



ORIGINAL ARTICLE

GC/MS and LC-MS/MS phytochemical evaluation of the essential oil and selected secondary metabolites of *Ajuga orientalis* from Jordan and its antioxidant activity



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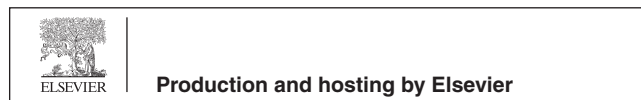
Ajuga orientalis;
Essential oil;
Antioxidant activity;
LC-ESI-MS/MS;
Total flavonoid content;
Total phenol content

Abstract The current investigation aimed to shed light in the volatile and non-volatile secondary metabolites of *Ajuga orientalis* L. from Jordan. GC/MS and GC/FID analysis of the hydrodistilled essential oil obtained from aerial parts of the plant revealed tiglic acid (18.90 %) as main constituent. Each of the methanol and butanol fractions of *A. orientalis* were screened for their total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity determined by DDPH and ABTS methods. The extracts were then analyzed by LC-ESI-MS/MS to unveil their chemical constituents, especially phenols and flavonoids. Results showed that the AO-B extract had the highest TPC (217.63 ± 2.65 mg gallic acid/g dry extract), TFC (944.41 ± 4.77 mg quercetin

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/g dry extract), highest DPPH and ABTS antioxidant activity ($(4.00 \pm 0.20) \times 10^{-2}$; $(3.00 \pm 0.20) \times 10^{-2}$ mg/mL, respectively) as compared to the AO-M extract. LC-ESI-MS/MS analysis of both extracts revealed the presence of several phenolics, flavonoids and nonphenolic acids.

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1. Introduction

Ajuga is one of the largest genera of the Lamiaceae (previously known as Labiateae) family (Amin 1991; Jalili and Jamzad 1999). Several species of this genus are well recognized as herbal remedies for the treatment of many ailments including gastrointestinal disorders, fever, dysentery, rheumatism, gout, asthma, diabetes, malaria, toothache and are reported to possess diuretic, antipyretic, tonic, diaphoretic and astringent properties (Chen et al., 1996; Ben Jannet et al., 2006; Israili and Lyoussi., 2009) in addition to their antibacterial, antitumor, antifeedant, antioxidant and neuroprotective effects (Turkoglu et al., 2010; Zerroug et al., 2011; Guo et al., 2011; Makni et al., 2013). *Ajuga* plants are known for their sundry of volatile and nonvolatile phytoconstituents including terpenoids, iridoids, sterols, flavonoids and many others (Teismann 2000; Küçükbay et al., 2013; Al-Qudah et al., 2014; Al-Qudah et al., 2017a,b).

Three *Ajuga* species were reported in the Flora of Jordan, these are *Ajuga chia* Schreber., *Ajuga Iva* L., and *A. orientalis* L. (Alhamad 2006., Oran 2015). *Ajuga orientalis* L. is a perennial herb that is 20–40 cm length characterized by its basal, erect wooly stems and blue violet colors. The plant is known to grow wild in humid places of Ajloun, Salt, Amman and Al-Karak. Flowering occurs in the spring season, during April and May (Al-Eisawi, 1998). Previous studies on phytochemical investigation of volatile constituents (Küçükbay et al., 2013; Sajjadi and Ghannadi 2004) and non-volatile secondary metabolites were limited (Oran et al., 2022), especially from Jordanian origin. Accordingly, the current study was designed to investigate the chemical composition of the hydro-distilled essential oil obtained from the aerial parts of *A. orientalis* (AO-HDEO) from Jordan and its antioxidant activity. Moreover, extracts of different polarities obtained from the aerial parts of the plant material were screened for their total phenols content (TPC), total flavonoids content (TFC) and antioxidant activities (by DPPH and ABTS methods). The presence of selected phenolic acids, flavonoids and other constituents in these extracts was determined by LC-ESI-MS/MS technique.

2. Experimental

2.1. General

Gas chromatography-Mass spectrometry (GC-MS) analysis was performed using Agilent 6890 series II – 5973 mass spectrometers interfaced with HP chemstation. UV-vis spectra were recorded on Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer. Detection of the selected phenolic acids, flavonoids and nonphenolic acids and compounds was done utilizing a Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Daltonik Elute UHPLC system (Bremen, Germany). *n*-Hexane (GC-grade), the *n*-alkanes (C₈-C₂₀) standard mixture, 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS, purity > 99 %), 2,2-diphenyl-2-picrylhydrazyl (DPPH, purity > 99 %), ascorbic acid (purity > 98 %), methanol, potassium persulfate, sodium carbonate, Folin and Ciocalteu's Phenol reagent, sodium nitrite, aluminum chloride, and sodium hydroxide were all products of Sigma-Aldrich.

2.2. Plant material

Fresh aerial parts of *A. orientalis* were collected at full flowering stage in April/2018 from Ajloun city, north of Jordan (N 32.363932; E 35.775043). The plant identity was confirmed by Prof. Dr. Jamil Laham, Yarmouk University, Irbid, Jordan. A voucher specimen (AO/L/2018) was deposited in Prof. Mahmoud A. Al-Qudah Laboratory, Department of Chemistry, Faculty of Science, Yarmouk University, Irbid, Jordan.

2.3. Hydro-distillation of essential oil and extracts preparation

Fresh aerial parts of *A. orientalis* (200 g) were minced, suspended in 250 mL distilled water and the mixture was subjected to hydro-distillation in a Clevenger type apparatus for 4 h. The obtained yellow oil was dissolved in *n*-hexane (GC-grade), dried over anhydrous sodium sulfate, and then stored in amber glass vial at 4–6 °C until analysis.

Extraction and fractionation of the aerial parts of *A. orientalis* was performed according to the procedure listed in the literature (Al-Jaber et al., 2012). Each of the aqueous methanol (AO-A) and the butanol (AO-B) fractions were then assayed for their TPC, TFC, antioxidant activity (by the DPPH and ABTS assay methods) and then were subjected to LC-ESI-MS/MS analysis for the detection of selected phenolic acids, flavonoids and nonphenolic compounds.

2.4. Determination of essential oil constituents and their % concentration

The chemical constituents of *A. orientalis* hydro-distilled essential oil (AO-HDEO) and their relative percentage composition were determined according to the procedure listed in the literature in the literature using the same instruments and under identical chromatographic conditions (Abu-Orabi et al., 2020).

Identification of chemical constituents was achieved by comparing their calculated Kovats retention index (KI) values relative to (C₈-C₂₀) *n*-alkanes literature values measured with columns of identical polarity, or by matching their recorded mass spectra with the built-in mass spectral libraries (NIST, Gaithersburg, MD, USA, and Wiley Co., Hoboken, NJ, USA) in addition to mass spectrum matching to the available authentic standards.

2.5. Total phenols and total flavonoids contents

The total phenols and total flavonoids contents of the AO-A and AO-B extracts were determined by Folin-Ciocalteu method and aluminum chloride assay, respectively as previously described (Al-Humaidi., 2017).

2.6. Antioxidant activity

The antioxidant activity of the AO-HDEO and each AO-A and AO-B fractions was determined by the DPPH and ABTS methods according to the procedure listed in the literature (Al-Qudah 2016; Al-Qudah et al., 2015; Al-Qudah et al., 2014; Teismann and Ferger., 2000). The ability of the AO-HDEO/fractions to scavenge radicals was calculated using the following equation:

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

Where A_c is the absorbance of the blank and A_s is the absorbance in the presence of essential oil/extract.

2.7. LC-MS analysis of secondary metabolites

Analysis of selected secondary metabolites was performed on Bruker Daltonik (Bremen, Germany) Impact II ESI-Q-TOF System equipped with Bruker Daltonik Elute UHPLC system (Bremen, Germany) in both positive (M + H) and negative (M–H) electrospray ionization modes. Chromatographic separation was performed on a C-18 reversed phase column (100 × 2.1 mm, 2.0 μm) from Bruker Daltonik (Germany) at 40 °C, with an autosampler temperature of 8 °C. The elution gradient consisted of mobile phase A: water with 0.05 % formic acid and mobile phase B: acetonitrile. The gradient elution program was: linear gradient 5–80 % B (0–27 min); 95 % B (27–29 min); 5 % B (29.1–35.0 min). The flow rate of the solvent was 0.51 mL/min and the injection volume of the sample was 3.0 μL. Mass spectrum was operating at the following conditions: the capillary voltage was 2500 V, the nebulizer gas was 2.0 bar, dry gas (N₂) gas flow was 8.0 L/min and the dry temperature was 200 °C. The mass accuracy was < 1 ppm; the mass resolution was 50,000 FSR (Full Sensitivity Resolution) and the TOF repetition rate was up to 20 kHz.

A stock solution containing standard compounds (0.5 mg/mL) was prepared in HPLC-grade. Plant samples were dissolved with 2.0 mL DMSO, the volume was completed to 50 mL by acetonitrile, then each sample was centrifuged at 4000 rpm for 2 min and 3.0 μL was injected. The composition of the samples was identified based on the identification of m/z ratio with reference to the retention time of the used standards.

3. Results and discussion

3.1. Essential oil

Hydro-distillation of the fresh aerial parts of *A. orientalis* afforded a yellow oil (yield 0.05 %, w/w). GC–MS analysis of the obtained HD-AOEO (Fig. 1) resulted in the identification of a total of 92 compounds amounting to 90.49 % of the total oil content (Table 1). The HD-AOEO was dominated by different classes of terpenoids, aliphatic hydrocarbons and their derivatives (Table 1), mainly oxygenated sesquiterpenoids (27.29 %). Individual main components included tiglic acid (18.90 %), ageratochromene (8.09 %), α -thujene (6.20 %), and 5-cedranone (5.82 %). Moreover, the obtained HDEO was assayed for its antioxidant activity using the DPPH and ABTS methods, results (Table 2) indicated a relatively high activity as compared to the employed positive controls (DPPH: $(6.92 \pm 0.22) \times 10^{-3}$ mg/mL; ABTS: $6.44 \pm 0.18) \times 10^{-3}$ mg/mL).

The chemical composition of the essential oils of *A. orientalis* from Turkey (Küçükbay et al., 2013) and from Iran (Sajjadi and Ghannadi, 2004); was quite different when compared to current results. The essential oil obtained from Turkish *A. orientalis* was dominated by phytol (36.7 %) while Iranian *A. orientalis* EO was dominated by germacrene (24.2 %). Fig. 2 shows the main variations among the different classes of constituents detected in the essential oils *A. orientalis* from Jordan (current study), Iran, and Turkey. This variation in composition could be attributed to the different climatic conditions, different soil properties in addition to other factors like time of collection and different extraction procedures (Mercy and David Udo., 2018).

3.2. TPC, TFC, antioxidant activity

In the current study, each of the aqueous methanol (AO-A) and butanol (AO-B) fractions were investigated for their TPC, TFC and antioxidant activity using two assay methods and according to the procedures listed in the literature ((Al-Qudah, 2016; Al-Qudah et al., 2014, 2015; Govindan et al., 2016; Sanchez-Moreno, 2002). As could be deduced from the results shown in Table 2, AO-B fraction had the highest

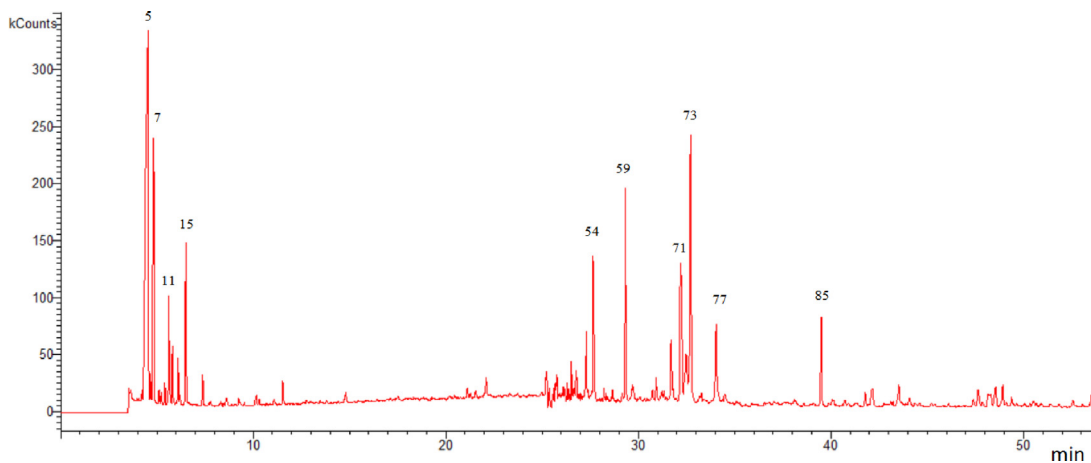


Fig. 1 GC–MS, peaks were numbered as reported in Table 1.

Table 1 Identified *A. orientalis* essential oil constituents and their % composition.

No	KI		Compound	% Composition	Identification mode KI ^b , MS ^c , Col ^d
	Lit. ^a	exp. ^b			
1	862	861	2E-Hexenol	0.10	MS, RI
2	867	866	2Z-Hexenol	0.13	MS, RI
3	870	869	<i>n</i> -Hexanol	0.09	MS, RI
4	900	902	<i>n</i> -Nonane	0.23	MS, RI
5	912	914	Tiglic acid	18.90	MS, RI, Col
6	923	919	2-Methyl-4-heptanone	0.43	MS, RI
7	930	926	α -Thujene	6.20	MS, RI, Col
8	937	937	Tetrahydro citronellene	0.34	MS, RI
9	938	941	Allyl isovalerate	0.20	MS, RI
10	930	948	Cumene	0.31	MS, RI
11	960	956	Thuja-2,4(10)-diene	2.31	MS, RI
12	965	964	2-Methyl-(3E)-octen-5-yne	0.90	MS, RI
13	967	975	Verbenene	0.82	MS, RI
14	978	979	Hexanal, dimethyl acetal	0.16	MS, RI
15	995	991	Mesitylene	2.61	MS, RI, Col
16	1025	1019	<i>psi</i> -Cumene	0.58	MS, RI
17	1037	1046	E- β -Ocimene	0.10	MS, RI
18	1069	1053	<i>m</i> -Tolualdehyde	0.28	MS, RI, Col
19	1081	1072	<i>p</i> -Tolualdehyde	0.24	MS, RI
20	1090	1080	Dehydro linalool	0.07	MS, RI
21	1096	1098	Linalool	0.31	MS, RI, Col
22	1104	1102	2-Isopropyl-5-methyl-(2E)-hexenal	0.14	MS, RI
23	1121	1121	<i>exo</i> -Fenchol	0.14	MS, RI
24	1138	1132	Benzeneacetonitrile	0.53	MS, RI
25	1213	1212	Octanol acetate	0.31	MS, RI
26	1361	1361	γ -Nonalactone	0.26	MS, RI
27	1362	1364	Hydroxy citronellol	0.11	MS, RI
28	1375	1370	α -Copaene	0.17	MS, RI
29	1386	1384	δ -Nonalactone	0.56	MS, RI
30	1460	1459	Allo-aromadendrene	0.71	MS, RI
31	1463	1463	<i>cis</i> -Cadina-1(6),4-diene	0.34	MS, RI
32	1465	1469	<i>cis</i> -Muurolo-4(14),5-diene	0.50	MS, RI
33	1472	1471	Dauca-5,8-diene	0.55	MS, RI
34	1476	1473	<i>trans</i> -Cadina-1(6),4-diene	0.76	MS, RI
35	1477	1476	γ -Gurjunene	0.43	MS, RI
36	1479	1477	γ -Muurolene	0.17	MS, RI
37	1481	1479	Amorpha-4,7(11)-diene	0.25	MS, RI
38	1481	1481	Germacrene D	0.48	MS, RI
39	1482	1483	Widdra-2,4(14)-diene	0.42	MS, RI
40	1484	1486	α -Amorphene	0.36	MS, RI
41	1488	1488	Aristolochene	0.37	MS, RI
42	1494	1491	<i>epi</i> -Cubebol	0.78	MS, RI
43	1495	1494	γ -Amorphene	0.31	MS, RI
44	1499	1495	4- <i>epi-cis</i> -Dihydroagarofuran	0.22	MS, RI
45	1500	1497	α -Muurolene	1.03	MS, RI
46	1500	1500	β -Himachalene	0.14	MS, RI
47	1502	1502	<i>trans</i> - β -Guaiene	0.24	MS, RI
48	1505	1504	α -Cuprenene	0.19	MS, RI
49	1505	1506	β -Bisabolene	0.22	MS, RI
50	1512	1510	δ -Amorphene	1.76	MS, RI
51	1513	1513	γ -Cadinene	0.35	MS, RI
52	1513	1516	<i>trans</i> -Cycloisolongifol-5-ol	0.12	MS, RI
53	1522	1518	<i>trans</i> -Calamenene	0.27	MS, RI
54	1523	1520	δ -Cadinene	3.96	MS, RI, Col
55	1538	1534	α -Cadinene	0.14	MS, RI
56	1545	1537	α -Calacorene	0.22	MS, RI
57	1548	1546	Italicene epoxide	0.24	MS, RI
58	1565	1558	β -Calacorene	0.19	MS, RI
59	1567	1563	Maaliol	4.67	MS, RI
60	1575	1572	Germacrene D-4-ol	0.67	MS, RI
61	1583	1582	Caryophyllene oxide	0.1	MS, RI

Table 1 (continued)

No	KI		Compound	% Composition	Identification mode KI ^b , MS ^c , Col ^d
	Lit. ^a	exp. ^b			
62	1590	1588	Globulol	0.09	MS, RI
63	1592	1593	Viridiflorol	0.09	MS, RI
64	1594	1599	Carotol	0.44	MS, RI
65	1607	1604	β-Oplophenone	0.76	MS, RI
66	1619	1611	1,10-di- <i>epi</i> -Cubenol	0.43	MS, RI
67	1623	1615	10- <i>epi</i> -γ-Eudesmol	0.32	MS, RI
68	1623	1618	α-Corocalene	0.14	MS, RI
69	1628	1624	1- <i>epi</i> -Cubenol	1.94	MS, RI
70	1631	1627	Eremoligenol	0.29	MS, RI
71	1628	1638	5-Cedranone	5.82	MS, RI
72	1650	1645	β-Eudesmol	2.52	MS, RI
73	1660	1652	Ageratochromene	8.09	MS, RI
74	1661	1664	<i>cis</i> -Calamene-10-ol	0.19	MS, RI
75	1665	1667	Junicedranone	0.27	MS, RI
76	1676	1671	Cadalene	0.16	MS, RI
77	1685	1687	5-neoCedranol	3.11	MS, RI
78	1700	1696	Amorpha-4,9-dien-2-ol	0.11	MS, RI
79	1702	1699	10- <i>nor</i> -Calamene-10-one	0.57	MS, RI
80	1760	1757	Benzyl benzoate	0.08	MS, RI
81	1763	1763	Aristolone	0.26	MS, RI
82	1807	1800	2-Ethyl hexyl salicylate	0.12	MS, RI
83	1805	1802	2-α-Acetoxy-amorpha-4,7(11)-diene	0.10	MS, RI
84	1811	1815	β-Chenopodiol	0.09	MS, RI
85	1864	1842	<i>cis</i> -Thujopsenic acid	2.45	MS, RI
86	1865	1859	Benzyl salicylate	0.25	MS, RI
87	1881	1878	Cyclohexyl anthranilate	0.16	MS, RI
88	1912	1909	Kudtdiol	0.46	MS, RI
89	1921	1920	Methyl hexadecanoate	1.02	MS, RI
90	1939	1951	11-Acetoxyeudesman-4-α-ol	0.18	MS, RI
91	1960	1964	Hexadecanoic acid	0.92	MS, RI
92	1993	1981	Ethyl hexadecanoate	0.37	MS, RI
Classes detected (no. of compounds/class)					
Oxygenated hemiterpenoids				19.1 (2)**	
Monoterpene hydrocarbons				9.77 (5)	
Oxygenated monoterpenes				0.77 (5)	
Sesquiterpene hydrocarbons				14.83 (28)	
Oxygenated sesquiterpenes				27.29 (28)	
Esters				3.13 (9)	
Phenolic compounds				12.64 (7)	
Non-phenolic compounds				2.96 (8)	
Total identified				90.49 %	

*^a(Lit.): Literature Kovats index; ^b(Exp.): Experimentally calculated Kovats index using C₈–C₂₀ *n*-alkanes on HP-5MS capillary column. ^cMS: Identification by mass spectrum (NIST and our local generated libraries were used for all MS comparisons). ^dCol: Co-Injection with an authentic compound, **: no of compounds detected in each class.

Table 2 Total phenolic (mg gallic acid/g dry extract), total flavonoids content (mg quercetin/g dry extract), and IC₅₀ (mg/mL) values of the *in-vitro* DDPH and ABTS antioxidant activities of the HDEO, AO-M and AO-B fractions of *A. orientalis* from Jordan.

Extracts	TPC	TFC	IC ₅₀ (mg/mL)	
			DDPH	ABTS
HDEO	–	–	(6.92 ± 0.22) × 10 ⁻³	(6.44 ± 0.18) × 10 ⁻³
AO-M	52.35 ± 1.35	281.24 ± 1.50	(14.00 ± 0.60) × 10 ⁻²	(7.00 ± 0.10) × 10 ⁻²
AO-B	217.63 ± 2.65	944.41 ± 4.77	(4.00 ± 0.20) × 10 ⁻²	(3.00 ± 0.20) × 10 ⁻²
Ascorbic acid	–	–	1.58 × 10 ⁻³ ± 3.0 × 10 ⁻⁵	1.78 × 10 ⁻³ ± 6.0 × 10 ⁻⁵
α-tocopherol	–	–	1.79 × 10 ⁻³ ± 1.0 × 10 ⁻⁵	2.33 × 10 ⁻³ ± 4.0 × 10 ⁻⁵

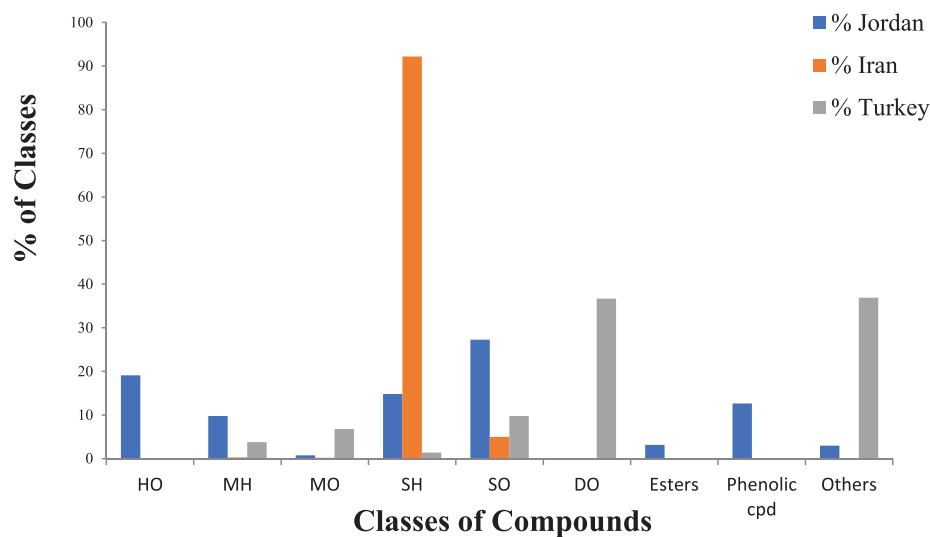
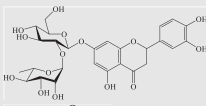
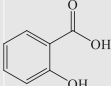
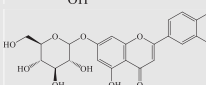
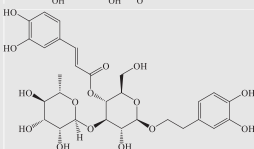
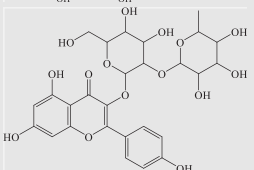
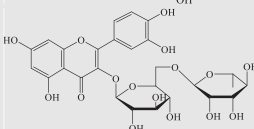
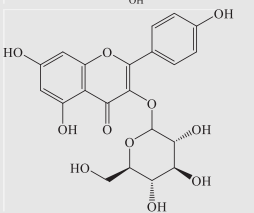
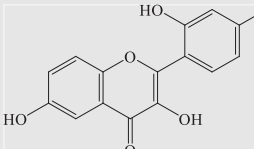
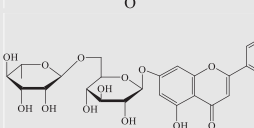
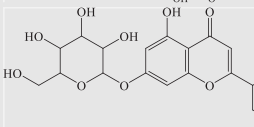
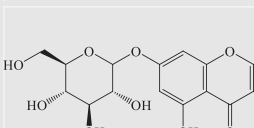
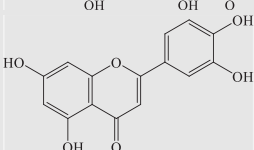


Fig. 2 A classification of the constituents of the *A. orientalis* L. and their % composition from Jordan, Iran, and Turkey. Hemiterpenoids oxygenated (HO), monoterpene hydrocarbons (MH), monoterpenes oxygenated (OM), sesquiterpene hydrocarbons (SH), sesquiterpenes oxygenated (SO), diterpene oxygenated (DO).

Table 3 Compounds identified in the AO-M and AO-B extracts from *A. orientalis* from Jordan.

No.	R_f	Name	Structure	Molecular Formula	m/z meas.	Mwt	Compounds*	
							AO-M	AO-B
1	0.99	Succinic acid		$C_4H_6O_4$	117.0193	118.0266	+	+
2	1.8	2,5-Dihydroxybenzoic acid		$C_7H_6O_4$	153.0192	154.0265	+	+
3	2.81	Caffeic Acid		$C_9H_8O_4$	179.0343	180.0415	+	+
4	3.16	Vanillic acid		$C_8H_8O_4$	167.0351	168.0423	+	+
5	4.4	<i>p</i> -Coumaric acid		$C_9H_8O_3$	163.0399	164.0471	+	+
6	4.44	Ethyl gallate		$C_9H_{10}O_5$	197.0457	198.053	+	+
7	4.82	3,5-Dimethoxy-4-hydroxyacetophenone		$C_{10}H_{12}O_4$	195.0644	196.0716	+	+
8	5.45	Vitexin		$C_{21}H_{20}O_{10}$	431.0976	432.1049	-	+

Table 3 (continued)

No.	R_f	Name	Structure	Molecular Formula	m/z meas.	Mwt	Compounds*	
							AO-M	AO-B
9	5.51	Eriodictyol-7-neohesperidoside		$C_{27}H_{32}O_{15}$	595.167	596.1743	+	+
10	5.89	Salicylic acid		$C_7H_6O_3$	137.0243	138.0315	+	+
11	5.89	Luteolin 7-O-glucoside (Cynaroside)		$C_{21}H_{20}O_{11}$	447.0931	448.1004	+	+
12	5.95	Acteoside = Verbascoside		$C_{29}H_{36}O_{15}$	623.1981	624.2056	+	+
13	6.05	3-O-Neohesperidoside Kaempferol		$C_{27}H_{30}O_{15}$	593.1511	594.1583	+	+
14	6.17	Rutin		$C_{27}H_{30}O_{16}$	609.145	610.1523	+	+
15	6.77	Kaempferol-3-O-glucoside		$C_{21}H_{20}O_{11}$	447.0931	448.1004	+	+
16	6.78	3,6,2',4'-Tetrahydroxyflavone		$C_{15}H_{10}O_6$	285.0399	286.0472	+	+
17	7.00	Diosmin		$C_{28}H_{32}O_{15}$	607.166	608.1733	+	+
18	7.09	7-Glu-Chrysoeriol		$C_{22}H_{22}O_{11}$	461.1085	462.1157	+	+
19	7.23	Kaempferol-7-O-glucoside		$C_{21}H_{20}O_{11}$	447.0928	448.1	+	+
20	8.55	Luteolin		$C_{15}H_{10}O_6$	285.0403	286.0475	+	+

(continued on next page)

Table 3 (continued)

No.	R_t	Name	Structure	Molecular Formula	m/z meas.	Mwt	Compounds*	
							AO-M	AO-B
21	10.24	Hispidulin		$C_{16}H_{12}O_6$	299.0557	300.063	+	+
22	13.73	Caffeic acid phenethyl ester		$C_{17}H_{16}O_4$	283.1011	284.1084	+	+
23	14.65	8-Prenylnaringenin		$C_{20}H_{20}O_5$	339.1235	340.1308	+	-
24	16.46	3-Gal(1-2)GluA Soyasapogenol B		$C_{42}H_{68}O_{14}$	795.4529	796.4602	+	-
25	21.63	Hederagenin		$C_{30}H_{48}O_4$	471.3472	472.3545	+	-
26	22.58	Glc-octadecatrienoyl- <i>sn</i> - glycerol		$C_{27}H_{46}O_9$	513.3099	514.3172	+	+
27	26.4	(<i>Z</i>)-3-Hydroxyoctadec-7-enoic acid		$C_{18}H_{34}O_3$	297.2435	298.2508	+	+
28	26.7	Myristic acid		$C_{14}H_{28}O_2$	227.2012	228.2085	+	-
29	28.78	Pentadecanoic acid		$C_{15}H_{30}O_2$	241.2176	242.2249	+	+

* (+): Detected; (-): not detected; samples were verified against authentic samples isolated in our labs or purchased from Sigma-Aldrich.

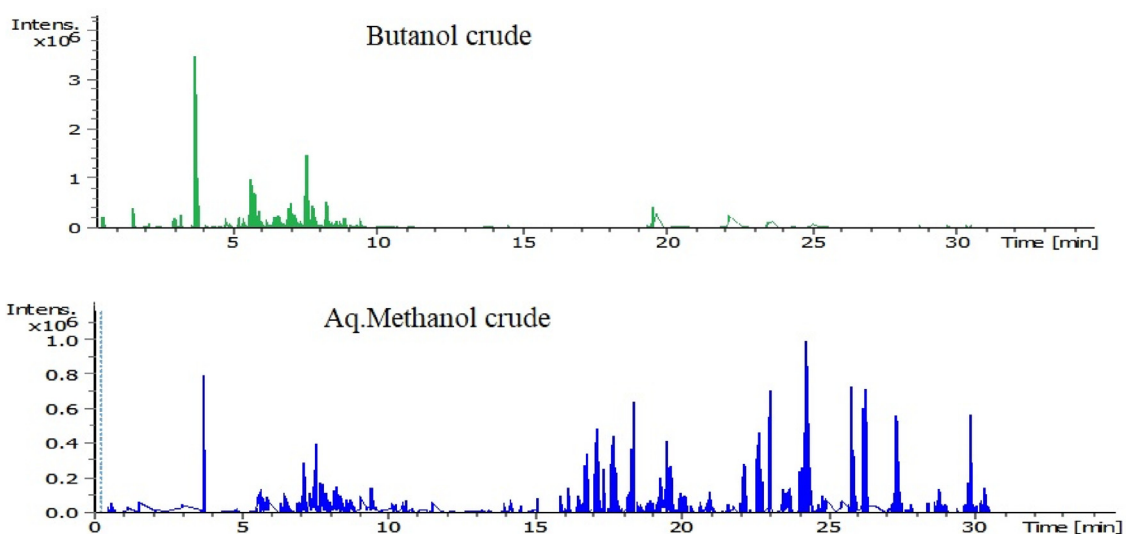


Fig. 3 LC-MS/MS chromatograms of AO-B and AO-M fractions of *A. orientalis* from Jordan.

TPC and TFC (217.63 ± 2.65 mg gallic acid/g extract; 944.41 ± 4.77 mg quercetin/g extract, respectively). This extract had also the highest antioxidant activity as measured by the DPPH ($(4.00 \pm 0.20) \times 10^{-2}$ mg/mL) and ABTS ($(3.00 \pm 0.20) \times 10^{-2}$ mg/mL) assay methods.

3.3. LC-MS/MS profiling of selected secondary metabolites

In the current investigation, AO-M and AO-B fractions were screened for the presence of a selected set of secondary metabolites by LC-ESI-MS/MS using both, the positive and negative ionization modes. The list of the 33 different phenolic and nonphenolic compounds detected in both extracts are shown in Table 3, chromatograms are shown in Fig. 3. Both extracts were found to contain acetoiside as a major phenolic acid derivative. It was noticed that each of 8-Prenylaringenin, 3-gal(1-2)gluA soyasapogenol B, hederagenin, myristic acid and (Z)-3-hydroxyoctadec-7-enoic acid were detected in the AO-A fraction only. The phenolic and flavonoids profiles detected in the extracts of *A. orientalis* from Jordan in our current study were completely different from those reported for the plant from Turkish origin (Göger et al., 2015; Zengin et al., 2018).

4. Conclusions

Different extracts of *A. orientalis* from Jordan were investigated for their TPC, TFC and antioxidant activities using the DPPH and ABTS assay methods. Results of the current study revealed that *A. orientalis* fractions had a relatively high TPC, TFC and good antioxidant activity as determined by the two assay methods (DDPH and ABTS), especially the butanol (AO-B) fraction. The detection of several phenolic and flavonoids compounds could justify the observed activity.

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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