Chromatographia (2017) 80:967–973
DOI 10.1007/s10337-017-3304-4

SHORT COMMUNICATION

Metabolomic Analysis of the Lichen *Everniopsis trulla* Using Ultra High Performance Liquid Chromatography-Quadrupole-Orbitrap Mass Spectrometry (UHPLC-Q-OT-MS)

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Received: 20 December 2016 / Revised: 15 March 2017 / Accepted: 17 March 2017 / Published online: 3 April 2017

Abstract A new depside was identified in the methanolic extract from the lichen *Everniopsis trulla* based on the metabolomics UHPLC–DAD–MS analysis and HESI–MS–MS fragmentation patterns along with thirty-two known compounds for the first time. The compounds were structurally characterized by UV and high resolution quadrupole orbitrap mass spectra and by comparison with literature. According to the characteristic fragmentation patterns, the presence of two simple aromatic compounds, six lipid derivatives, eight depsidones, thirteen depsides, a chromone, two diphenylethers, and a dibenzofuran were identified. To our knowledge, this is the first study of the lichen *E. trulla* by liquid chromatography hyphenated with tandem mass spectrometry.

Keywords Depsides · Depsidones · UHPLC–ESI–MS · *Everniopsis trulla* · Lichens · Orbitrap

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Introduction

Lichens are symbiotic associations of fungi with microalgae and/or cyanobacteria. They are organisms with remarkable tolerance to diverse atmospheric conditions including extreme temperature, desiccation, salinity, and UV radiation [1, 2]. More than 17,000 species of lichens have been reported so far [3]. Lichens are well known as source of phenolics (orcinol and β-orcinol), quinones (parietin), dibenzofurans (usnic acid), depsides (atranorin), depsidones (lobaric acid), depsones (picrolichenic acid), xanthones (lichexanthone), pulvinic acids (vulpinic acid) and γ-lactones (protolichesterinic acid) [1, 2]. Up to date, many lichen substances have been reported to have a wide range of biological activities including analgesic, antioxidant, antibiotic, anti-inflammatory, antiproliferative, antibacterial, antifungal, antitumor, enzyme inhibition, gastroprotective, antiprotozoal, antiviral, antidiabetic [2, 4–6]. The most important use of lichens in traditional medicine is based on the fact that they contain bioactive substances. Due to this fact, lichens attract great attention as source of new bioactive substances [2, 3].

On the other hand, ultra high performance liquid chromatography-diode array detection (UHPLC-DAD) coupled to an electrospray ionization tandem mass spectrometer (ESI–MS–MS) has emerged as a powerful technique for identification and elucidation of metabolites in complex extracts [7–10]. The Q-Exactive Focus is a newly released

fast hybrid high resolution mass spectrometer used to detect and quantify small organic compounds (up to 2000 amu). The hyphenated Q-Exactive Focus instrument is a HRAM instrument (high resolution accurate mass) which combines UHPLC-DAD with an orbitrap, a quadrupole (Q) and a higH −resolution collision cell (HCD), which allows high resolution MS fragments [7–10].

Continuing our investigation of lichens, we describe for the first time the phytochemical profile and a new depside from the lichen *Everniopsis trulla* based on UHPLC-DAD coupled with high resolution electrospray ionization tandem mass spectrometry (Q-orbitrap).

Experimental

Lichen Material

The lichen specimen *E. trulla* (100 g) was collected at "Canchas city", in Asunción, Huaraz, Perú, in 2011 at an altitude of 3427 m. A voucher specimen (ET-15032011) was deposited in the Museo de Historia Natural from the Universidad Mayor de San Marcos (Lima, Perú) and Prof. Dr. Magda Chanco confirmed its identity.

UHPLC-Orbitrap-ESI–MS–MS

Sample Preparation

The lichen *E. trulla* (10 g) was extracted with methanol for 30 min in the dark using an ultrasonic bath at room temperature (100 mL, three times). After filtration, the combined extracts (300 mL) were immediately concentrated in vacuo at 40 °C and a dark brown gum was obtained (90 mg).

Instrument

A Thermo Scientific Dionex Ultimate 3000 UHPLC system equipped with a quaternary Series RS pump and a Thermo Scientific Dionex Ultimate 3000 Series TCC-3000RS column compartments with a Thermo Fisher Scientific Ultimate 3000 Series WPS-3000RS autosampler and a rapid separations PDA detector controlled by Chromeleon 7.2 Software (Thermo Fisher Scientific, Waltham, MA and Dionex Softron GmbH Part of Thermo Fisher Scientific, Germany) hyphenated with a Thermo high resolution Q Exactive focus mass spectrometer (Thermo, Bremen, Germany) were used for analysis. The chromatographic system was coupled to the MS with a Heated Electrospray Ionization Source II (HESI II). Nitrogen (purity >99.999%) obtained from a Genius NM32LA nitrogen generator (Peak Scientific, Billerica, MA, USA) was employed as both the collision and damping gas. Mass calibration for Orbitrap was performed once a week, in both negative and positive modes, to ensure a working mass accuracy lowers than or equal to 5 ppm. Cafeine and N-butylamine (Sigma Aldrich, Saint Louis, Mo, USA) were the calibration standards for positive ions and buspirone hydrochloride, sodium dodecyl sulfate, and taurocholic acid sodium salt (Sigma Aldrich, Saint Louis, Mo, USA) were used to calibrate the mass spectrometer. These compounds were dissolved in a mixture of acetic acid, acetonitrile, water and methanol (Merck Darmstadt, Germany) and were infused using a Chemyx Fusion 100 syringe pump (Thermo Fisher Scientific, Bremen, Germany). XCalibur 2.3 software (Thermo Fisher Scientific, Bremen, Germany) and Trace Finder 3.2 (Thermo Fisher Scientific, San José, CA, USA) were used for UHPLC control and data processing, respectively. Q Exactive 2.0 SP 2 from Thermo Fisher Scientific was used to control the mass spectrometer.

LC Parameters

An UHPLC C18 column (Acclaim, 150 mm × 4.6 mm ID, 5 m, Thermo Fisher Scientific, Bremen, Germany) operated at 25 °C was employed. The detection wavelengths were 254, 280, 320 and 440 nm. PDA was recorded from 200 to 800 nm, and mobile phases were 1% formic aqueous solution (A) and acetonitrile (B). The gradient program [time (min), % B] was: (0.00, 5); (5.00, 5); (10.00, 30); (15.00, 30); (20.00, 70); (25.00, 70); (35.00, 5) and 12 min for column equilibration before each injection. The flow rate was 1.00 mL min^{-1}, and the injection volume was 10 μL. Standards and lichen extracts dissolved in methanol were kept at 10 °C inside the autosampler.

MS Parameters

The HESI parameters were as follows: sheath gas flow rate 75 units; aux. gas unit flow rate 20; capillary temperature 400 °C; aux gas heater temperature 500 °C; spray voltage 2500 V (for ESI−); and S lens RF level 30. Full scan data in positive and negative was acquired at a resolving power of 70,000 FWHM (full width half maximum) at *m/z* 200. For the compounds of interest, a scan range of *m/z* 100–1000 was chosen; the automatic gain control (AGC) set at $3e^6$ and the injection time set to 200 ms. Scan-rate was set at 2 scans s^{-1}. External calibration was performed using a calibration solution in positive and negative modes. For confirmations purposes, a targeted MS/MS analysis was performed using the mass inclusion list, with a 30 s time window, with the Orbitrap spectrometer operating both in positive and negative mode at 17,500 FWHM (*m/z* 200). The AGC target was set to $2e^5$, with the max. injection time of 20 ms. The precursor ions are filtered by the quadrupole which operates at an isolation window of *m/z* 2.

The fore vacuum, high vacuum and ultrahigh vacuum were maintained at approximately 2 mbar, from 105 and below 1010 mbar, respectively. Collision energy (HCD cell) was operated at 30 kv. Detection was based on calculated exact mass and on retention time of target compounds, as shown in Table 1. The mass tolerance window was set to 5 ppm for the two modes.

Results and Discussion

Thirty-three compounds were identified for the first time in the methanolic extract from *E. trulla*. All of them were elucidated on the basis of their ESI–MS–MS fragmentation patterns and UV data [8–11]. On the basis of the examined information (Fig. 1) we identified two aromatic compounds (peaks 1 and 3), six lipid derivatives (peaks 2, 5, 9, 11, 14 and 29), eight depsidones (peaks 4, 6, 8, 10, 12, 20, 27 and 28), thirteen depsides (peaks 7, 13, 15, 16, 18, 19, 21, 23, 24–26, 31 and 33), a chromone (peak 17), two diphenylethers (peaks 22 and 32) and a dibenzofuran (peak 30).

All compounds were detected in negative mode using UHPLC-Q-Orbitrap-ESI–MS–MS (Table 1). Peak 1 was identified as orsenillic acid ($[M - H -]^-$ at *m/z* 167.0345) whose fragmentation produced a diagnostic MS ion at *m/z* 123.0437. Peak 2 was identified as 4,5-dihydroxy-2-nonenoic acid based on its high resolution MS spectrum at *m/z* 187.0976 ($[M - H -]^-$ peak) and their daughter MS ions at *m/z* 145.0498 and 101.0239. Peak 3 was identified as atranol, which showed a $[M - H -]^-$ peak at *m/z* 151.0395 and also produced a daughter ion at *m/z* 123.0437. Peak 4 was identified as salazinic acid (molecular anion at *m/z* 387.0359). The fragmentation of this peak produced ions at 269.0464 $[M - H - C_3H_3O_5]^-$, 241.0504 $[M - H - C_4H_2O_6]^-$, 151.0394 $[C_8H_7O_3]^-$, and 123.0445 $[C_7H_7O_2]^-$ confirming this depsidone. Peak 5 showing a high resolution $[M - H -]^-$ ion at *m/z* 329.2328 and a daughter ion at *m/z* 183.1383 was identified as 12,13,15-trihydroxy-9-octadecenoic acid. Peak 6 was assigned to depsidone siphulellic acid based on its molecular anion at *m/z* 401.0516 ($[M - H -]^-$ peak) and their daughter ions at 253.0505 $[M - H - 2CO_2 - CH_3COOH]^-$, 149.0238 $[C_8H_5O_3]^-$, and 123.0444 $[C_7H_7O_2]^-$. Peak 7 was identified as lecanoric acid, which showed an $[M - H -]^-$ ion at m/z 317.0667. Major diagnostic daughter MS ions of this depside were $[M - H - C_8H_6O_3]^-$, $[M - H - C_8H_8O_4-]^-$ and $[C_7H_7O_2]^-$ (167.0343, 149.0237 and 123.0444 a.m.u., respectively). Peak 8 was assigned to galbinic acid whose $[M - H -]^-$ ion was at *m/z* 429.0464 and diagnostic daughter MS ions of this depsidone were at *m/z* 269.0454 $[M - H - C_8H_6O_3]^-$, 149.0238 $[M - H - C_8H_8O_4-]^-$ and 123.0443 $[C_7H_7O_2]^-$. Peak 9 showing a $[M - H -]^-$ ion at *m/z* 403.3065 and a daughter ion at *m/z* 247.1545 was identified as 9,10,12,13-tetrahydroxydocosanoic ($C_{22}H_{43}O_6$). Norstictic acid was identified as peak 10. The fragmentation of this depsidone produced diagnostic ions at *m/z* 327.0509 $[M - H- CO_2]^-$, 151.0393 $[M - H - C_{10}H_4O_6]^-$, and 123.0444 $[M - H - C_{11}H_4O_7]^-$confirming this compound. Peak 11 was assigned the structure 9,10,12,13-tetrahydroxytricosanoic acid based on its molecular anion at *m/z* 417.3216 ($[M - H -]^-$ peak) and their daughter ions at *m/z* 247.1546 $[C_{12}H_{23}O_5]^-$, and 173.1178 $[C_9H_{17}O_3]^-$. Peak 12 was identified as hypoconstictic acid, which showed an $[M - H -]^-$ ion at *m/z* 387.0723. This depsidone showed also two diagnostic daughter MS ions at *m/z* 343.0822 $[M - H - CO_2]^-$, and 299.0923 $[M - H - 2CO_2]^-$. Peak 13 was tentatively identified as a gyrophoric acid derivative according to SciFinder research, showing an UV absorbance at λ_{max} 243, 287, 326 nm, which is a characteristic of depside related compounds. The deprotonated molecule ($[M - H -]^-$) was evidenced at *m/z* 449.0880 and its fragmentation showed daughter ions at m/z 151.0395, 149.0237, and 123.0446. Considering that peak 13 showed very similar diagnostic losses to that of gyrophoric acid and based on biosynthetic considerations, we tentatively identified peak 13 as 4-((4-((2,4-dihydroxy-6-methylbenzoyl)oxy)-2-hydroxy-6-methylbenzoyl)oxy)-2-hydroxy-6-methylbenzoic acid lactone (Fig. 2). Peak 14 was identified as 6-ethyl-6-n-pentylpentadecan-4,5,7,8,15-pentol-15-acetate based on its high resolution MS spectrum at *m/z* 431.3379 ($[M - H -]^-$ ion) and its daughter ion at *m/z* 161.0811 $[C_7H_{13}O_4]^-$. The depside 2′-O-methylevernic acid was identified as Peak 15 ($[M - H -]^-$ at *m/z* 345.0981). The fragmentation of this ion produced ions at *m/z* 181.0501 $[M - H - C_9H_8O_3]^-$ and 137.0600 $[M - H - C_{10}H_8O_5]^-$ confirming this depside. Peak 16 with a $[M - H -]^-$ ion at *m/z* 467.0984 was assigned to gyrophoric acid, which was identified by spiking experiments with an authentic standard. Peak 17 was identified as lepraric acid, showing a molecular anion at *m/z* 361.0931. The fragmentation of peak 17 produced also ions at *m/z* 235.0609 $[M - H - C_6H_6O_3]^-$, 195.0292 $[M - H - C_9H_{10}O_3]^-$, and 149.0238 $[M - H - C_{10}H_{12}O_5]^-$ confirming this structure. Peak 18 was identified as evernic acid (molecular anion at *m/z* 331.0824). Main daughter ions of peak 18 was at *m/z* 167.0344 $[M - H - C_9H_8O_3]^-$, 149.0238 $[M - H - C_9H_{10}O_4-]^-$ and 123.0444 $[C_7H_7O_2]^-$. Peak 19 with a $[M - H -]^-$ ion at *m/z* 359.0767 was identified as 4-O-demethylbaeomycesic acid and their major diagnostic daughter MS ions were $[M - H - C_9H_6O_4-]^-$, $[M - H - C_9H_8O_5]^-$, and $[M - H - C_{10}H_6O_6]^-$ (181.0501, 163.0395 and 137.0601 a.m.u., respectively). The depsidone α-Alectoronic acid was identified as Peak 20 ($[M - H -]^-$ at *m/z* 511.1974). The fragmentation of this compound produced two ions at *m/z* 247.0975 $[C_{14}H_{15}O_4]^-$ and 149.0239 $[C_8H_5O_3]^-$ confirming its structure. Peak 21

Table 1 Identification of metabolites in *E. trulla* by UHPLC–ESI–MS–MS

Peak	Tentative identification	$[M-H]^-$	Retention time (min)	Theoretical mass (*m*/*z*)	Measured mass (*m*/*z*)	Accuracy (ppm)	Metabolite type	MS ions (ppm)
1	Orsellinic acid	$C_8H_7O_4$	11.40	167.0344	167.0345	0.6	A	123.0443
2	4,5-Dihydroxy-2-nonenoic acid	$C_9H_{15}O_4$	12.21	187.0976	187.0972	2.1	L	145.0498; 101.0239
3	Atranol	$C_8H_7O_3$	14.82	151.0395	151.0395	0.0	A	123.0443
4	Salazinic acid	$C_{18}H_{11}O_{10}$	15.11	387.0352	387.0359	1.8	D	269.0464; 241.0504 151.0394; 123.0445
5	12,13,15-Trihydroxy-9-octadecenoic acid	$C_{18}H_{33}O_5$	18.29	329.2328	329.2333	1.5	L	183.1383
6	Siphulellic acid	$C_{19}H_{13}O_{10}$	19.27	401.0509	401.0516	1.7	D	253.0505; 149.0238 123.0444
7	Lecanoric acid	$C_{16}H_{13}O_7$	19.44	317.0661	317.0667	1.9	d	167.0343; 149.0237 123.0444
8	Galbinic acid	$C_{20}H_{13}O_{11}$	19.65	429.0458	429.0464	1.4	D	269.0454; 149.0238 123.0443
9	9,10,12,13-Tetrahydroxydocosanoic acid	$C_{22}H_{43}O_6$	19.84	403.3065	403.3067	0.6	L	247.1545
10	Norstictic acid	$C_{18}H_{11}O_9$	20.05	371.0403	371.0409	1.6	D	327.0509 151.0393; 123.0444
11	9,10,12,13-Tetrahydroxytricosanoic acid	$C_{23}H_{45}O_6$	20.31	417.3216	417.3222	1.4	L	247.1546; 173.1178
12	Hypoconstictic acid	$C_{19}H_{15}O_9$	20.54	387.0716	387.0723	1.8	D	343.0822; 299.0923
13	Gyrophoric acid derivative	$C_{24}H_{17}O_9$	20.70	449.0873	449.0880	1.9	d	151.0397; 149.0237 133.0287; 123.0441
14	6-Ethyl-6-*n*-pentylpentadecan-4,5,7,8,15-pentol-15-acetate	$C_{24}H_{47}O_6$	20.80	431.3373	431.3379	1.4	L	161.0811
15	2-O-Methylevernic acid	$C_{18}H_{17}O_7$	21.02	345.0974	345.0981	2.1	d	181.0501; 137.0600
16	Gyrophoric acid[a]	$C_{24}H_{19}O_{10}$	21.31	467.0978	467.0984	1.3	d	317.0667; 167.0345 149.0238; 123.0443
17	Lepraric acid	$C_{18}H_{17}O_8$	21.69	361.0923	361.0931	2.2	C	235.0609; 195.0292 149.0238
18	Evernic acid	$C_{17}H_{15}O_7$	21.81	331.0818	331.0824	1.8	d	167.0344; 149.0238 123.0444
19	4-O-Demethylbaeomycesic acid	$C_{18}H_{15}O_8$	22.43	359.0767	359.0774	1.9	d	181.0501; 163.0395 137.0601

Table 1 continued

Peak	Tentative identification	$[M-H]^-$	Retention time (min)	Theoretical mass (*m/z*)	Measured mass (*m/z*)	Accuracy (ppm)	Metabolite type	MS ions (ppm)
20	α-Alectoronic acid	$C_{28}H_{31}O_9$	22.57	511.1968	511.1974	1.8	D	247.0975; 149.0239
21	Baeomycesic acid	$C_{19}H_{17}O_8$	22.89	373.0923	373.0931	2.1	d	193.0514; 177.0188 167.0341; 123.0443
22	β-Alectoronic acid	$C_{28}H_{31}O_9$	23.46	511.1968	511.1974	1.2	DE	369.1346; 247.0973 163.0396
23	Methyl 8-hydroxy-4-*O*-demethylbarbatate	$C_{19}H_{19}O_8$	23.60	375.1080	375.1086	1.6	d	343.0823; 163.0395
24	2-0-Methylstenosporic acid	$C_{24}H_{29}O_7$	23.87	429.1913	429.1919	1.7	d	223.0972; 179.1072
25	8-Hydroxybarbatic acid	$C_{19}H_{19}O_8$	24.11	375.1085	375.1089	2.4	d	195.0654; 181.0499
26	Barbatic acid	$C_{19}H_{19}O_7$	24.29	359.1131	359.1137	1.7	d	181.0501; 163.0394 137.0600
27	α-Collatolic acid	$C_{29}H_{33}O_9$	24.62	525.2125	525.2130	0.9	D	263.1281
28	Lobaric acid	$C_{25}H_{27}O_8$	25.04	455.1711	455.1713	0.4	D	411.1815; 367.1909 352.1681; 296.1048
29	9,10-Dihydroxy-12,13-dioxononadecanoic acid	$C_{19}H_{33}O_6$	25.43	357.2283	357.2285	2.2	L	173.1180
30	Usnic acid[a]	$C_{18}H_{15}O_7$	26.14	343.0818	343.0824	1.2	DBF	328.0591; 259.0609; 231.0661
31	Atranorin	$C_{19}H_{17}O_8$	26.38	373.0923	373.0929	1.6	d	177.0190; 163.0386
32	β-Collatolic acid	$C_{29}H_{33}O_9$	26.77	525.2125	525.2128	0.3	DE	265.1077
33	Chloroatranorin	$C_{19}H_{16}O_8Cl$	28.92	407.0534	407.0540	1.5	d	228.9906; 210.9800 163.0394

A aromatic, *L* lipid, *D* depsidone, *d* depside, *DE* diphenylether, *DBF* dibenzofuran, *C* chromone

[a] Identified by spiking experiments with an authentic compound

was identified as baeomycesic acid, which showed a [M – H −]$^-$ peak at *m/z* 373.0931. Major diagnostic daughter MS ions were $[C_{10}H_9O_4]^-$, $[C_9H_5O_4-]^-$, $[C_8H_7O_4-]^-$ and $[C_7H_7O_2]^-$ (193.0514, 177.0188, 167.0341 and 123.0443 a.m.u., respectively). Peak 22 was assigned to β-alectoronic acid base on its high resolution molecular anion at *m/z* 511.1974 ([M – H −]$^-$ peak) and their daughter ions at 369.1346 $[C_{21}H_{21}O_6]^-$, 247.0973 $[C_{14}H_{15}O_4]^-$, and 163.0396 $[C_9H_7O_3]^-$. Peak 23 was assigned to methyl 8-hydroxy-4-O-demethylbarbatate whose [M – H −]$^-$ ion was at *m/z* 375.1086 and daughter ions at *m/z* 343.0823 [M – H − CH_4O]$^-$, and 163.0395 $[C_9H_7O_3]^-$. Peak 24 was identified as 2-*O*-methylstenosporic acid, which showed a [M – H −]$^-$ peak at *m/z* 429.1919 and their daughter ions at *m/z* 223.0972 $[C_{12}H_{15}O_4]^-$, and 179.1072 $[C_{11}H_{15}O_2-]^-$. In the same manner, peak 25 was identified as 8-hydroxybarbatic acid based on its high resolution MS spectrum and UV data (247; 278; 318 nm). Peak 26 was identified as barbatic acid whose [M – H −]$^-$ ion was evidenced at *m/z* 359.1137. This depside was elucidated based on their fragmentation patterns at *m/z* 181.0501 $[C_9H_9O_4]^-$, 163.0394 $[C_9H_7O_3]^-$, and 137.0600 $[C_9H_7O_3]^-$. Peaks 27 and 32 had the same [M – H −]$^-$ ion at *m/z* 525.2130 with different retention time on UHPLC at 24.62 and 26.77 min, and thus were tentatively identified as α- and β-Collatolic acid, respectively.

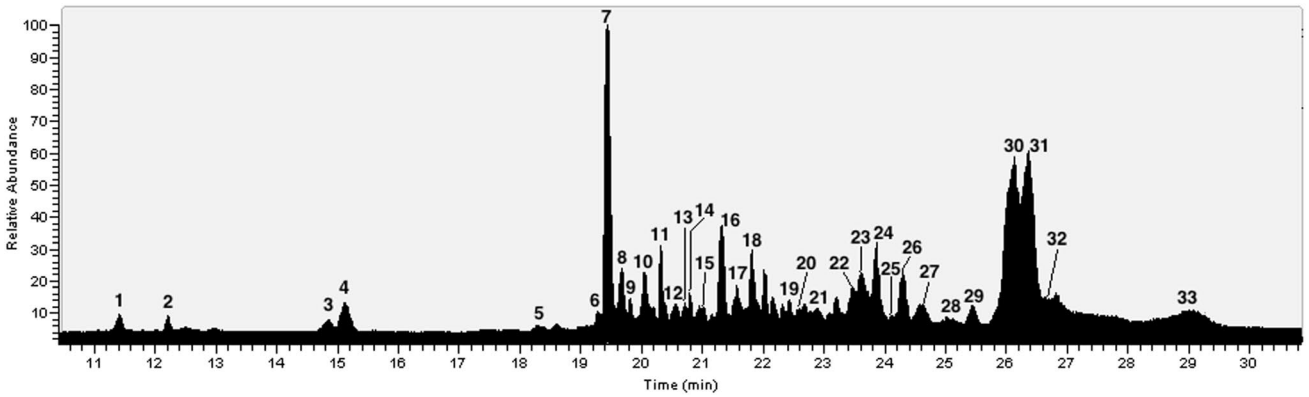

Fig. 1 Chromatogram of *E. trulla* on negative mode

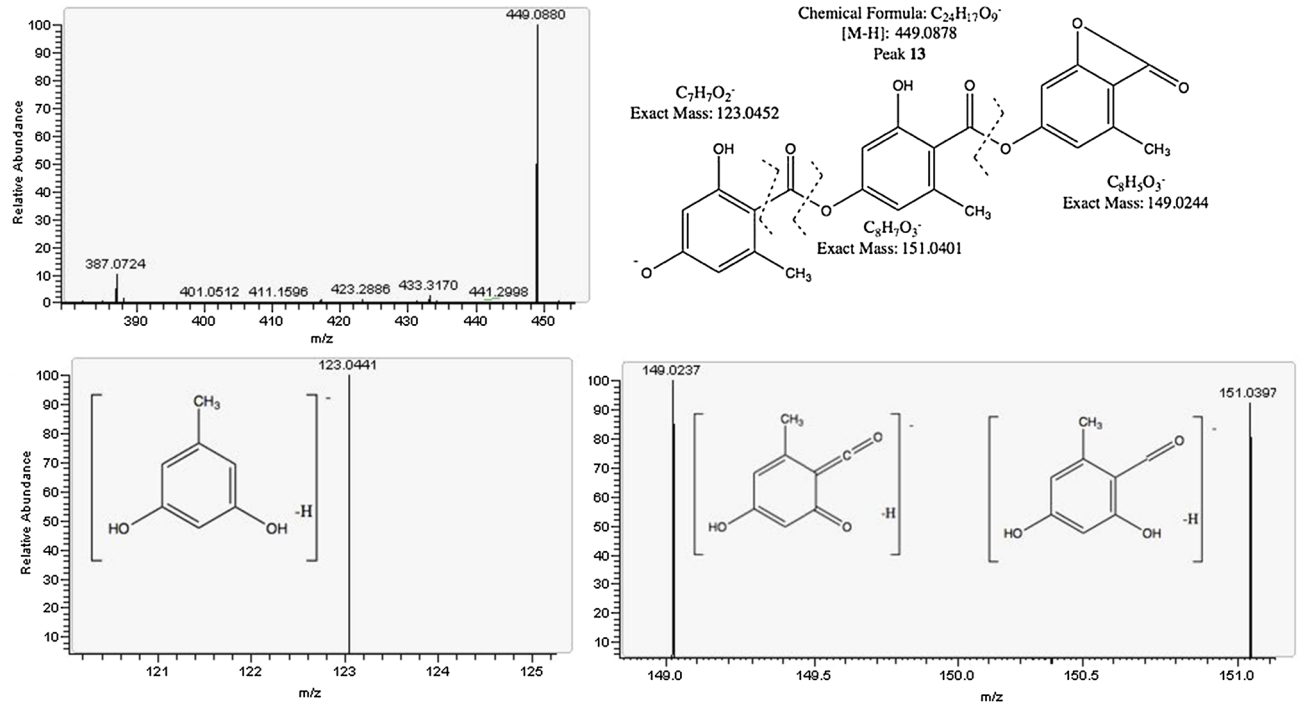

Fig. 2 Chemical structure of new compound tentatively identified by UHPLC-ESI–MS–MS and its proposed fragmentation pathway

Peak 28 was identified as lobaric acid (molecular anion at *m/z* 455.1713). The fragmentation of peak 28 also produced ions at 411.1815 $[M - H - CO_2]^-$, 367.1909 $[M - H - 2CO_2]^-$, 352.1681 $[M - H - 2CO_2 - CH_3]^-$, and 296.1048 $[M - H - 2CO_2 - C_5H_{11}]^-$ confirming this depsidone. Peak 29 was identified as 9,10-dihydroxy-12,13-dioxononadecanoic acid according to its high resolution MS spectrum and its fragment ion at *m/z* 173.1180. Usnic acid with a $[M - H -]^-$ ion at *m/z* 343.0824 was evidenced as peak 30. The main daughter ions of peak 30 were $[M - H - CH_3]^-$, $[M - H - C_4H_3O_2\text{-}]^-$ and $[M - H - C_5H_3O_3]^-$ (328.0583, 259.0612 and 231.0663 a.m.u., respectively). Peak 31 was identified as atranorin based on its high resolution mass spectrum ($[M - H -]^-$ ion at *m/z* 373.0929) and their daughter fragments at *m/z* 177.0190 $[M - H - C_{10}H_{12}O_4]^-$, and 163.0386 $[M - H - C_{10}H_{10}O_5]^-$. Finally, peak 33 was assigned to chloroatranorin. Its high resolution mass spectrum showed a $[M - H -]^-$ ion at *m/z* 407.0540. The fragmentation of peak 33 produced ions at *m/z* 228.9906 $[C_9H_6ClO_5]^-$, 210.9800 $[C_9H_4ClO_4]^-$, and 163.0394 $[C_9H_7O_3]^-$ confirming the structure.

To date, limited phytochemical studies regarding to the lichen have been reported. *E. trulla* from Perú was studied by Ramut et al. in the late 1978 [12], who demonstrated the presence of atranorin, chloroatranorin and usnic acid. At 2012, Rodriguez et al. reported that an extract of *E. trulla* was able to degrade prions based on its serine protease activity [13]. However, several HPLC–MS analysis of lichen extracts have been published [8–10, 14, 15]. From *Ophioparma ventosa* the phenolic thamnolic acid, divaricatic acid, and usnic acid were reported using LC–MS–MS in negative mode while usnic acid, stictic acid, norstictic acid, psoromic acid haemoventosin, atranorin, thamnolic acid, decarboxythamnolic acid, and divaricatic acid were reported using DART–MS [14]. Tomasi et al. 2013 reported a study of three chemotypes of *Ramalina cuspidate* and *R. siliquosa* using LC–ESI–MS–MS approach. Ten compounds (conhypoprotocetraric acid, salazinic acid, peristictic acid, cryptostictic acid, protocetraric acid, stictic acid, norstictic acid, hypoprotocetraric acid, usnic acid and O-demethylbarbatic acid) were detected in Ramalina chemotypes [15]. In another study, Cornejo et al. 2016 reported the presence of 22 compounds in the Antarctic lichen *Ramalina terebrata* using UHPLC-Q-Orbitrap-ESI–MS–MS. This work confirmed the presence of parietin, lobaric acid, placodiolic acid, arthoniaic acid, pseudoplacodiolic acid, gyrophoric acid, 3-hydroxyumbilicaric acid, 4-O-dimethylbaemycesic acid, and thirteen hydroxylipids for the first time. On the other hands, Musharraf et al. 2015 reported the presence of thirteen metabolites from *Parmotrema grayana* and nine compounds from *Heterodermia obscurata* based on negative mode electrospray quadrupole time-of-flight mass spectrometry (HR-ESI-Qq-TOF–MS–MS). Among them orcinol, 4-hydroxy-1,3-benzenedicarboxaldehyde, orsellinic acid, lecanoric acid, gyrophoric acid, divaricatinic acid, 3-methoxy-5-propylphenol, methyl-β-orcinolcarboxylate, atranorin, placodiolic acid, divaricatic acid, chloroatranorin, sekikaic acid, atranol, and methylorsellinate were detected [9]. Furthermore, Boustie et al. 2015 compared the versatility of laser desorption-ionization time-of-flight mass spectrometry (LDI-MS) applied to lichens with those obtained by direct ESI–MS detection as well as LC–ESI–MS. This technique was applied for the study of *Diploicia canescens*, *Evernia prunastri*, *Ophioparma ventosa*, *Pseudevernia furfuracea*, *Roccella fuciformis*, and *Xanthoria parietina* showing better results than ESI–MS. For instance, from *Diploicia canescens* diploicin, dechlorodiploicin, secalonic acids, and chloroatranorin, were detected using LDI-MS while using ESI–MS only diploicin and dechlorodiploicin were detected [8]. In the present study we confirmed the presence of several unreported compounds based on UHPLC-Q-Orbitrap-ESI–MS–MS confirming this technique as an important tool for the field of lichen metabolomics.

Conclusions

In the present study, a total of 33 compounds were identified for the first time using UHPLC-DAD-Orbitrap-ESI–MS–MS from the lichen *E. trulla*. One of them, peak 13, is reported for the first time in lichens using the proposed method. This work indicated that this technique is rapid, effective and accurate for structural characterization of phytochemical constituents in lichens. Besides, the UHPLC–MS fingerprinting could be very useful for the chemotaxonomy of this species.

Acknowledgements The work was supported by FONDECYT REGULAR 1150745. We are grateful to Prof. Magda Chanco for the identification of the lichen under study. Mario Simirgiotis acknowledge FONDEQUIP EQM 140002.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interests.

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