Revista Brasileira de Farmacognosia xxx (2016) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
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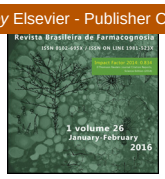

Original Article

UHPLC high resolution orbitrap metabolomic fingerprinting of the unique species *Ophryosporus triangularis* Meyen from the Atacama Desert, Northern Chile

Mario J. Simirgiotis[a,*], Cristina Quispe[b], Andrei Mocan[c], José Miguel Villatoro[a], Carlos Areche[d], Jorge Bórquez[e], Beatriz Sepúlveda[f], Carlos Echiburu-Chau[g,h]

[a] *Instituto de Farmacia, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile*
[b] *Instituto de Etnofarmacología, Facultad de Ciencias de la Salud, Universidad Arturo Prat, Iquique, Chile*
[c] *Department of Pharmaceutical Botany, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania*
[d] *Departamento de Química, Facultad de Ciencias, Universidad de Chile, Santiago, Chile*
[e] *Departamento de Química, Facultad de Ciencias Básicas, Universidad de Antofagasta, Antofagasta, Chile*
[f] *Departamento de Ciencias Químicas, Universidad Andres Bello, Campus Viña del Mar, Viña del Mar, Chile*
[g] *Centro de Investigaciones del Hombre en el Desierto (CIHDE), Arica, Chile*
[h] *Facultad de Ciencias de la Salud, Universidad de Tarapacá, Arica, Chile*

ARTICLE INFO

Article history:
Received 20 May 2016
Accepted 28 October 2016
Available online xxx

Keywords:
UHPLC
Orbitrap high resolution mass spectrometry
Flavonoids
Chilean plants
Ophryosporus
Paposo Valley

ABSTRACT

High-resolution mass spectrometry is currently used to determine the mass of biologically active compounds in plants and UHPLC-Orbitrap is a relatively new technology that allows fast fingerprinting and metabolomics analysis. In this work, several phenolic compounds including eleven phenolic acids, two fatty acids, two chromones and fourteen flavones were rapidly identified in the methanolic extracts of aerial parts and flowers of the unique Chilean species *Ophryosporus triangularis* Meyen, Asteraceae, growing in the Atacama Desert by means of ultrahigh resolution liquid chromatography orbitrap MS analysis (UHPLC-PDA-OT-MS) for the first time. The UHPLC-MS fingerprint generated can be used for the authentication of this endemic species. The methanolic extracts of the aerial parts and flowers showed also antioxidant capacities (65.34 ± 1.32 and 52.41 ± 1.87 μg/ml in the DPPH assay, 184.88 ± 13.22 and 196.80 ± 13.28 μmol TE/g dry weight in the ferric reducing power assay and 56.17 ± 3.03 and 65.41 ± 1.96% in the superoxide anion scavenging assay, respectively).

Introduction

Ophryosporus is a genus of South American flowering plants belonging to the sunflower (Asteraceae, order Asterales, tribe *Eupatorieae*) family. The tribe *Eupatorieae* Cass has two genus growing in Chile: *Eupatorium* and *Ophryosporus*. The genus *Ophryosporus* Meyen belongs to the subtribe *Critoniinae* R. M. King & H. Rob, including 41 South American species which grow along the cordillera de los Andes distributed in Argentina, Brazil, Bolivia, Chile, Ecuador and Peru. The aerial parts (leaves and stems) of species belonging to this genus produce several phenolic compounds including flavonoids, chromenones, acetophenones, coumarins, chromenes, benzofurans, and terpenoids such as eudesmanolides, sesquiterpenes and *ent* and *seco*-labdane diterpenoids which are of taxonomical relevance (Bohlmann and Zdero, 1979; Bohlmann et al., 1984; Zdero et al., 1990; deLampasona et al., 1997; Favier et al., 1997, 1998; Sigstad et al., 1992, 1993, 1996; Barrero et al., 2006; Garneau et al., 2013). The essential oils from some of the species were also studied. For instance, the essential oils from leaves and stems of *Ophryosporus piquerioides* show mainly (*E*)-nerolidol, caryophyllene oxide, camphene and β-caryophyllene as principal components, while the essential oils from *O. hepthantus* showed mainly precocene and *p*-cimene (Arze et al., 2004). Furthermore, several compounds isolated from these species show interesting biological activities. For instance, from the acetone extract of leaves from *O. axilliflorus*, known by the local name of charrua in Argentina, seven acetophenones were isolated which presented anti-inflammatory activity in a carragenan-induced mouse paw edema assay (Favier et al., 1998) while from the butanol extract of leaves of the species *O. heptanthus* fourteen compounds, including terpenoids and flavonoids were isolated which presented antioxidant activities (Barrero et al., 2006). In

* Corresponding author.
E-mail: mario.simirgiotis@uantof.cl (M.J. Simirgiotis).

http://dx.doi.org/10.1016/j.bjp.2016.10.002

addition, the ethanolic extract of leaves and stems of *O. peruvianus* showed antibacterial activity against bacteria and fungi including *Bacillus subtilis*, *Candida albicans*, *Trichophyton mentagrophytes* and *Sporothrix schenckii* (4.3, 17, 27 and 13 mm of growth inhibition zone, in a disk bioassay) (Rojas et al., 2003). Paposo is a small valley on the Chilean coast about 50 km of the town of Taltal and 200 km south of the city of Antofagasta. Coastal fogs in this location called locally camanchacas allow the growing of several species in this special ecosystem (Squeo et al., 1998) including *Ophryosporus triangularis* Meyen, locally called rabo de zorro (foxtail), in Chile. This is a unique endemic species growing in a relatively small area in northern Chile, in the Atacama Desert, in the Norte grande region, from Arica to Coquimbo. From *O. triangularis* growing in the surroundings of La Serena, Chile, several benzofurans, polymethoxylated flavones and chromenes were isolated including the compounds 6-acetyl-2,2-dimethyl-chromen-4-one, 5,4-dihydroxy-6-methoxyflavone, 6,5,4′-trihydroxyflavone, 6-acetyl-8-senecioyl-2,2-dimethylchromene, 5-acetyl-6-hydroxy-2-isopropenyl-benzofuran, and its *O*-methylated-derivative (Alarcon et al., 1993.) among others. However, to the best of our knowledge, no medicinal uses or bioactivities were reported so far for this species.

HPLC hyphenated with mass spectrometry is today a key tool in the analysis of plant samples for chemotaxonomy or metabolic profiling. Among the instruments in the market, the Q-exactive focus mass spectrometer combines rapid ultra-high performance liquid chromatography (UHPLC) separation, diode array detection (PDA or DAD) and high mass spectrometry (MS) performance of an orbital trap with the help of a quadrupole and an outstanding diagnostic power using a high energy collision dissociation (HCD) cell, which delivers high resolution MS^n fragments. This methodology is useful for the fast analysis of small compounds, including toxins, pesticides, phenolics and terpenes (Zhao and Jiang, 2015; Martínez-Domínguez et al., 2016; Simirgiotis et al., 2016b; Yang et al., 2016). In the continuing efforts made by our laboratory in the search for new interesting metabolites from Paposo flora (Simirgiotis et al., 2015, 2016b) several phenolic compounds were rapidly detected in the methanolic extract from flowers and aerial parts (leaves and stems) of *O. triangularis* (Fig. 1) using high resolution Q-orbitrap technology coupled to an PDA detector. The methanol extracts from flowers and aerial parts were also submitted to antioxidant activity assays (DPPH, FRAP and superoxide anion scavenging assays), plus measurements of total phenolic and flavonoid content by spectroscopy.

Fig. 1. Picture of one herborized sample of *Ophryosporus triangularis* collected in Quebrada de Paposo, Atacama Desert, in April 2011.

Material and methods

Chemicals and plant material

HPLC–MS Solvents, LC–MS formic acid and GR acetonitrile and methanol were purchased from Merck (Santiago, Chile). Ultrapure water was obtained from a Millipore water purification system (Milli-Q Merck Millipore, Chile). HPLC standards, Isorhamnetin, caffeic acid, quercetin-3-*O*-glucose, *p*-coumaroyl galactose (all standards with purity higher than 95% by HPLC) were purchased either from Sigma–Aldrich (Saint Louis, MO, USA), ChromaDex (Santa Ana, CA, USA), or Extrasynthèse (Genay, France). Folin–Ciocalteu phenol reagent (2 N), reagent grade Na_2CO_3, $AlCl_3$, HCl, $FeCl_3$, $NaNO_2$, NaOH, quercetin, trichloroacetic acid, sodium acetate, gallic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Trolox, nitroblue tetrazolium, xanthine oxidase and DPPH (1,1-diphenyl-2-picrylhydrazyl radical) were purchased from Sigma–Aldrich Chemical Co.

Three different specimens of *Ophryosporus triangularis* Meyen, Asteraceae (aerial parts and flowers) were collected in Quebrada de Paposo in June 2011 (geographical coordinates: lat: 25° 07′ 94″ S, long: 70° 27′ 27″ W, for specimen I, 25° 00′ 48″ S, long: 70° 26′ 81″ W for specimen II and 25° 09′ 52″ S, long: 70° 26′ 30″ W for specimen III). The samples were identified by the botanist Alicia Marticorena from the University of Concepción, Chile. Voucher herbarium specimens are kept at the Natural Products Laboratory of the Universidad de Antofagasta under reference number: OT110403-12, OT110403-13 and OT110403-14.

Extraction

Dried aerial parts (leaves and stems) and flowers of *O. triangularis* (100 mg each, for the three different specimens) were extracted separately with methanol for 30 min with sonication (5 ml) in order to obtain an extract. The extracts were immediately concentrated *in vacuo* below 40 °C to give two resulting brown syrups for each sample (10.3 and 15, 11.2 and 17 and 13.24 and 17.4 mg, for aerial parts and flowers of samples I, II and III, respectively).

UHPLC-DAD-MS instrument

The equipment used was a Thermo Scientific Dionex Ultimate 3000 UHPLC system hyphenated with a Thermo Q exactive focus machine. For the analysis the prepared extracts were filtered (PTFE filter) and 10 μl were injected in the instrument, with all specifications set as previously reported (Simirgiotis et al., 2016b).

LC parameters and MS parameters

Liquid chromatography was performed using a UHPLC C18 column (Acclaim, 150 mm × 4.6 mm ID, 2.5 μm, Thermo Fisher

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Scientific, Bremen, Germany) as reported previously (Simirgiotis et al., 2016b). The detection wavelengths were 254, 280, 330 and 354 nm, and DAD was recorded from 200 to 800 nm for peak characterization. Mobile phases were 1% formic aqueous solution (A) and acetonitrile (B). The gradient program was: 5% to 30% solvent B from 5 to 10 min, then, maintaining 30% B for 5 min, then to 70% B 5 min more, then maintaining 70 B in 5 more min, then returning to 5% B in 5 more min and 12 min for column equilibration before each injection. The flow rate was 1 ml min^{-1}, and the injection volume was 10 μl. The HESI II and Orbitrap spectrometer parameters were optimized as previously reported (Simirgiotis et al., 2016b).

Antioxidant assays

DPPH Free radical scavenging activity

The DPPH free radical scavenging activity was determined as previously described (Ramirez et al., 2015). Values are reported as IC_{50} (concentration of sample required to scavenge 50% of DPPH free radicals). If $IC_{50} \leq 50$ μg/ml the sample has high antioxidant capacity, if 50 μg/ml < $IC_{50} \leq 100$ μg/ml the sample has moderate antioxidant activity and if $IC_{50} > 200$ μg/ml the sample has no relevant antioxidant activity.

Ferric reducing antioxidant power

Ferric reducing ability (FRAP assay) or ferric reducing antioxidant power or of the extracts and standards was performed as recommended by (Benzie and Strain, 1996) with some modifications as described (Ramirez et al., 2015). The stock solutions prepared were 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. Plant extracts or standard methanolic Trolox solutions (150 μl) were incubated at 37 °C with 2 ml of the FRAP solution (25 ml acetate buffer, 5 ml TPTZ solution, and 10 ml $FeCl_3 \cdot 6H_2O$ solution) in the dark for 30 min. Absorbance of the ferrous tripyridyltriazine blue complex formed was then read at 593 nm. Quantification was performed using a standard calibration curve of antioxidant Trolox (from 0.2 to 2.5 μmol/ml, R^2: 0.995). Samples were analyzed in triplicate ($n = 3$ for each sample) and results are expressed in μmol TE/g dry mass.

Superoxide anion scavenging assay

The superoxide anion scavenging activities of extract and standards were measured as reported previously (Simirgiotis et al., 2016a). The superoxide anion (SAA) radical ($O_2^{\bullet -}$) produced by the enzyme xanthine oxidase reduces the nitro blue tetrazolium (NBT), producing a chromophore measured by spectroscopy at 520 nm. The SA scavengers reduce the speed of formation of this chromophore. Extracts were evaluated at 100 μg/ml. Values are presented as mean ± standard deviation of three determinations.

Polyphenol, flavonoid and anthocyanin contents

The total polyphenolic contents (TPC) were determined by spectroscopy using the Folin–Ciocalteau reagent (Simirgiotis et al., 2013b). An aliquot of each extract (200 μl, approx. 2 mg/ml) was added to the Folin–Ciocalteau reagent (2 ml, 1:10, *v*/*v* in purified water) and after 5 min of reaction at 25 °C, 2 ml of a 100 g/l solution of Na_2CO_3 was added. Then, after 60 min, the absorbance was measured at 710 nm. The calibration curve was performed with gallic acid (concentrations ranging from 16 to 500 μg/ml, $R^2 = 0.999$) and the results were expressed as mg gallic acid equivalents/g dry matter. Determination of total flavonoid content (TFC) of the methanolic extracts was performed as described using the $AlCl_3$ method (Simirgiotis et al., 2013b). Quantification was expressed by reporting the absorbance in the calibration graph of quercetin, used as a standard flavonoid (from 0.1 to 65 μg/ml, $R^2 = 0.994$). Results are expressed as mg quercetin equivalents/g dry weight. All measurements were performed using a Halo MPR-96 microplate reader (Dynamica GmbH, Dietikon, Switzerland) or a Spectroquant Pharo UV-Vis spectrophotometer (Merck, Darmstadt, Germany).

Statistical analysis

The statistical analysis was carried out using the software originPro 9.1 (Originlab Corporation, Northampton, MA, USA). Data are reported as mean ± SD for at least three replications. Analysis of variance was performed using ANOVA. Tukey's test was computed for all assays (95% significance level).

Results and discussion

Phenolic content and antioxidant capacities of Ophryosporus triangularis

Consumption of plants, fruits and vegetables with antioxidant activity has been related to a variety of beneficial biological activities such as anticancer, diabetes and heart disease prevention (Owen et al., 2000; Cao et al., 2014). In this study, the methanolic extracts of aerial parts and flowers of the endemic desert Chilean species *O. triangularis* showed good antioxidant activity. Several antioxidant tests with different mechanism of action were employed in this study, since some authors consider that different tests should be performed to assure the antioxidant capacity. For instance the Folin–Ciocalteau's assay frequently overestimates the phenolic content because the reagent is able to react with also proteins and inorganic ions. The extracts from flowers and aerial parts of three different specimens collected in Paposo Valley (Figs. S1 and S2, supplementary material) were tested in antioxidant activity assays (DPPH, FRAP, and superoxide anion scavenging assay, SAA, Table 1), plus measurements of total phenolic (TPC) and total flavonoid (TFC) contents (Table 1). There were only some small differences between the specimens (Table 1) however, the differences could be attributed to other antioxidant compounds rather than phenolics since the chromatograms for phenolic compounds were almost identical for the three specimens under study (Fig. S3, Supplementary material). Since it is difficult to compare the tests with others performed in different conditions, we have compared our values with previous antioxidant plant materials analyzed by us and also to the values published for the related species *O. hepthantus* (Barrero et al., 2006). Thus, the TPC values of the aerial parts for specimen I were lower to that reported for the leaves of the Chilean species *Cryptocaria alba*, (100.12 ± 0.83 mg GAE/g dry weight) but the values for the flowers were close to that value (Simirgiotis, 2013). The TPC of the aerial parts for specimen one were also almost half to that obtained for the aerial parts of *Luma apiculata* (179.83 ± 0.38 mg GAE/g) (Simirgiotis et al., 2013a), while the total flavonoid content of the aerial parts (Fig. 2) was eight times lower than those of the aerial parts of *L. apiculata* (139.70 ± 1.48 mg Q/g), but were higher than that of the extract of the peel of Chilean Pica lemon (23 mg Q/g dry weight) (Brito et al., 2014). The lower total flavonoid content found could be attributed to the high *O*-methylation pattern of the flavonoids detected in the extracts. In the DPPH assay (Table 1), the values were approximately five times lower than those reported for the ethyl acetate extract ($IC_{50} = 9.74$ μg/ml) and three times lower than the butanol extract ($IC_{50} = 17.6$ μg/ml) of the leaves of *O. hepthantus* (Barrero et al., 2006). The DPPH values measured for specimen one were close than those obtained for the polar (ethyl acetate) extracts of three Chilean *Nolana* species (from 30 to 120 μg/ml) (Simirgiotis et al., 2015) and close to those reported for methanolic extract of six antioxidant Chilean berries (from 2 to 12 μg/ml) (Ramirez et al.,

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Table 1
Scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ferric reducing antioxidant power (FRAP), superoxide anion scavenging activity (SAA), total phenolic content (TPC), total flavonoid content (TFC) of methanolic extracts of three different samples of *Ophryosporus triangularis* from the Paposo Valley (II Region) of Chile.

Plant parts	DPPH[−] [a]	FRAP[b]	SAA[c]	TPC[d]	TFC[e]	Yield[f]
O. triangularis aerial parts-1	65.34 ± 1.32[l]	184.88 ± 13.22[k]	56.17 ± 3.03[l]	74.25 ± 3.94[m]	17.89 ± 1.34[o]	10.30
O. triangularis flowers-1	52.41 ± 1.87[j]	196.82 ± 13.28[k]	65.41 ± 1.96	94.71 ± 3.50[n]	29.44 ± 1.62[r]	15.02
O. triangularis aerial parts-1I	61.22 ± 1.16	181.45 ± 11.10[k]	52.23 ± 4.10[l]	72.18 ± 3.23[m]	13.41 ± 1.22	11.20
O. triangularis flowers-1I	51.33 ± 1.28[j]	193.73 ± 11.21[k]	62.45 ± 1.86	91.29 ± 2-87[n]	25.19 ± 1.47	17.00
O. triangularis aerial parts-1II	69.79 ± 1.46	197.23 ± 12.87[k]	55.24 ± 2.87[l]	75.44 ± 3.12[m]	19.37 ± 136[o]	13.24
O. triangularis flowers-1II	63.72 ± 1.48[l]	201.84 ± 15.32[k]	69.68 ± 1.68	98.98 ± 3.43[n]	32.26 ± 1.54[r]	17.41
Quercetin[g]	31.43 ± 1.24	449.11 ± 11.31	80.05 ± 3.37	–	–	–
Gallic acid[g]	21.78 ± 1.39	565.49 ± 14.65	93.24 ± 2.63	–	–	–

Values in the same column marked with the same letter are not significantly different (at $p < 0.05$).
[a] Antiradical DPPH activities are expressed as IC_{50} in μg/ml for extracts and compounds.
[b] Expressed as μM trolox equivalents/g dry weight.
[c] Expressed in percentage scavenging of superoxide anion at 100 μg/ml.
[d] Total phenolic content (TPC) expressed as mg gallic acid/g dry weight.
[e] Total flavonoid content (TFC) expressed as mg quercetin/g dry weight.
[f] Yield is expressed as percentage in a dry weight basis.
[g] Used as standard antioxidants.

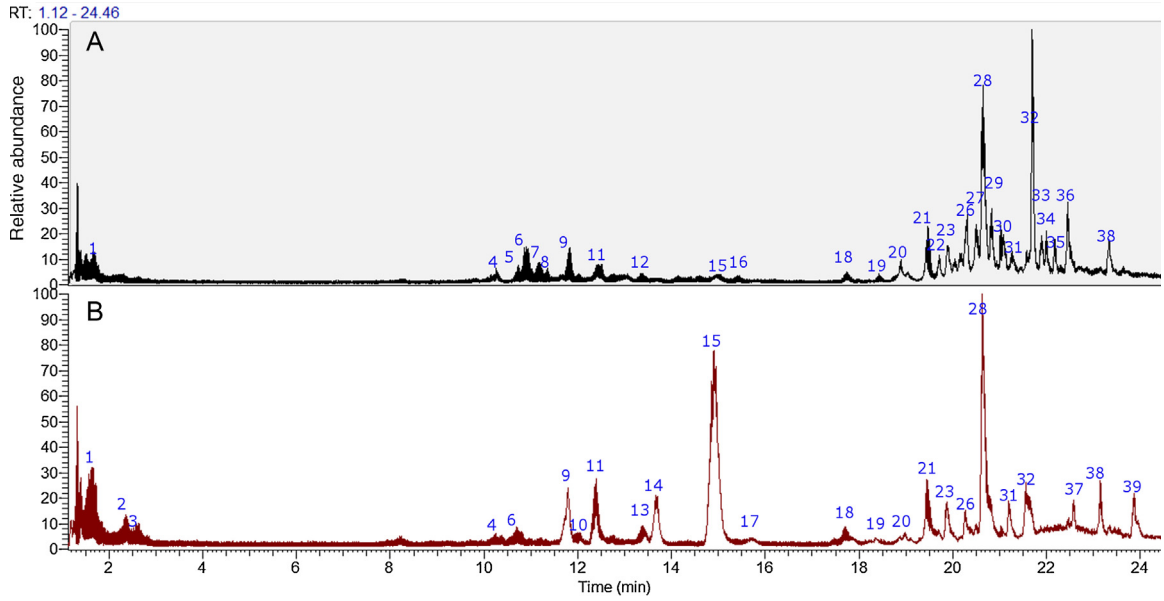

Fig. 2. TIC (total ion current, negative mode) UHPLC chromatograms of *Ophryosporus triangularis* extract. (A) Flowers and (B) aerial parts.

2015). The FRAP values (Table 1) of the flowers and aerial parts for specimen one were higher to that of the ethyl acetate extract of *Nolana ramosissima* (116.07 ± 3.42 μM Trolox equivalents/g dry weight) (Simirgiotis et al., 2015) and to that of the methanolic extract of the fruits of *Luma chequen* (76.2 ± 0.042 μM Trolox equivalents/g dry weight) (Simirgiotis et al., 2013a). The superoxide anion scavenging activity (SAA) of the aerial parts and flowers for specimen one was lower than those of the tested phenolic standards (93.24 and 80.05% for gallic acid and quercetin, respectively). The SAA of the flowers (65.41%) were also close than those reported for the methanolic extract of the blueberries *Vaccinium corimbosum* (72.61 ± 1.91%) (Ramirez et al., 2015).

Comprehensive identification of the compounds

Thirty-nine compounds were detected and twenty-eight identified or tentatively identified by means of high resolution orbitrap MS and PDA detection (Table 2) by comparison with HR-MS and PDA data found in literature. Some of the compounds were identified by co-elution with authentic standards. Among the twenty eight compounds identified, eleven were phenolic acids (peaks 2, 8–12, 14, 15, 18, 20 and 35), fourteen were flavonoids (peaks 23–36), two were chromones (peaks 31 and 39), two were sugars (peaks 6 and 7), and two were fatty acids (peaks 37 and 38). Some seven compounds remained unknown (peaks 3–5, 13, 16, 21, and 22). The UHPLC chromatogram for specimen one (Fig. 2) and detailed identification of the compounds are explained below.

Phenolic acids

Most of the phenolic acids are reported for the first time in this species. Peak 2 with a $[M-H]^-$ ion at *m/z* 191.0190 was identified as quinic acid ($C_7H_{11}O_6^-$) while peak 11 with a $[M-H]^-$ ion at *m/z* 179.0342 was identified as caffeic acid ($C_9H_7O_4^-$) (Simirgiotis et al., 2015). Accordingly, peak 12 with a $[M-H]^-$ ion at *m/z* 323.1340 producing a caffeic acid ion at *m/z* 179.0341 was identified as an unknown caffeic acid derivative ($C_{13}H_{23}O_9^-$). Peak 8 with a $[M-H]^-$ ion at *m/z* 473.2015 was identified as thymol-diglucoside (2-isopropyl-5-methylphenol-diglucose) as reported (Farag et al., 2014). Peaks 9 (Fig. 3A), 10 and 15 with $[M-H]^-$ ions at *m/z* 325.0923, 325.0924 and 325.0923 were identified as coumaroyl-hexose isomers (Farag et al., 2013), *p*-coumaroyl hexose (*trans*-2-hydroxy-glucosyl-cinnammate), *O*-coumaroyl hexose and

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Table 2
UHPLC-PDA and high resolution mass spectral data for *Ophryosporus triangularis* from the Atacama Desert.

Peak number	Retention time (min)	UV max (nm)	Theoretical mass (*m*/*z*)	Measured mass (*m*/*z*)	Other ions (*m*/*z*)	Formula	Identification
1	1.64	–	–	377.0891	191.0553 (quinic acid)	–	Unknown quinic acid derivative
2	2.36	–	191.0190	191.0190	111.0078	$C_7H_{11}O_6^-$	Quinic acid[a]
3	2.64	–	–	197.8073	115.0027	–	Unknown
4	10.25	–	–	283.1544	481.1835	–	Unknown
5	10.73	–	–	350.1370	211.0637, 170.0185, 161.0810	–	Unknown
6	10.81	–	161.0819	161.0810	113.0233	$C_7H_{14}O_4^-$	Cymarose
7	10.87	–	–	381.1313	161.0809	–	Unknown cymarose conjugate
8	11.32	269	473.2028	473.2015	463.1730	$C_{22}H_{33}O_{11}^-$	Thymol-*O*-diglucoside
9	11.76	227–277–320	325.0929	325.0923	651.1906 [2M−H]$^-$, 163.039 (coumaric acid), 119.0491	$C_{15}H_{17}O_8^-$	*O*-Coumaroyl glucose[a]
10	11.97	227–277–320	325.0929	325.0924	651.1907 [2M−H]$^-$, 163.0390 (coumaric acid), 119.0491	$C_{15}H_{17}O_8^-$	*p*-Coumaroyl glucose
11	12.40	244–290–324	179.0348	179.0342	135.0441	$C_9H_7O_4^-$	Caffeic acid[a]
12	12.66	244–290–324	–	323.1340	179.0341	$C_{13}H_{23}O_9$	Caffeic acid derivative
13	13.42	–	–	313.0712	131.0704	$C_{17}H_{13}O_6^-$	Unknown
14	13.67	265	327.1085	327.1076	165.0547 (phenyllactic acid)	$C_{15}H_{19}O_8^-$	Phenyllactic acid-2-*O*-glucoside
15	14.93	227–277–320	325.0929	325.0923	651.1907 [2M−H], 163.0391 (hydroxycinnamic acid), 119.0491	$C_{15}H_{17}O_8^-$	*p*-Coumaroyl galactose[a]
16	15.43	–	–	471.1861	461.1576	–	Unknown
17	15.72	257–355	447.0933	447.0959	241.0016, 205.0862	$C_{21}H_{19}O_{11}^-$	Quercetin-3-*O*-rhamnose
18	17.70	265	165.0557	165.0549	121.0650	$C_9H_9O_3^-$	Phenyllactic acid
19	18.43	257–348	463.0882	463.0876	301.0632	$C_{21}H_{19}O_{12}^-$	Quercetin-3-*O*-glucose[a]
20	18.89	235–325	515.1195	515.1185	353.0870, 191.0552, 179.0341	$C_{25}H_{23}O_{12}^-$	Dicaffeoyl-quinic acid
21	18.89	237–277–325	–	651.1984	493.0394, 330.0373	–	Unknown
22	19.71	237–277–325	–	695.1223	515.1183	–	Unknown diQA derivative
23	19.85	257–342	425.0184	425.0176	345.0606, 330.0372, 315.0139, 195.0290	$C_{17}H_{13}O_{11}S^-$	7,3′-di-*O*-Methylmyricetin-5′-*O*-sulfate
24	19.94	257–341	425.0184	425.0173	345.0605 (syringetin), 330.0372, 315.0501, 287.0189	$C_{17}H_{13}O_{11}S^-$	Syringetin-7-*O*-sulfate
25	20.15	258–342	477.1038	477.1028	447.0924, 284.0320	$C_{22}H_{21}O_{12}^-$	Isorhamnetin-3-*O*-glucoside
26	20.24	258–342	507.1144	507.1132	344.0530 (syringetin), 329.0295	$C_{23}H_{23}O_{13}^-$	Syringetin-3-*O*-galactoside
27	20.50	258–346	331.0459	331.0453	316.0216, 271.0140	$C_{16}H_{11}O_8^-$	3′-*O*-Methylmyricetin
28	20.66	258–346	439.0341	439.0331	359.3074, 329.0294, 316.0579, 195.0687	$C_{18}H_{15}O_{11}S^-$	3,3′,7-tri-*O*-Methyl-5′-sulfate myricetin
29	20.84	255–348	359.0772	359.0756	329.0652, 316.0216, 287.0188	$C_{18}H_{15}O_8^-$	3,3′-7-tri-*O*-Methylmyricetin
30	21.03	255–346	329.0667	329.0662	299.0189, 271.0240, 255.0291	$C_{17}H_{13}O_7^-$	3-*O*-Methylisorhamnetin
31	21.24	239–325	311.0561	311.0554	265.0449	$C_{17}H_{11}O_6^-$	Methyl-4[(7-hydroxy-4-oxo-4H-chromen-3-yl)oxy]benzoate
32	21.70	254, 354	345.0616	345.0608 (4.8)	330.0370, 315.0136, 285.0400	$C_{17}H_{13}O_8^-$	7,3′-di-*O*-Methylmyricetin
33	21.87	258–346	315.0510	315.0504	271.0241, 243.0291	$C_{16}H_{11}O_7^-$	Isorhamnetin[a]
34	21.98	258–346		345.0611	285.0399	$C_{17}H_{13}O_8$-	3′,5′-di-*O*-Methylmyricetin (Syringetin)
35	22.19		417.1555	417.1546	360.210	$C_{22}H_{25}O_8^-$	Syringaresinol
36	22.46	254, 354	345.0616	329.0660	299.0190, 271.0240, 227.0341	$C_{17}H_{13}O_8^-$	3,4′-Dimethylmyricetin
37	22.59	318	327.2177	327.2171	325.1836, 211.1331	$C_{18}H_{31}O_5^-$	Tri-hydroxyoctadecadienoic acid
38	23.45	275	329.2333	329.2327	325.1836, 2111331	$C_{18}H_{33}O_5^-$	Tri-hydroxyoctadecaenoic acid
39	23.95	239–325	363.1238	363.1231	325.1834	$C_{22}H_{19}O_5^-$	Chromone derivative

[a] Identified by spiking experiments with authentic compound.

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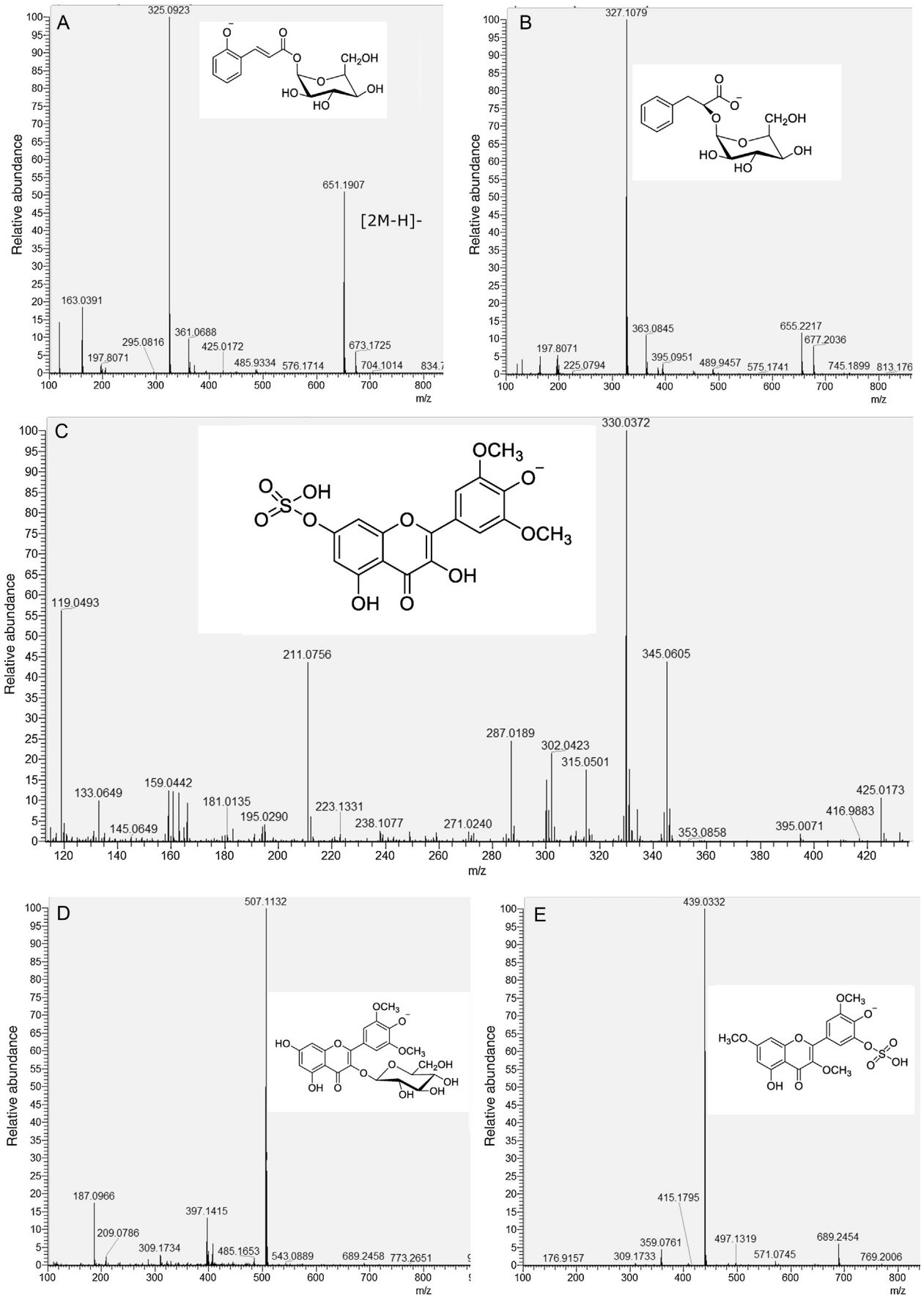

Fig. 3. (A–H) Examples of Full MS spectra and structures of metabolites for peaks 9, 14, 24, 26, 28, 31, 38 and 39, respectively.

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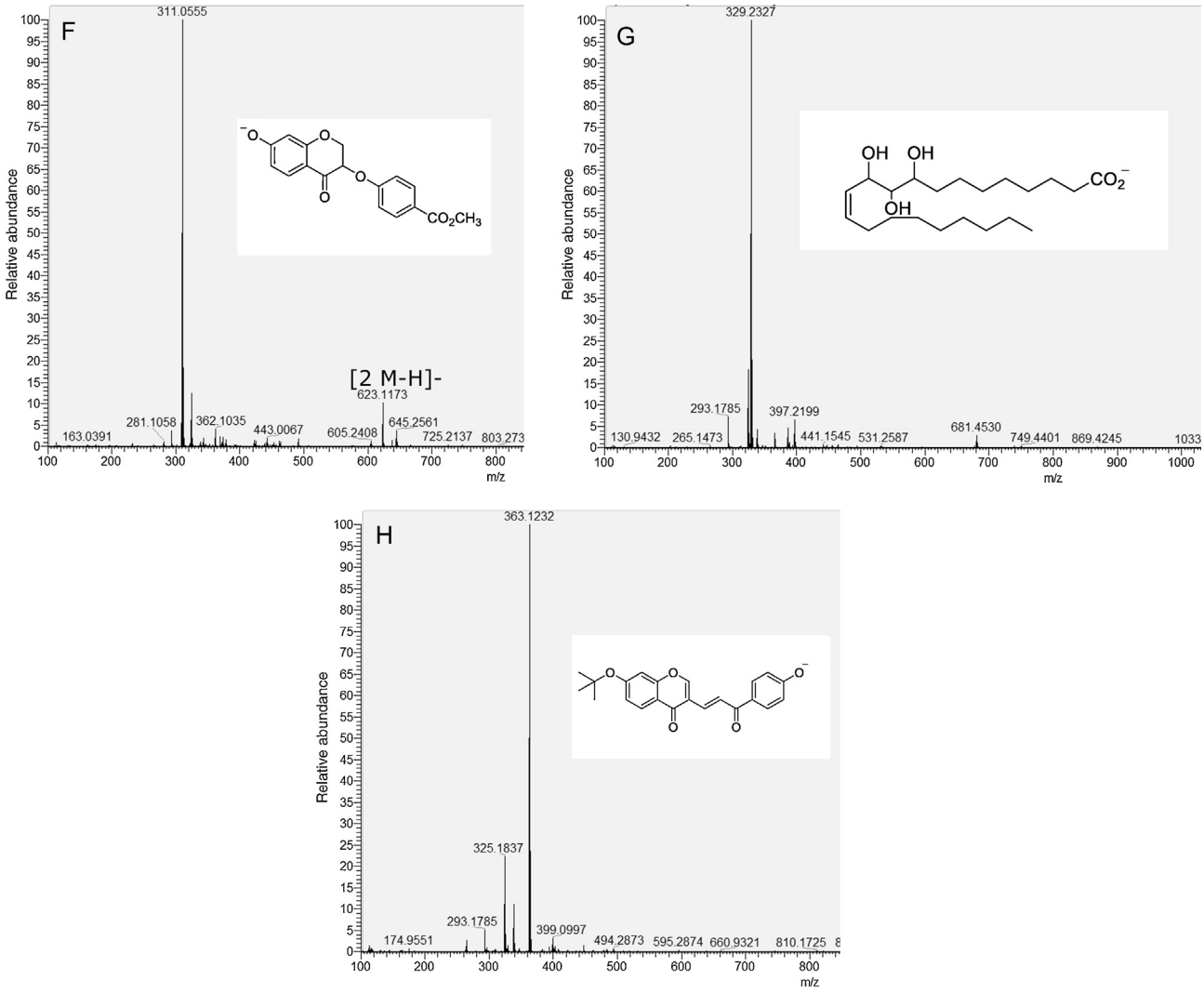

Fig. 3. (*Continued*)

p-coumaroyl hexose (*trans*-2-hydroxy-galactosyl-cinnammate), respectively. Peak 14 (Fig. 3B) with a $[M-H]^-$ ion at *m/z* 327.1076 ($C_{15}H_{19}O_8^-$) producing a phenyllactic acid MS^2 ion at around *m/z* 165.0547 ($C_9H_9O_3^-$) was identified as phenyllactic acid-2-*O*-glucoside (Beelders et al., 2014). Peak 18 was identified as the respective aglycone phenyllactic acid (Li et al., 2015) ($C_9H_9O_3^-$). Peak 35 with a $[M-H]^-$ ion at *m/z* 417.1546 was identified as the lignin syringaresinol (Sharp et al., 2001) ($C_{22}H_{25}O_8^-$). Peak 20 with a $[M-H]^-$ ion at *m/z* 515.1182 and daughter characteristic ions at *m/z* 353.0870, 191.0552 (quinic acid $C_7H_{11}O_6^-$) and 179.0341 was identified as dicaffeoyl-quinic acid (CQA) (Narváez-Cuenca et al., 2012). The CQA was also identified according to its UV spectra (λ_{max} at 235–314–325 nm).

Flavonols

Several *O*-methylated flavonoids have previously been detected in *O. triangularis* and their presence is of taxonomical relevance for this species and several species among this genus (Alarcon et al., 1993; Sigstad et al., 1996; Barrero et al., 2006). These types of compounds were also detected in the present work, confirming these flavonoids as chemotaxonomic markers. Isomer compounds detected by peaks 23 and 24 (Fig. 3C) with $[M-H]^-$ ions at *m/z* 425.0176 and *m/z* 425.0177 ($C_{17}H_{13}O_{11}S^-$) producing ions at *m/z* 345.0605 (di-*O*-methylmyricetin), 330.0372 (*O*-methylmyricetin), 315.0138 (myricetin), were identified as 3′,7-di-*O*-methylmyricetin-5′-*O*-sulfate and syringetin-7-*O*-sulfate (Harborne and Boardley, 1984), respectively. Peak 25 with an $[M-H]^-$ ion at *m/z* 477.1028 was identified as isorhamnetin glucoside ($C_{22}H_{21}O_{12}^-$) and peak 26 (Fig. 3D) producing a MS^2 ion at *m/z* 344.0530 (syringetin) was identified as syringetin 3-*O*-galactoside ($[M-H]^-$ ion at *m/z* 507.1132, $C_{23}H_{23}O_{13}^-$) (Su et al., 2016). Peak 28 (Fig. 3E) with an $[M-H]^-$ ion at *m/z* 439.0331 and MS fragments at *m/z* 359.3074 ($[M-H\text{-sulfate}]^-$), 329.0294, 195.0687 was tentatively identified as myricetin 3,3′7-tri-*O*-methyl-5′-*O*-sulfate (Harborne and Boardley, 1984) ($C_{18}H_{15}O_{11}S^-$), while peak 29 with an $[M-H]^-$ ion at *m/z* 359.0756 as the related compound 3,3′,7-tri-*O*-methylmyricetin ($C_{18}H_{15}O_8^-$), peak 32 with an $[M-H]^-$ ion at *m/z* 345.0608 was identified as 7,3′di-*O*-methylmyricetin ($C_{17}H_{13}O_8^-$) and peak 36 as its isomer syringetin (3′,5′di-*O*-methyl myricetin). Peak 33 with an $[M-H]^-$ ion at *m/z* 315.0504 was identified by co-elution with an authentic compound as isorhamnetin ($C_{16}H_{11}O_7^-$) (Makita et al., 2016) and peak 30 as its methylated derivative 3-*O*-methylisorhamnetin ($C_{17}H_{13}O_7^-$). Peak 27 with an $[M-H]^-$ ion at *m/z* 331.0453 yielding a MS^2 ion at *m/z* 316.0216 (myricetin) was identified as the 3′-*O*-methylmyricetin ($C_{16}H_{11}O_8^-$) (Makita et al., 2016). Peaks 19 and 17 with molecular anions at *m/z* 463.0876 and 447.0959 were identified as quercetin-3-*O*-glucoside ($C_{21}H_{19}O_{12}^-$) and rhamnoside ($C_{21}H_{19}O_{11}^-$), respectively (Panusa et al., 2015).

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Oxylipins

This is the first report of these antioxidant fatty acids in the genus. Peak 38 with an $[M-H]^-$ ion at *m/z* 327.2171 (Fig. 3G) was identified as the antioxidant fatty acid tri-hydroxyoctadecadienoic acid (Oxylipin) (Jiménez-Sánchez et al., 2016) and peak 37 with an $[M-H]^-$ ion at *m/z* 329.2327 was identified as the related tri-hydroxyoctadecaenoic acid (Jiménez-Sánchez et al., 2016).

Chromenes

Chromenes were also detected in other *Ophryosporus* species (Ferracini et al., 1989; Sigstad et al., 1992; Alarcon et al., 1993) and those compounds are also chemical markers for the genus. For *O. triangularis* collected in La Serena, Chile, Alarcon et al. reported several chromones and benzofuranes including 5-acetyl-6-methoxy-2-isopropenyl benzofurane, 6-acetyl-2,2-dimethyl-chromenone, 6-acetyl-5-methoxy-8-senecioyl-2,2-dimethylchromene, 6-acetyl-8-senecioyl-2,2-dimethylchromene, 6-acetyl-2,2-dimethyl-chromen-4-one (Alarcon et al., 1993). Peak 31 (Fig. 3F) with an $[M-H]^-$ ion at *m/z* 311.0554 ($C_{17}H_{11}O_6^-$) was identified as methyl-4[(7-hydroxy-4-oxo-4*H*-chromen-3-yl)oxy]benzoate while peak 39 (Fig. 3H) with an $[M-H]^-$ ion at *m/z* 363.1231 was identified as a related chromone (Mazzei et al., 2003) derivative of molecular formula $C_{22}H_{19}O_5^-$.

Sugars

The sugar cymarose (Franz, 1971) ($[M-H]^-$ ion at *m/z* 161.0810) was identified (peak 6, molecular formula $C_7H_{14}O_4^-$). An unknown compound (peak 7) producing a cymarose residue was also detected (peak 7, $[M-H]^-$ ion at *m/z* 381.1313).

Conclusions

The methanolic extracts of the aerial parts and flowers of an endemic species from the Atacama Desert showed antioxidant capacities (65.34 ± 1.32 and 52.41 ± 1.87 μg/ml in the DPPH assay, 184.88 ± 13.22 and 196.80 ± 13.28 μmol TE/g dry weight in the ferric reducing power (FRAP) assay and 56.17 ± 3.03 and 65.41 ± 1.96% in the superoxide anion scavenging assay, respectively). Thirty-nine compounds responsible for the antioxidant activity were detected in the methanolic extract of the aerial parts and twenty-four were detected in the methanolic extract of the flowers using a hybrid UHPLC-PDA-OT-MS instrument in three different samples collected in Paposo Valley, Atacama Desert. Several compounds were identified for the first time in this species. The flowers and aerial parts of *O. triangularis* can be useful for the preparation of nutraceuticals or phytomedicines. Furthermore, the hyphenated UHPLC-Q-exactive focus machine equipped with orbitrap-PDA detectors and high resolution collision cell provide a powerful method for accurate and fast metabolomics analysis of the Atacama Desert flora and the UHPLC–MS fingerprint generated can be used for the authentication of this species while several compounds including some *O*-methylated flavones and phenolic acids such as *p*-coumaroyl-glucoside can be used as chemotaxonomic markers. Further studies on this Chilean plant should be undertaken with more samples to obtain a more complete picture on the constituents and potential of this species.

Author's contributions

MS and AM wrote and revised the manuscript, CQ, JMV and CA analyzed the data, BS and JB performed the antioxidant assays, CEC helped in the collection of the plant material an analysis of the data.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors appreciate financial support by FONDECYT (Grant 1140178) and fondequip (EQM140002). We thank CONAF (Corporación Nacional Forestal de Chile) for the support in the collection of Paposo Valley flora, in the national protected area.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2016.10.002.

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