also injected for confirmation.

3.8. LC-MS/MS profiling of phenolic acids and flavonoids

A Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Daltonik Elute UHPLC system (Bremen, Germany) was used for screening flavonoids and phenolics compounds of interest in both positive (M + H)

and negative (M – H) electrospray ionization modes. The Full Sensitivity Resolution (FSR) was 50000, the mass accuracy was < 1 ppm, and the TOF repetition rate was up to 20 kHz. Chromatographic separation was conducted using a 120, C18 reversed phase column, 100 × 2.1 mm, 1.8 µm (120 Å°) from Bruker Daltonik (Bremen, Germany) at 30 °C, autosampler temperature 8.0 °C with total run time 20.0 min using gradient grade. The elution gradient consisted of mobile phase A (water / methanol (90:10 %)) with 5 mM ammonium formate and 0.1 % formic acid) and solvent B (methanol with 5 mM ammonium formate and 0.1 % formic acid). The gradient program with the following proportions of solvent B was applied (%B, min): 40–90 % B (0.00–6.00 min), isocratic 90 % (6.00–10.00 min), isocratic 40 % (10.01–15.00 min). The solvent flow rate was 0.5 mL/min and the injection volume was 10 µL. MS/MS analysis was performed in negative ion mode with an ion spray voltage of – 4,500 V. Nitrogen gas at a pressure of 60 psi was used as the nebulizing and drying gas. The mass spectra were obtained over the *m/z* range of 100–1,000 amu. Each crude extract was dissolved in 2.0 mL DMSO and then completed to 50 mL using acetonitrile. After that, each sample was centrifuge at 4000 rpm for 2.0 min. 1.0 mL was taken and transfer to the autosampler in order to inject 3.0 µL.

3.9. Determination of antioxidant activity

The antioxidant activity of the essential oils was determined by DPPH, ABTS radical scavenging, and Ferrous Ion Chelating (FIC) assay methods using the procedure described in the literature (Al-Jaber et al. 2014; Al-Qudah et al. 2014; Al-Qudah 2016; Al-Jaber et al. 2018; Al-Qudah et al. 2018a; Al-Qudah et al. 2018b; Al-Humaidia et al. 2019; Abu-Orabi et al. 2020; Al-Qudah et al. 2020a). The percentage of scavenging activity was calculated using the equation:

$$\text{Scavenging activity(\%)} = [(A_c - A_s)/A_c] \times 100,$$

where A_c is the absorbance of the control and A_s is the absorbance in the presence of either extracts or control substance.

Non-linear regression analysis of GraphPad Prism 6 (GraphPad Software, San Diego, California, USA) was applied for the determination of IC_{50} in all of the antioxidant assays from the sigmoidal curve which was obtained by plotting the percentages of scavenging relative to the control *versus* logarithmic concentration of test essential oils. Each concentration was tested three times in 3 independent experiments.

4. Conclusion

In this study, the chemical composition of the essential oils obtained from the leaves and flowers of *A. cotula* L. grown in Jordan were analysed by GC/MS has been reported here for the first time. Additionally, fractions of different polarities were also screened for the presence of selected phenolic acids and flavonoids using LC-ESI-MS/MS. Sesquiterpenes hydrocarbons (SH) were the dominant components in the oils extracted from both organs. All investigated extracts were generally rich in phenolic acids and flavonoids with notable qualitative and quantitative differences. The high phenolic acids and flavonoids content contributed to the high antioxidant activities observed for all investigated extracts.

Declaration of Competing Interest

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.

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