Matrix-free UV-laser desorption ionisation mass spectrometry as a versatile approach for accelerating dereplication studies on lichens

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ABSTRACT

This supporting material contains place and date of harvest and voucher specimen number of lichens described in this study (Table S-1). Then are displayed molecular formulas associated with all molecules detected as either single compounds and/or within lichen extracts (Figure S-1). Further are depicted LDI-MS (Figures S-2 – S-43) and ESI-MS spectra (Figures S-44 – S-81) associated with all studied single molecules and lichen acetone extracts. All spectra are acquired in negative-ion mode except for mycosporine serinol which was ionized using positive mode.

EXPERIMENTAL SECTION

Mass spectrometry and instrument settings

LDI experiments were carried out on a Bruker Biflex III time-of-flight (TOF) mass spectrometer (bruker Daltonik, Bremen, Germany) equipped with a 337-nm pulsed nitrogen laser (model VSL-337i, Laser Sciences Inc., Boston, MA). Spectra were acquired in the linear positive and negative mode within mass range of 20 to 2000 Da. The acceleration voltage was 19 kV, pulse ion extraction was 200 ns and laser frequency was 5 ns. Experiments were performed within a laser energy range of 30-80% (46.2 µJ-103.2 µJ), depending on sample requirements. For LDI analyses, single molecules and extracts were prepared at a concentration of 10 $mg.mL^{-1}$ in dichloromethane (DCM) or a mixture of DCM and pyridine 9:1 (v/v), depending on sample solubility. Mycosporine serinol was dissolved in distilled water.

For direct injection, ESI-MS solutions of single compounds and lichen extracts were prepared in DCM at concentrations of 0.5 mg mL^{-1} and 1.0 mg mL^{-1} respectively. These samples were introduced into an expression CMS single quadrupole (Advion) using a mixture of MeOH/ water (+ 0.1% formic acid) 9:1 (v/v) at a flow rate of 200 µL min^{-1}.

Full scan mass spectra were recorded in the negative ion mode in a mass range of 100 and 1200 Da applying the following parameters: detector gain 1200, ESI voltage 3.5 kV, capillary voltage 180 V, source voltage 20 V, source voltage dynamic 20 V, nebulizer gas pressure 60 psig, desolvation gas flow rate 4L min^{-1}, capillary temperature 250°C and source gas temperature 50°C. Full scan mass spectra were recorded in the positive ion mode in a mass range of 100 and 1200 Da using the following parameters: detector gain 1200, ESI voltage 3.5 kV, capillary voltage 180 V, source voltage 40 V, source voltage dynamic 0 V, nebulizer gas pressure 60 psig, dissolvation gas flow rate 4L min^{-1}, capillary temperature 250°C and source gas temperature 20°C. Data processing and evaluation for MS measurement was performed with the Data and Mass Express 2.2.29.2 software (Advion).

The LC-ESI and ESI mass spectra were all obtained from the same single quadrupole analyzer (Advion, Ithaca, USA). The MS system was coupled to a HPLC system (Prominence Shimadzu, Marne La Vallée, France) equipped with a Kinetex C18 HPLC column (100 x 4.6 mm – 2,6 µ - 6A, Phenomenex) and consisting of a quaternary pump (LC20ADSP), a surveyor autosampler (SIL-20AHT), and a diode array detector (SPD-M20A). HPLC analyses were performed by gradient elution using the following parameters: A (0.1% formic acid in water) and B (acetonitrile). T: 0 min, 20 % B; 0-25 min, 80 % B linear; 25-30 min, 100 % B linear ; 30-35 min 100% B ; 35-40 min 20% B linear. The flow rate was 0.2 mL min^{-1}.

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Table S-1 : Place and date of harvest and voucher specimen number of lichens described in this study.

Lichens	Voucher specimen	Place and date of harvest	Reference
Cladonia portentosa	JB/05/48	Cressensac (Lot) - 2005	[1]
Diploicia canescens	JB/06/10	Dinard (Ille-et-Vilaine) - 2006	[2]
Evernia prunastri	JB/06/51	Cressensac (Lot) -2006	
Flavocetraria nivalis	JB/02/37	Font-Romeu (Pyrénées Orientales) - 2002	[3]
Lecidella asema	2014/JYM/04	Pointe du Raz (Finistère) - 2014	
Ophioparma ventosa	JB/09/58	Obergurgl (Austria) - 2009	[4]
Pertusaria amara	JB/07/108	Liffré (Ille-et-Vilaine) - 2007	[5]
Pseudevernia furfuracea	JB/00/04	Meymac (Corrèze) - 2000	
Ramalina siliquosa* var. *crassa	JB/11/e496	Carantec (Finistère) - 2011	[6]
Ramalina cuspidata* var. *stenoclada	JB/11/e495	Trégastel (Finistère) - 2011	[6]
Roccella fuciformis	JB/05/57	Ploumanach (Côtes d'Armor) - 2005	[7]
Roccella phycopsis	JB/05/46	Ile de Bréhat (Côtes d'Armor) - 2005	[7]
Tephromela atra	JB/05/e56	La Guimorais (Ille-et-Vilaine) - 2006	[8]
Usnea filipendula	JB/00/78	Ventadour (Corrèze) - 07/2000	
Vulpicida pinastri	JB/14/198	Jausier (Alpes Maritimes) - 04/2014	[3]
Xanthoria parietina	JB/06/59	Dinard (Ille-et-Vilaine) - 01/2006	

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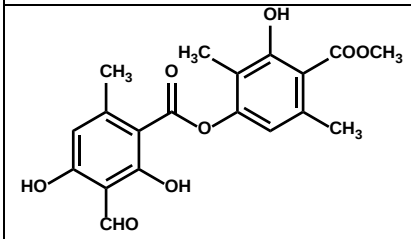
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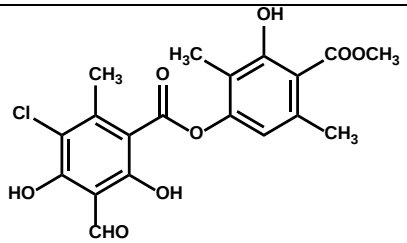
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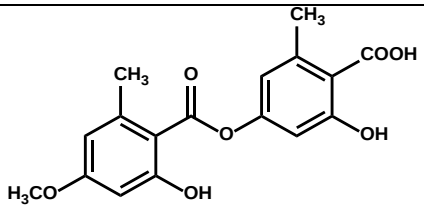
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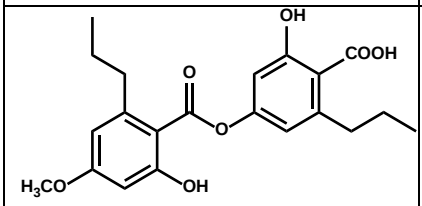
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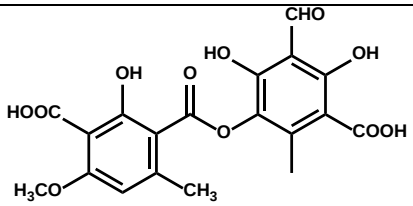
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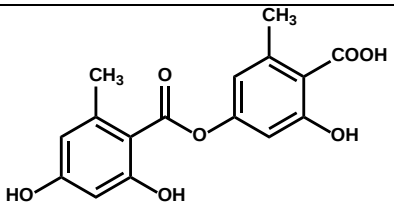
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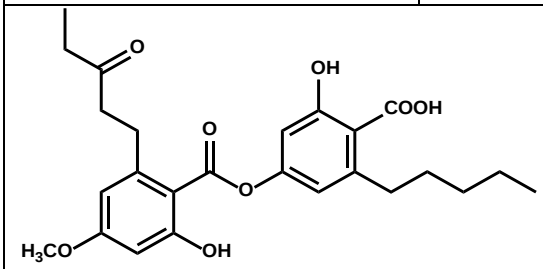
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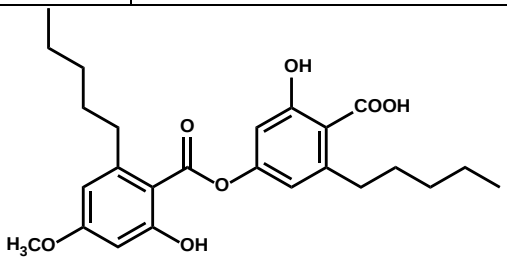
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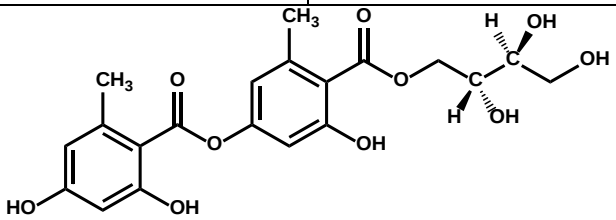
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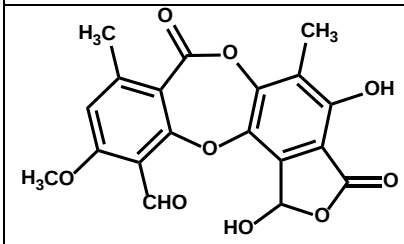
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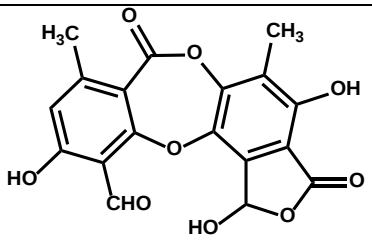
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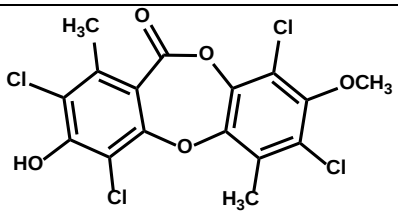
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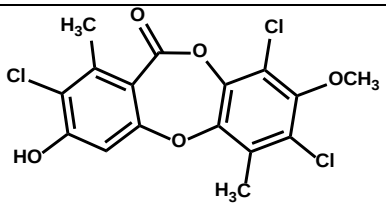
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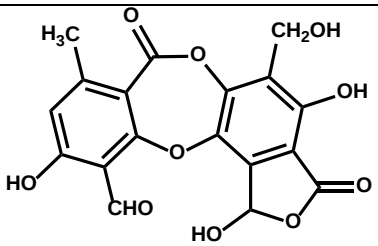
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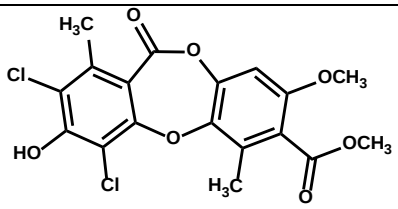
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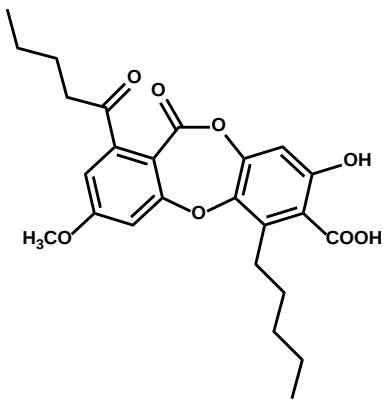
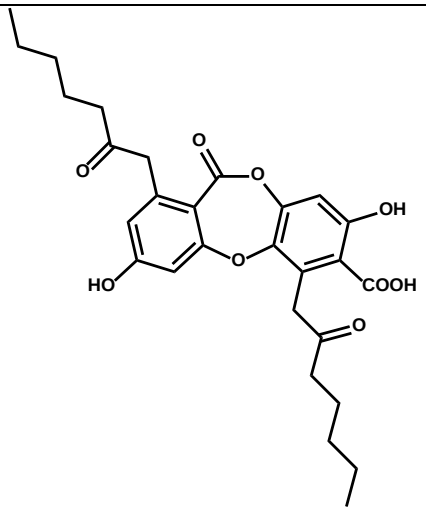
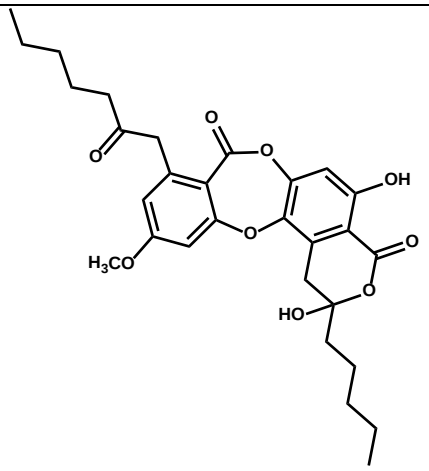
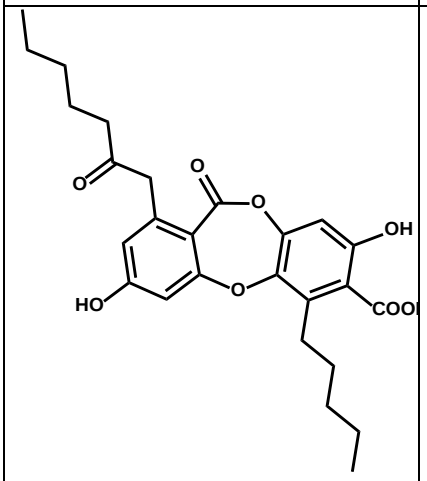
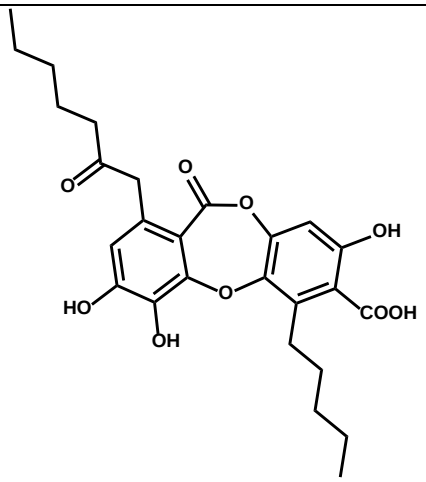
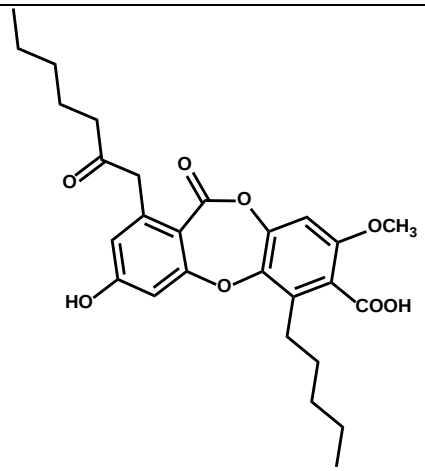
 Lobaric acid	 α-alectoronic acid	 α-collatolic acid
 Physodic acid	 3-hydroxyphysodic acid	 2'-O-methylphysodic acid
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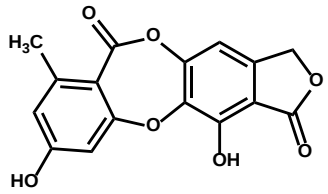
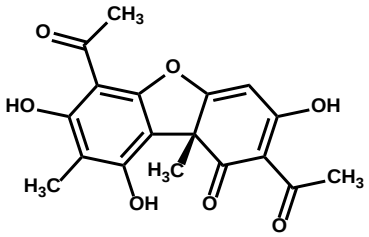
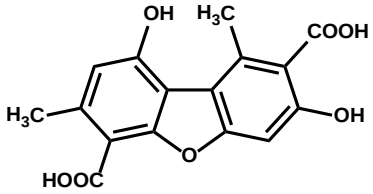
 Variolaric acid	 (+)-Usnic acid	 Pannaric acid
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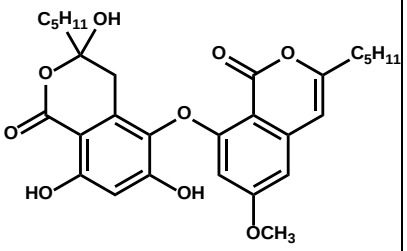
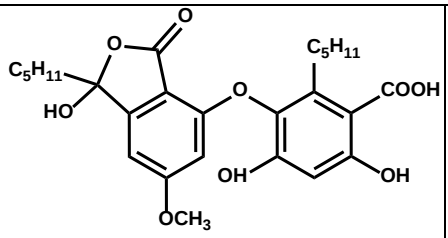
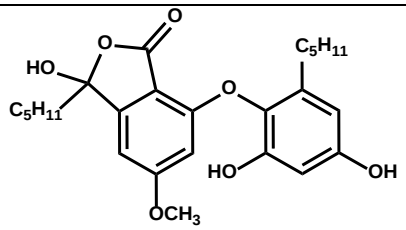
 β-collatolic acid	 Lobarin	 Sakisacaulon A

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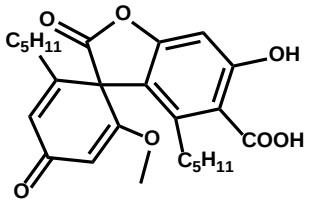
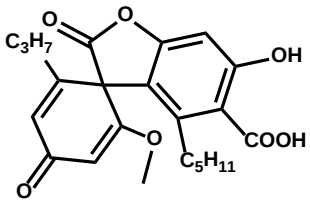
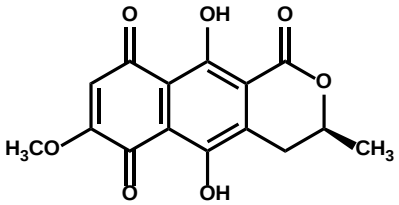
 Picrolichenic acid	 Subpicrolichenic acid	 Haemoventosin
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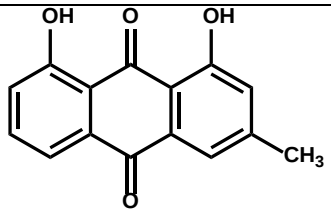
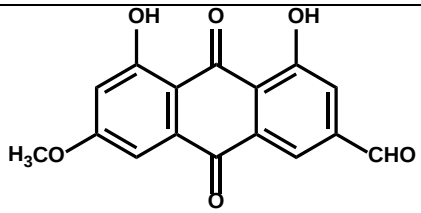
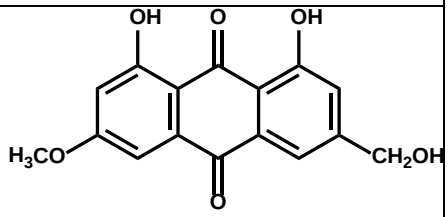
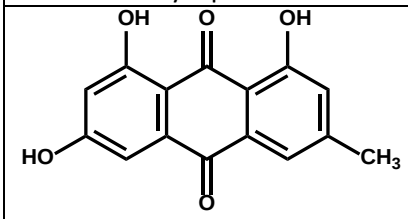
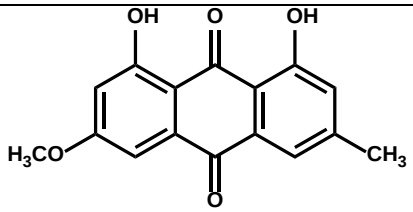
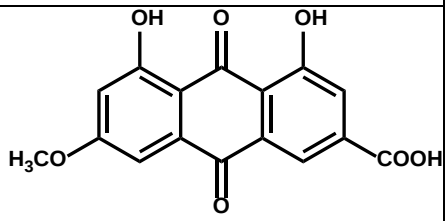
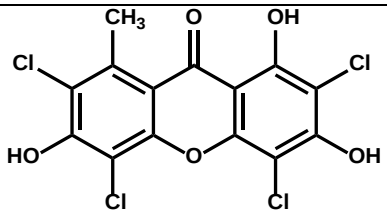
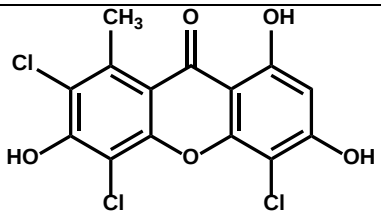
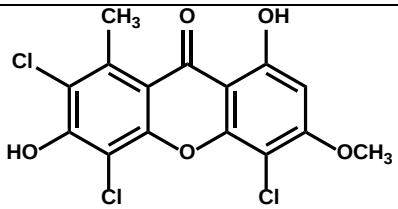
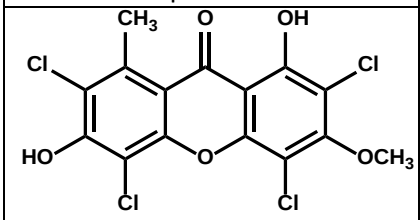
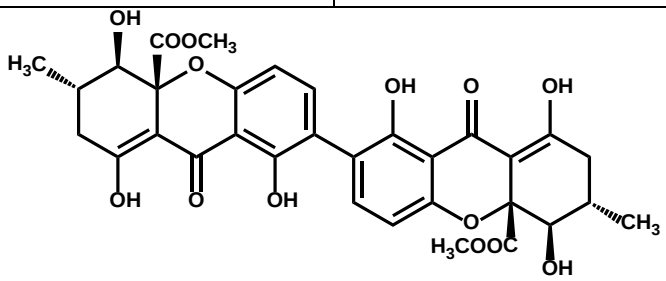
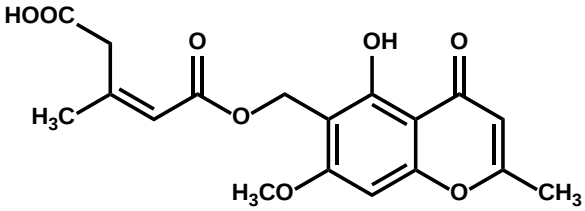
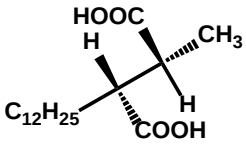
 Chrysophanol	 Fallacinal	 Fallacinol
 Emodin	 Parietin	 Parietinic acid
XANTHONES		
 Thiophanic acid	 Asemone	 3-O-methylasemone
 3-O-methylthiophanic acid	 Secalonic acid D	
CHROMONE		**ALIPHATIC ACID**
 Lepraric acid		 Roccellic acid

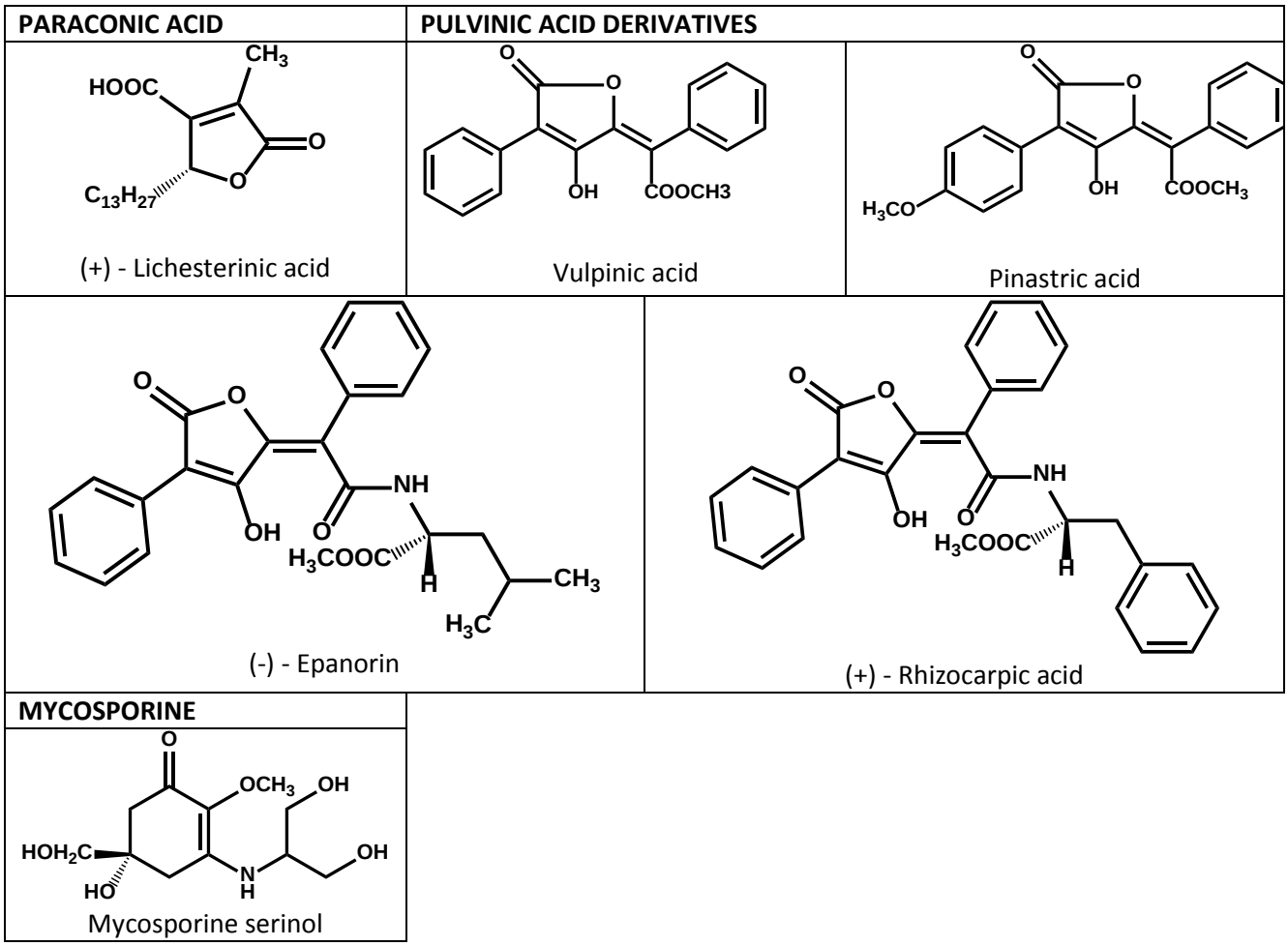


Figure S- 1: Structures of studied molecules, either as single compounds or within lichen extracts.

LDI-MS SPECTRA

SINGLE MOLECULES

DEPSIDES

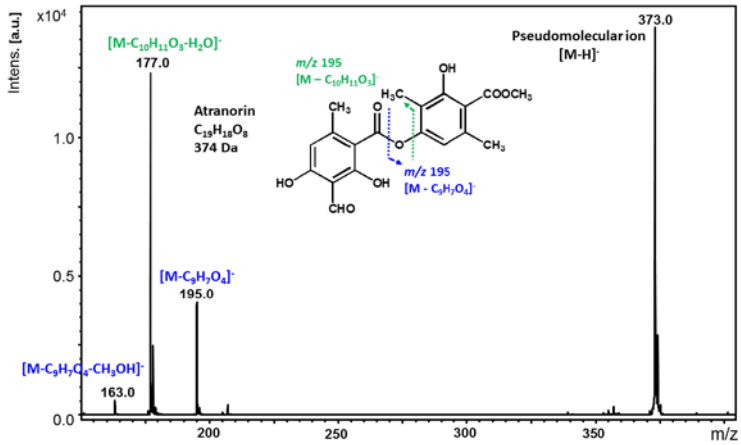


Figure S- 2: Negative LDI mass spectrum of atranorin

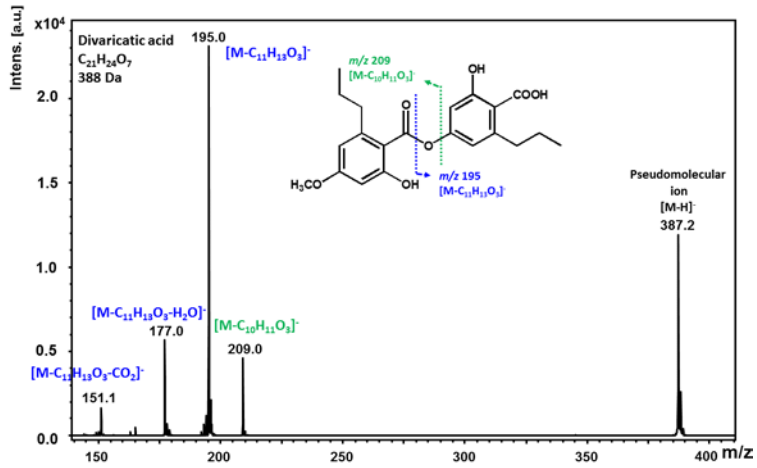


Figure S- 3: Negative LDI mass spectrum of divaricatic acid

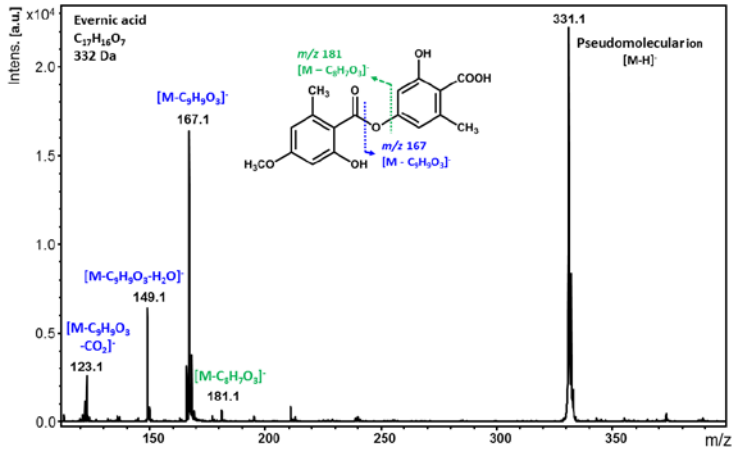


Figure S- 4: Negative LDI mass spectrum of evernic acid

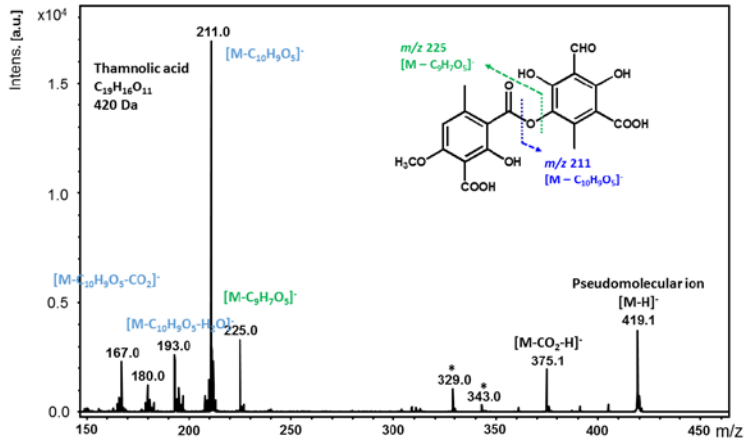


Figure S- 5: Negative LDI mass spectrum of thamnolic acid

DEPSIDONES

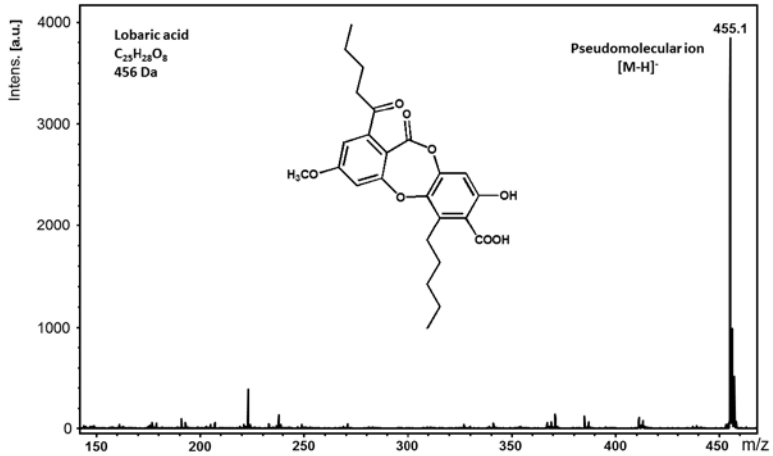


Figure S- 6: Negative LDI mass spectrum of lobaric acid

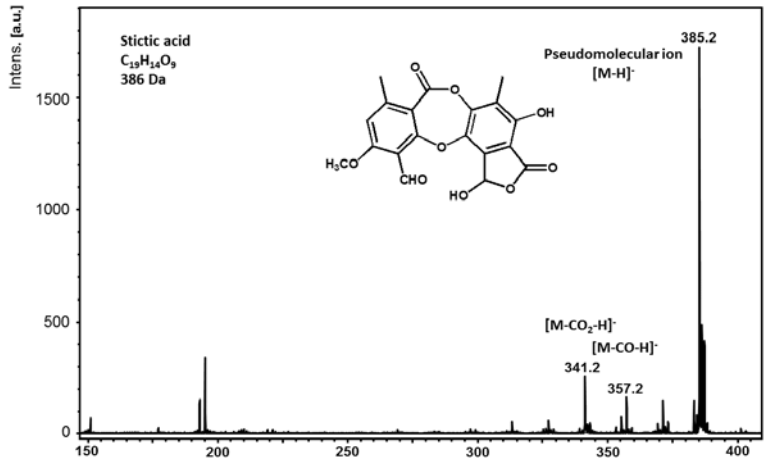


Figure S- 7: Negative LDI mass spectrum of stictic acid

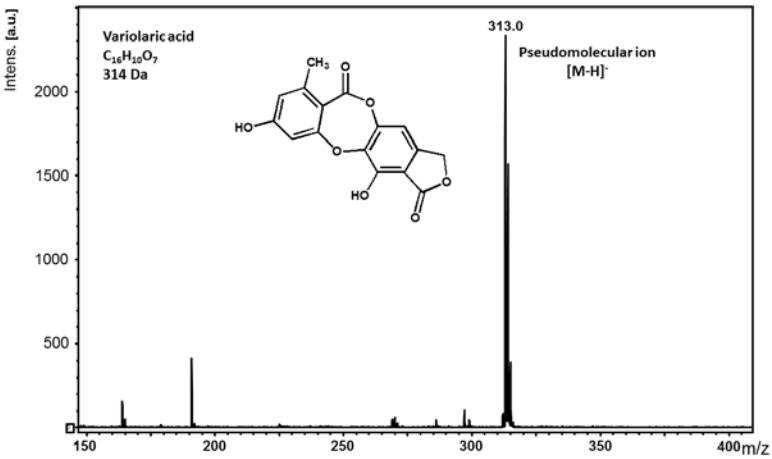


Figure S- 8: Negative LDI mass spectrum of variolaric acid

DIBENZOFURAN AND RELATED

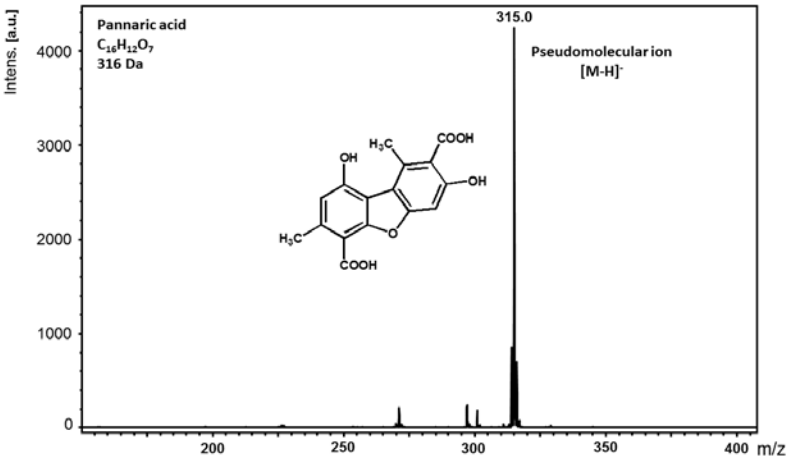


Figure S- 9: Negative LDI mass spectrum of pannaric acid

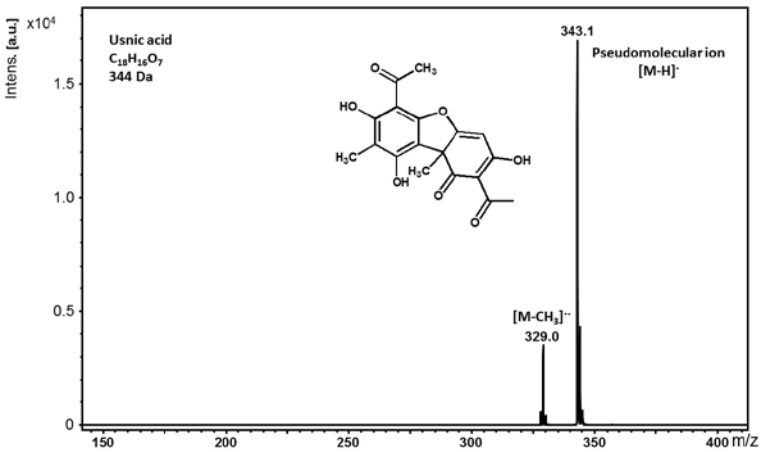


Figure S- 10: Negative LDI mass spectrum of usnic acid

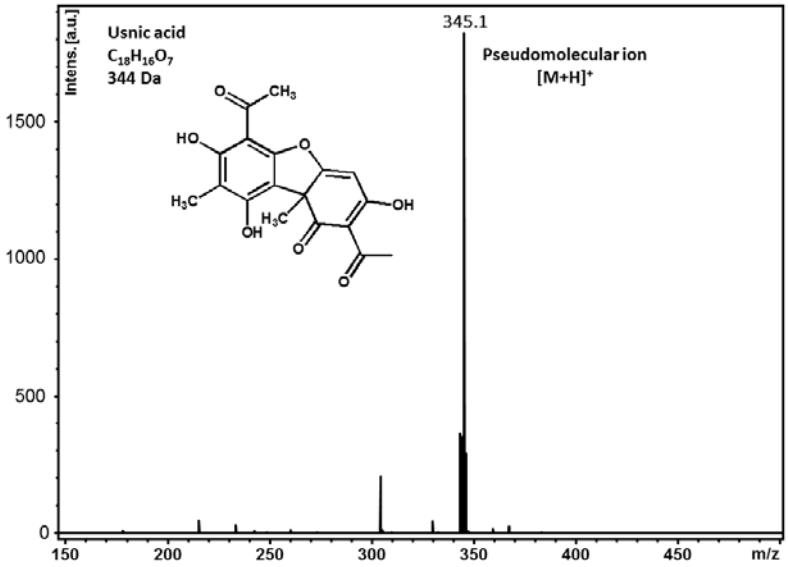


Figure S- 11 : Positive LDI mass spectrum of usnic acid

DIPHENYLETHERS

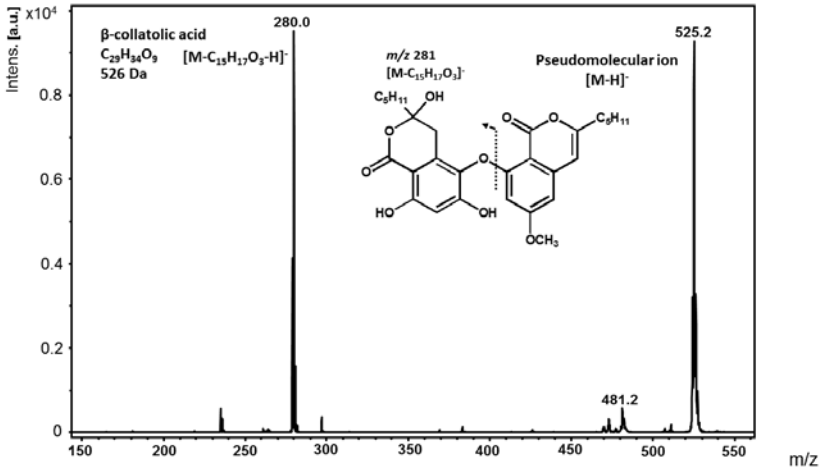


Figure S- 12: Negative LDI mass spectrum of β-collatolic acid

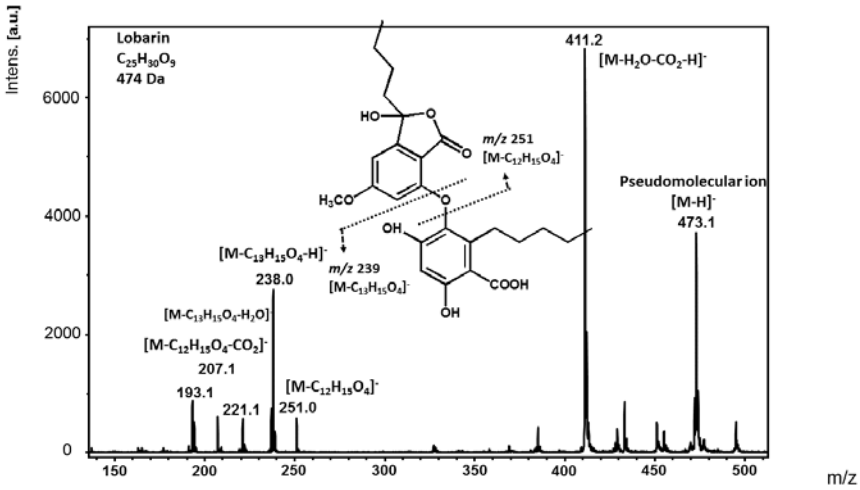


Figure S- 13 : Negative LDI mass spectrum of lobarin

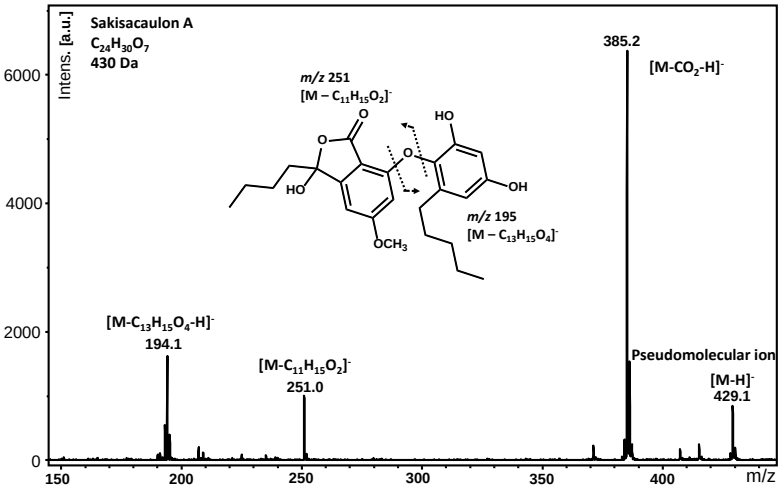


Figure S- 14: Negative LDI mass spectrum of sakisacaulon A

QUINONES

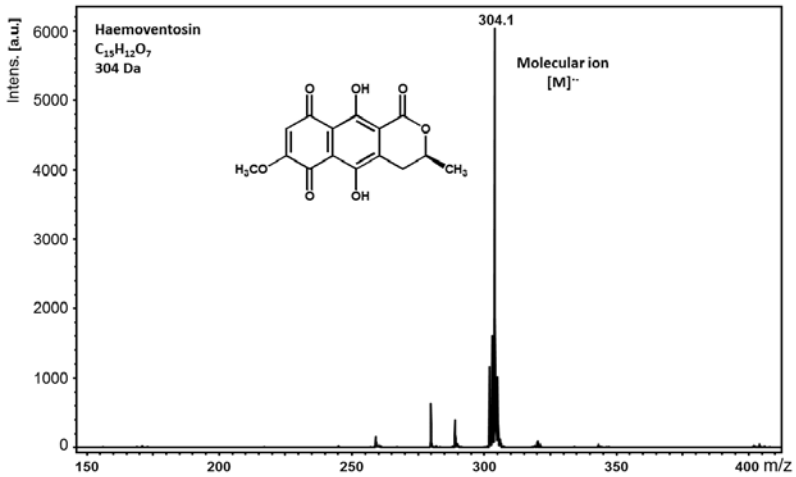


Figure S- 15: Negative LDI mass spectrum of haemoventosin

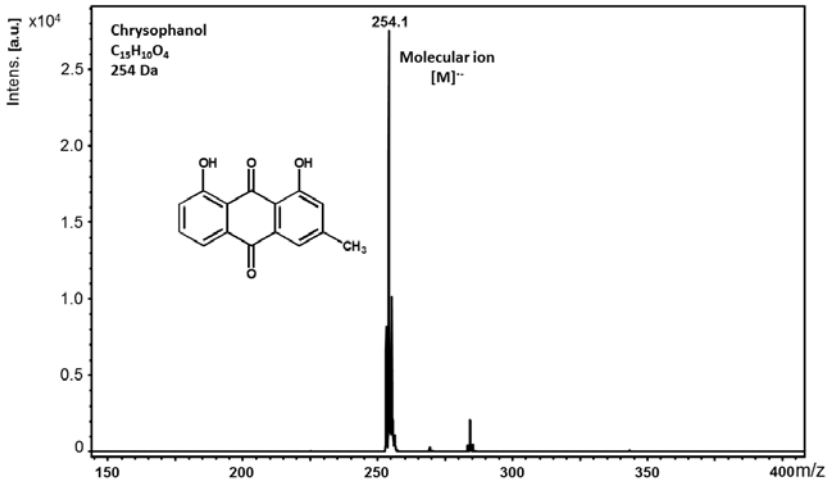


Figure S- 16: Negative LDI mass spectrum of chrysophanol

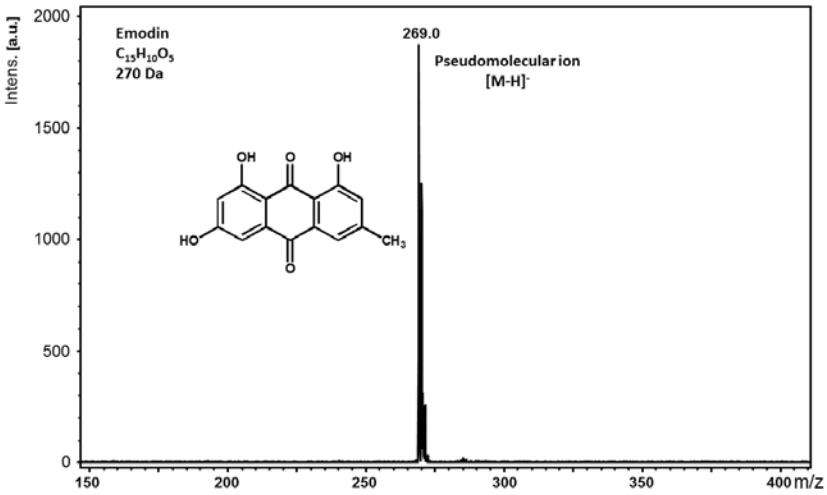


Figure S- 17: Negative LDI mass spectrum of emodin

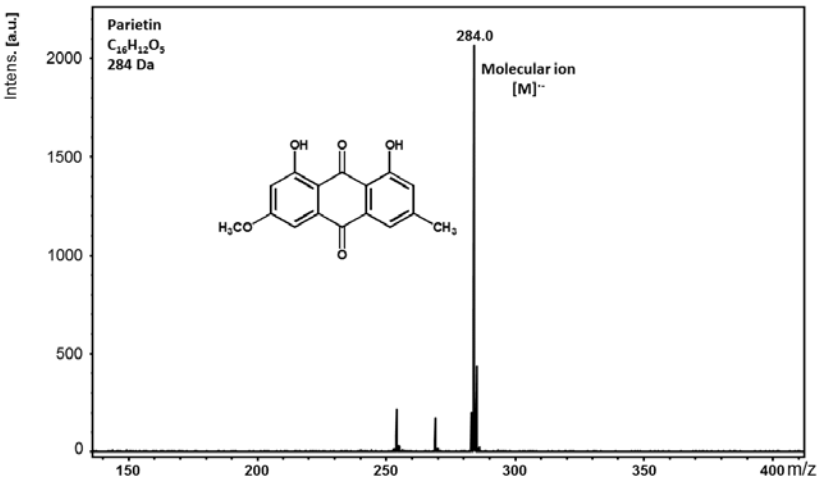


Figure S- 18 : Negative LDI mass spectrum of parietin

PULVINIC ACID DERIVATIVES

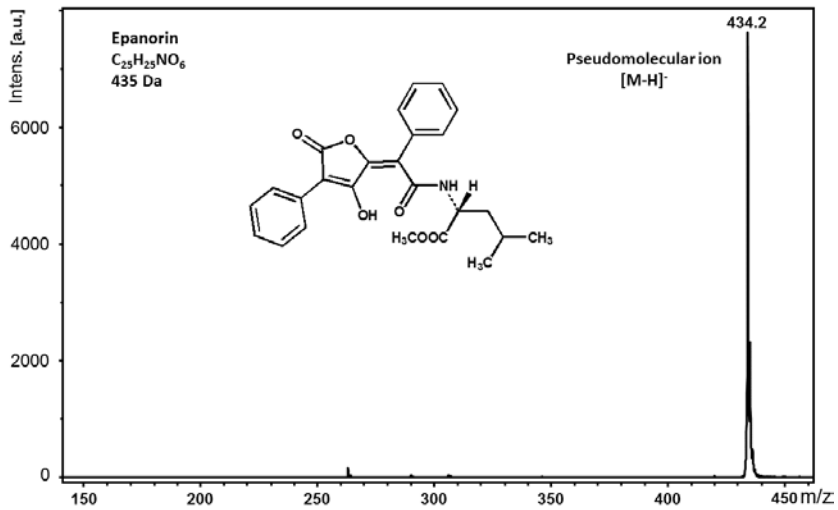


Figure S- 19: Negative LDI mass spectrum of epanorin

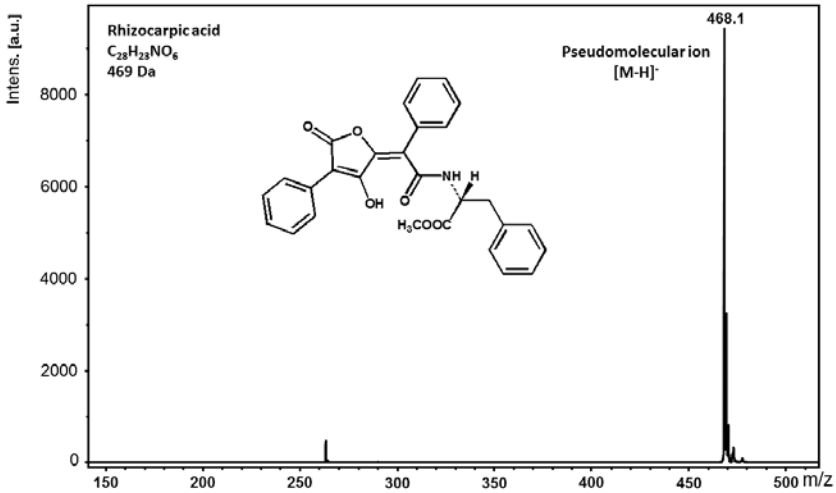


Figure S- 20: Negative LDI mass spectrum of rhizocarpic acid

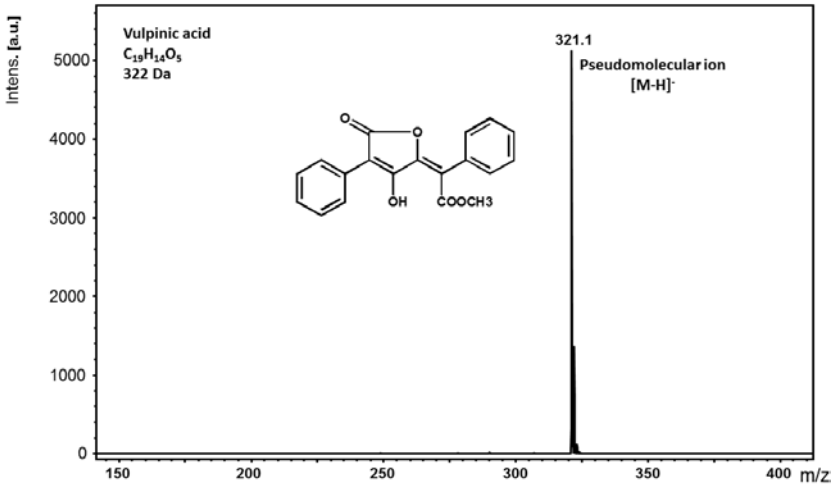


Figure S- 21: Negative LDI mass spectrum of vulpinic acid

XANTHONES

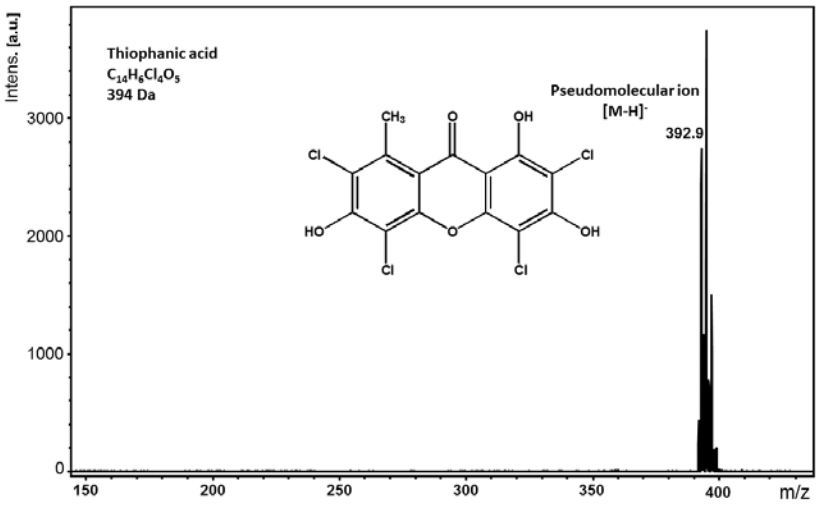


Figure S- 22: Negative LDI mass spectrum of thiophanic acid

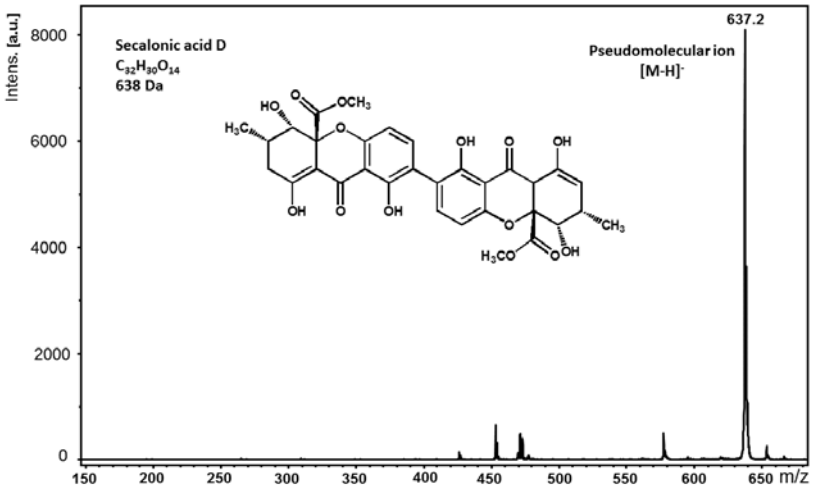


Figure S- 23: Negative LDI mass spectrum of secalonic acid D

ALIPHATIC AND PARACONIC ACIDS

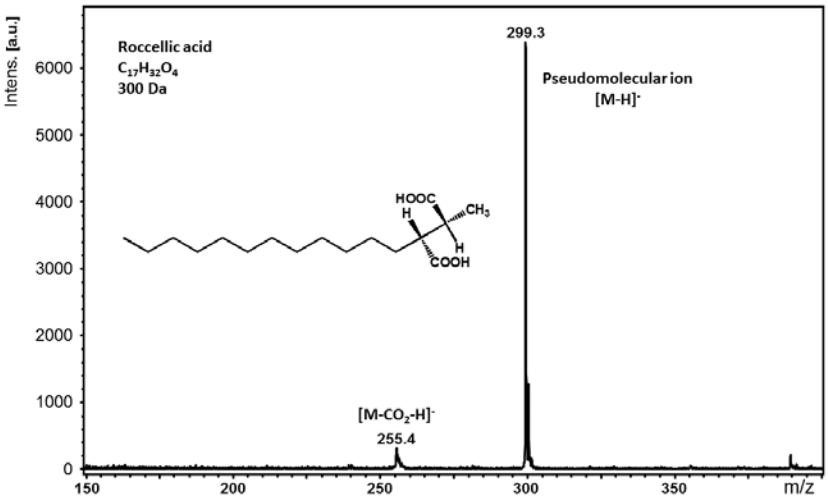


Figure S- 24: Negative LDI mass spectrum of roccellic acid

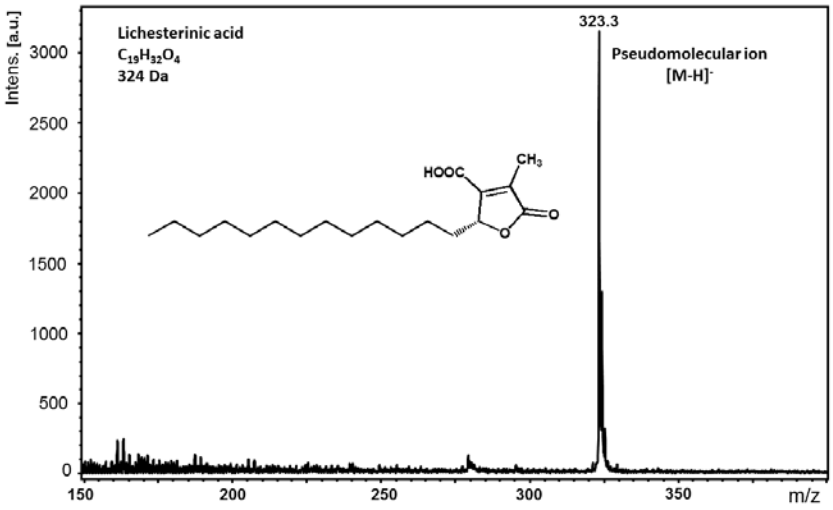


Figure S- 25: Negative LDI mass spectrum of lichesterinic acid

CHROMONES

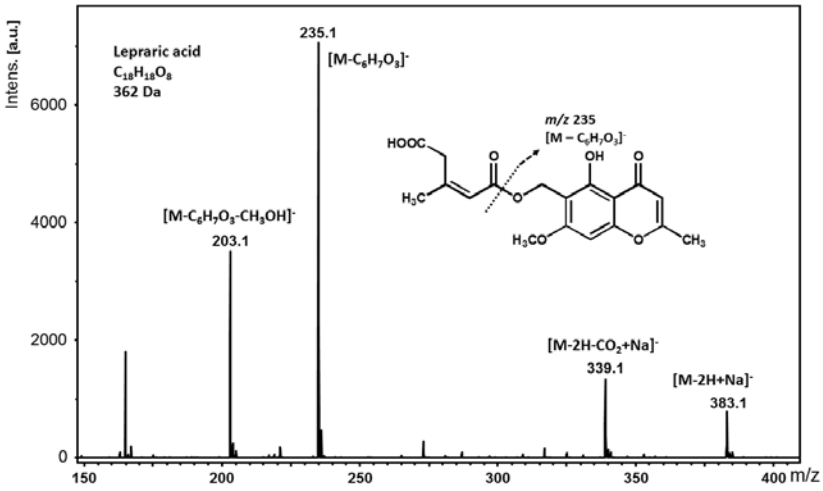


Figure S- 26: Negative LDI mass spectrum of lepraric acid

MYCOSPORINE

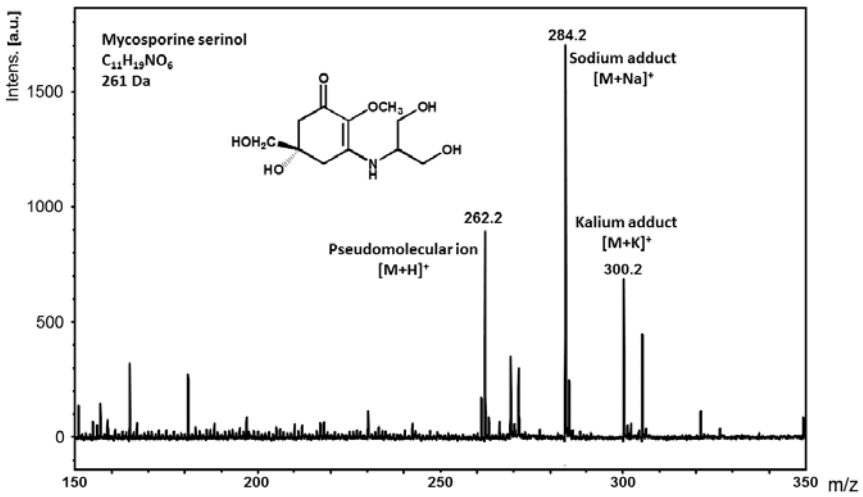


Figure S- 27: Positive LDI mass spectrum of mycosporine serinol

EXTRACTS

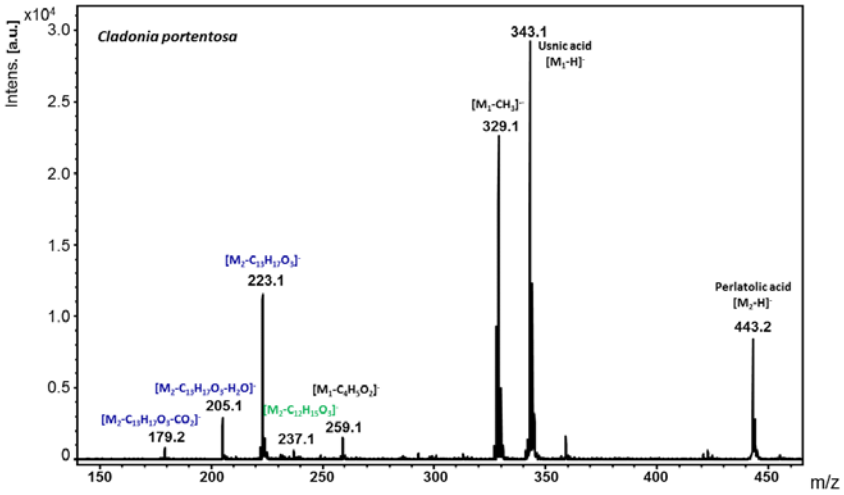


Figure S- 28: Negative LDI mass spectrum of the acetone extract of *Cladonia portentosa*

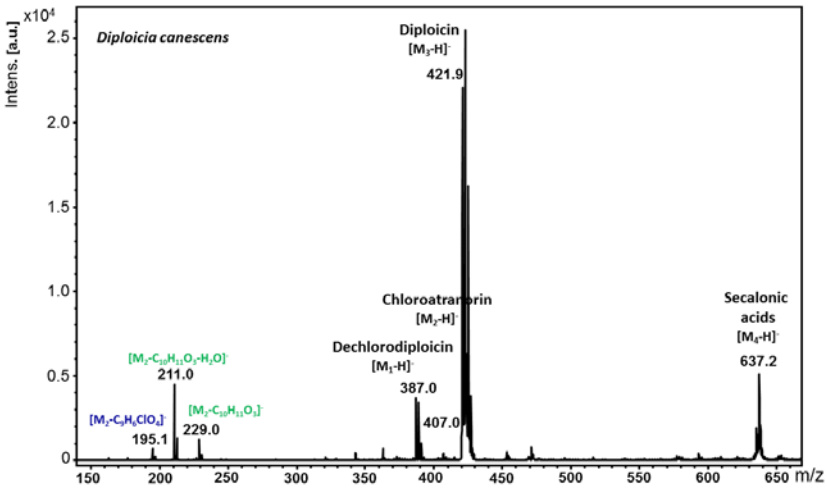


Figure S- 29: Negative LDI mass spectrum of the acetone extract of *Diploicia canescens*

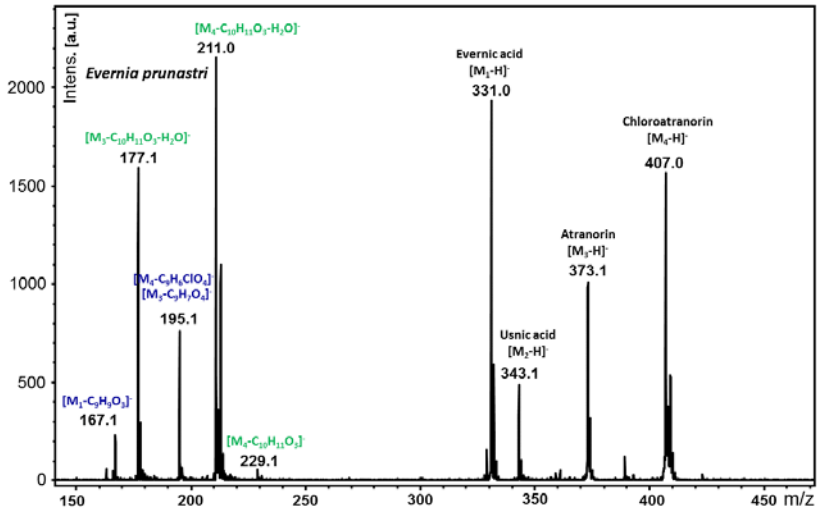


Figure S- 30: Negative LDI mass spectrum of the acetone extract of *Evernia prunastri*

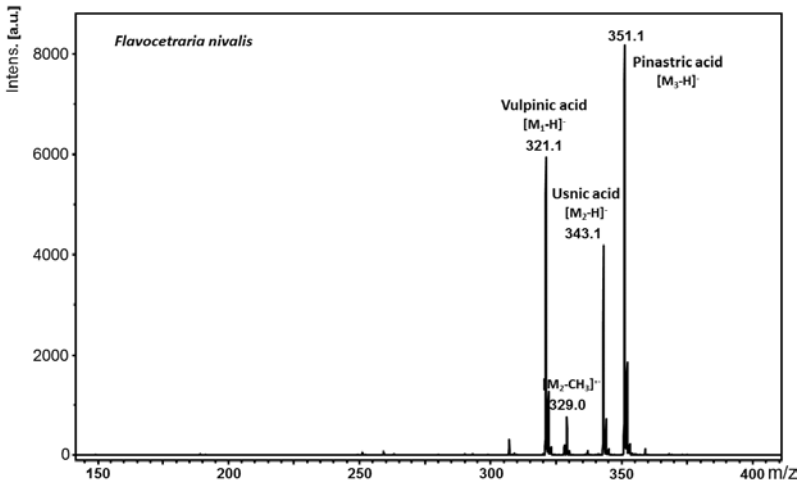


Figure S- 31: Negative LDI mass spectrum of the acetone extract of *Flavocetraria nivalis*

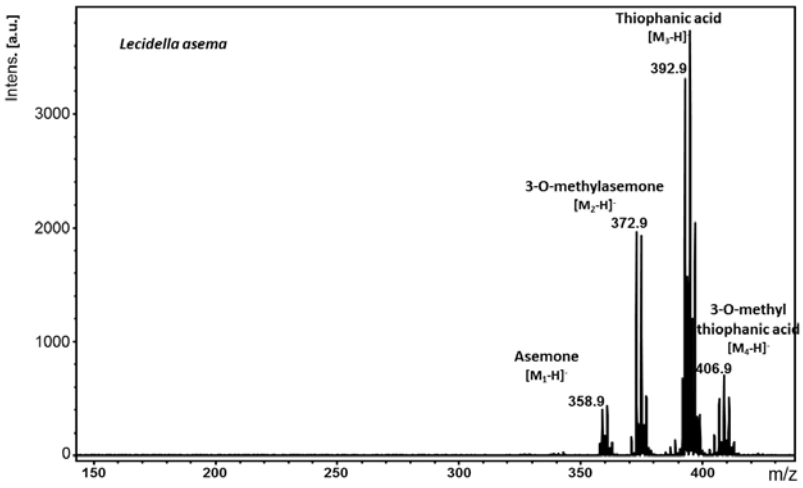


Figure S- 32: Negative LDI mass spectrum of the acetone extract of *Lecidella asema*

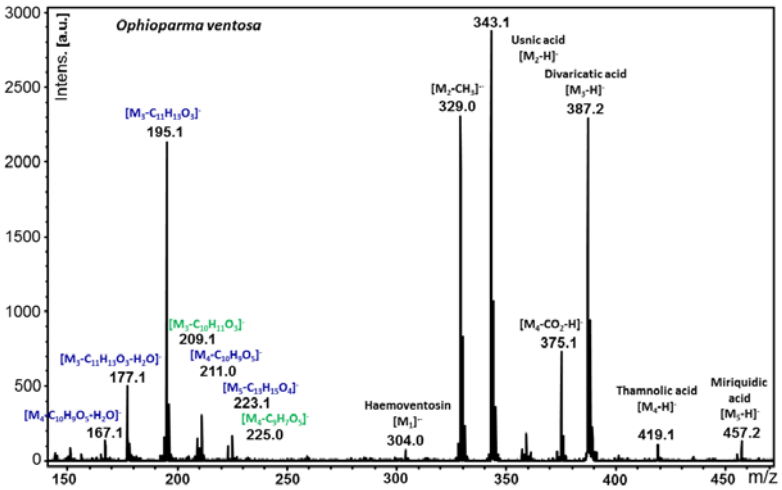


Figure S- 33: Negative LDI mass spectrum of the acetone extract of *Ophioparma ventosa*

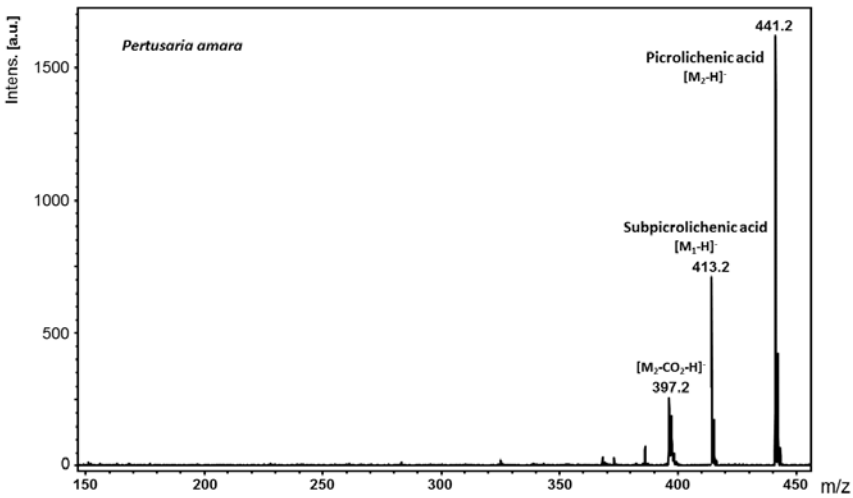


Figure S- 34: Negative LDI mass spectrum of the acetone extract of *Pertusaria amara*

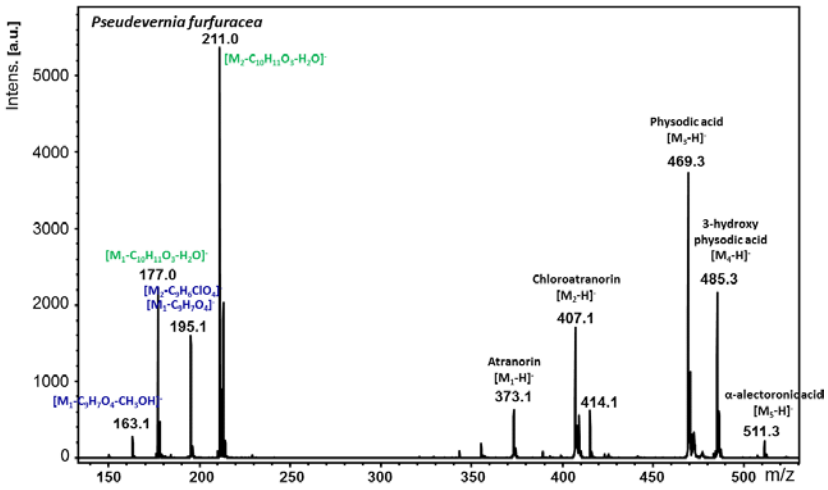


Figure S- 35: Negative LDI mass spectrum of the acetone extract of *Pseudevernia furfuracea*

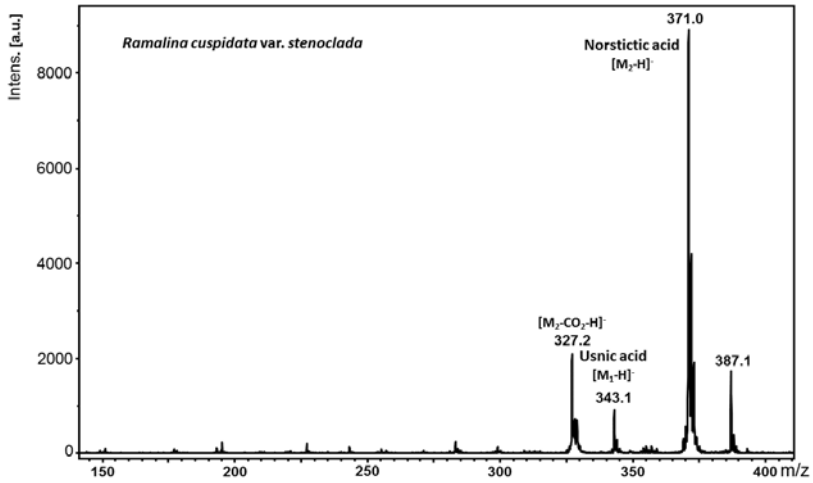


Figure S- 36: Negative LDI mass spectrum of the acetone extract of *Ramalina cuspidata* var. *stenoclada*

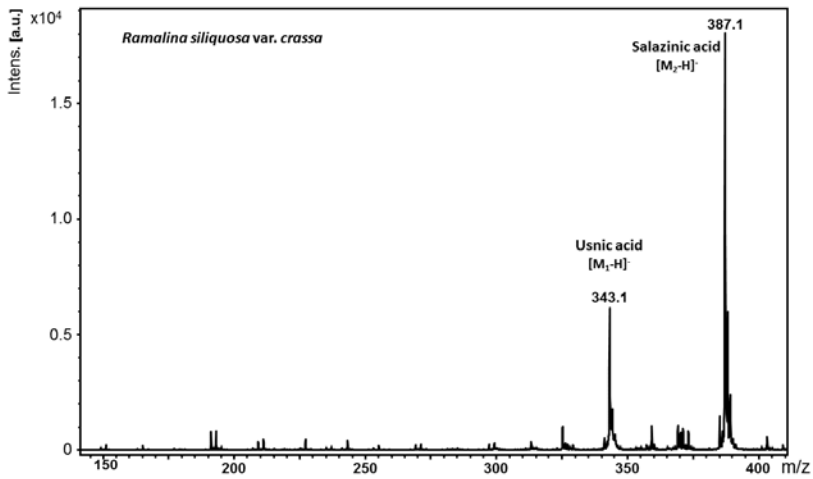


Figure S- 37: Negative LDI mass spectrum of the acetone extract of *Ramalina siliquosa* var. *crassa*

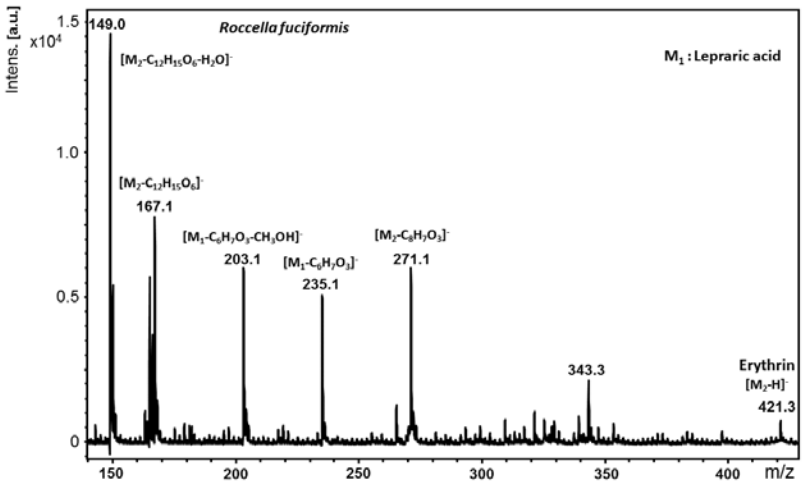


Figure S- 38: Negative LDI mass spectrum of the acetone extract of *Roccella fuciformis*

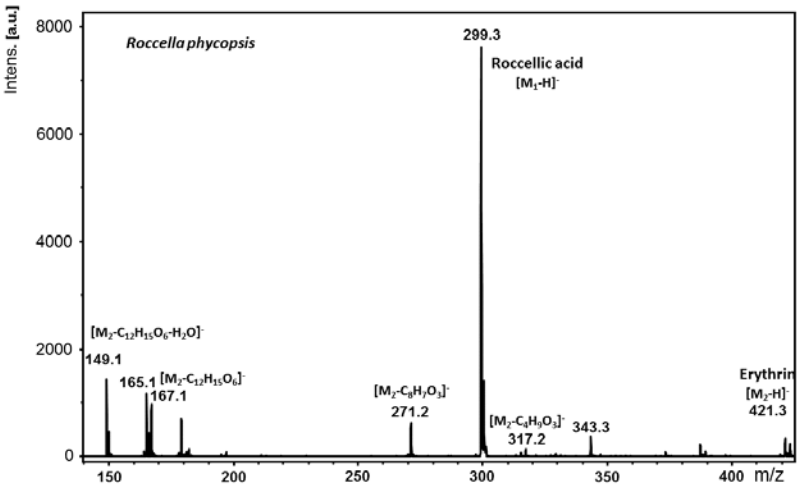


Figure S- 39: Negative LDI mass spectrum of the acetone extract of *Roccella phycopsis*

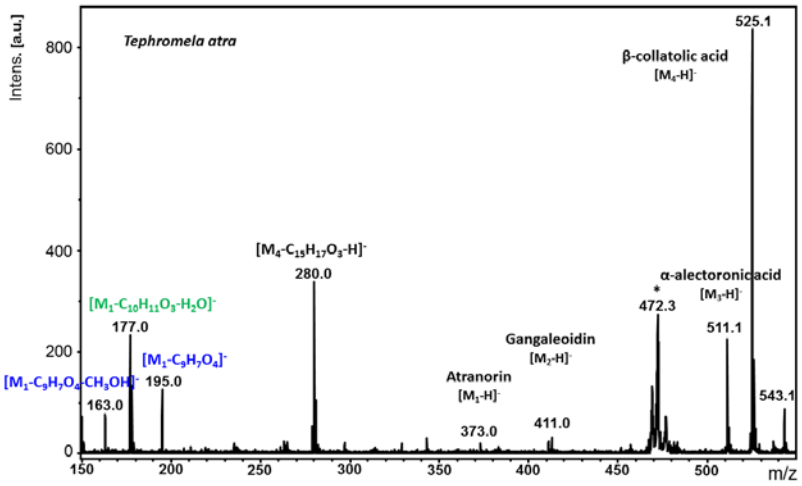


Figure S- 40: Negative LDI mass spectrum of the acetone extract of *Tephromela atra*

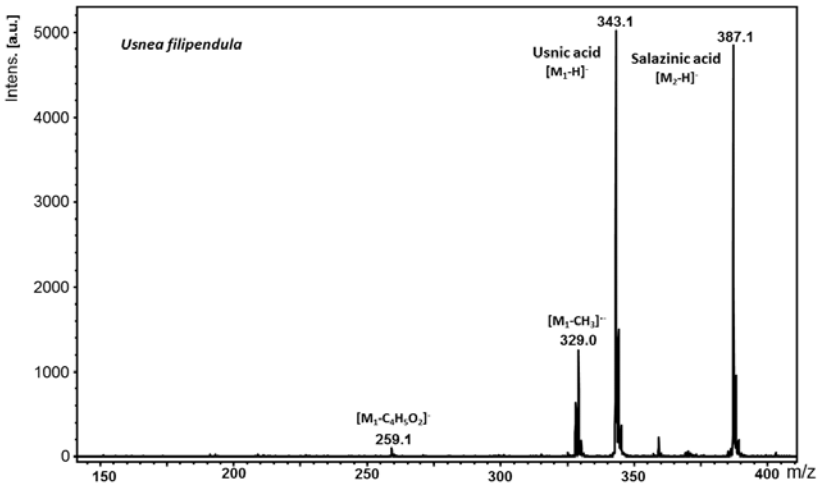


Figure S- 41: Negative LDI mass spectrum of the acetone extract of *Usnea filipendula*

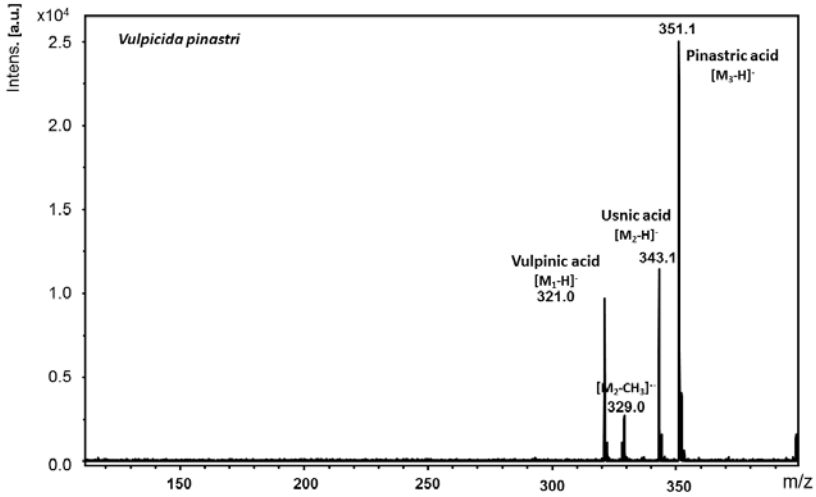


Figure S- 42: Negative LDI mass spectrum of the acetone extract of *Vulpicida pinastri*

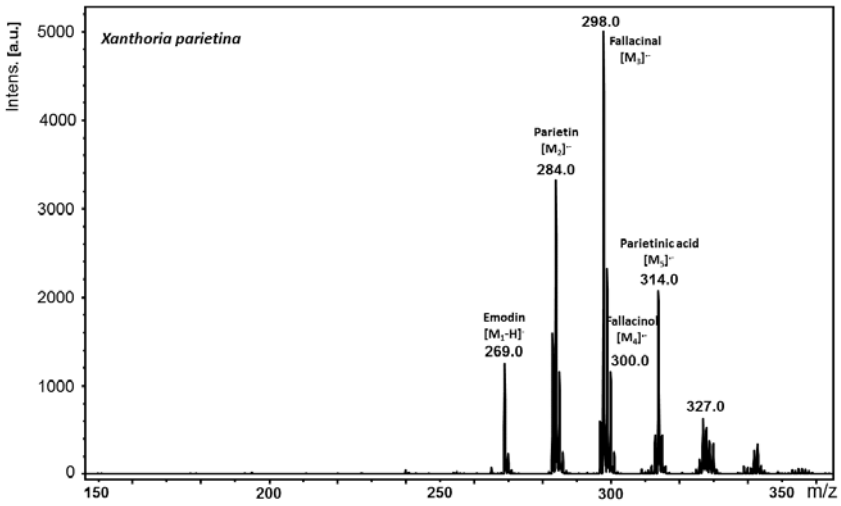


Figure S- 43: Negative LDI mass spectrum of the acetone extract of *Xanthoria parietina*

ESI-MS SPECTRA

SINGLE MOLECULES

DEPSIDES

Atranorin

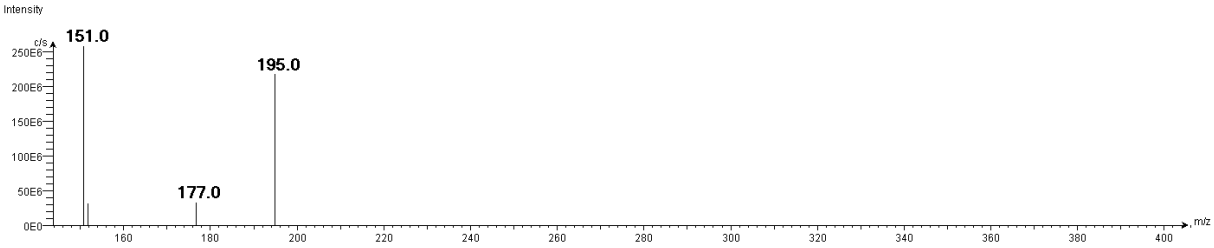


Figure S- 44: Negative ESI mass spectrum of atranorin

Divaricatic acid

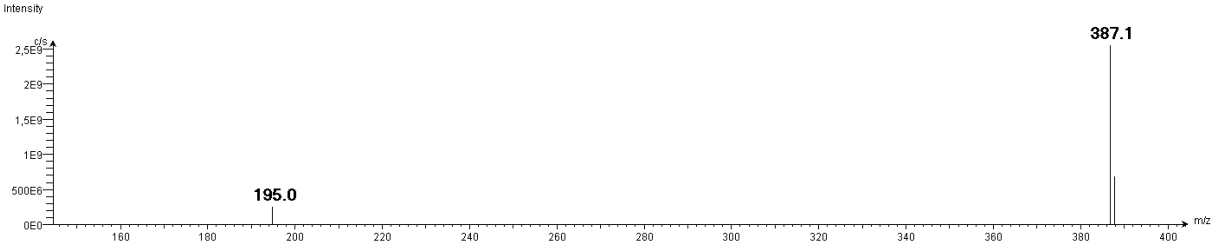


Figure S- 45 : Negative ESI mass spectrum of divaricatic acid

Evernic acid

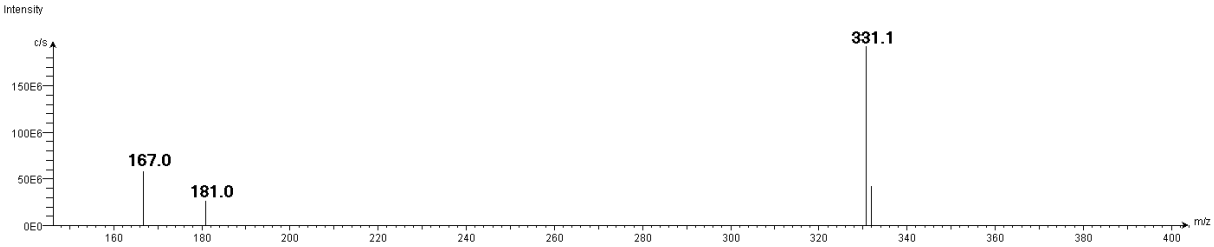


Figure S- 46 : Negative ESI mass spectrum of evernic acid

Thamnolic acid

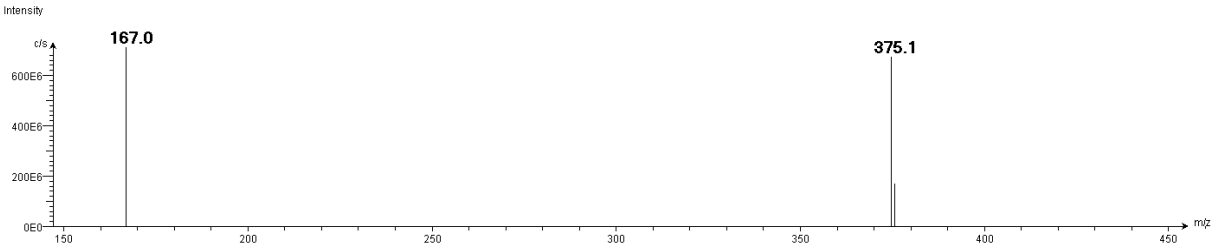


Figure S- 47 : Negative ESI mass spectrum of thamnolic acid

DEPSIDONES

Lobaric acid

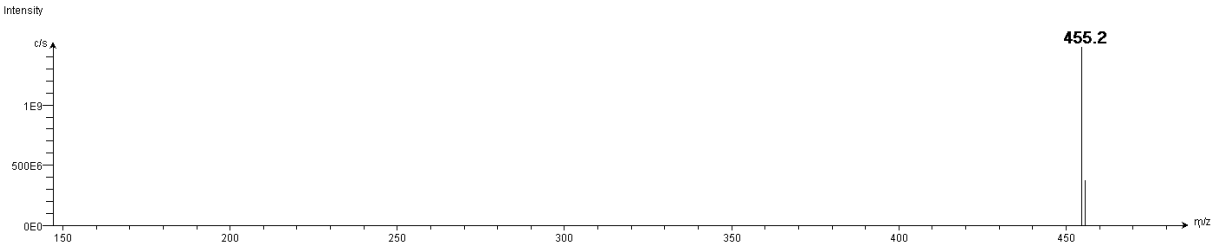


Figure S- 48: Negative ESI mass spectrum of lobaric acid

Stictic acid

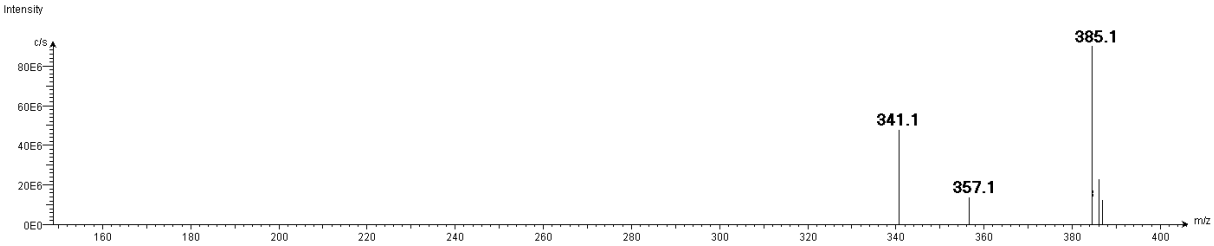


Figure S- 49 : Negative ESI mass spectrum of stictic acid

Variolaric acid

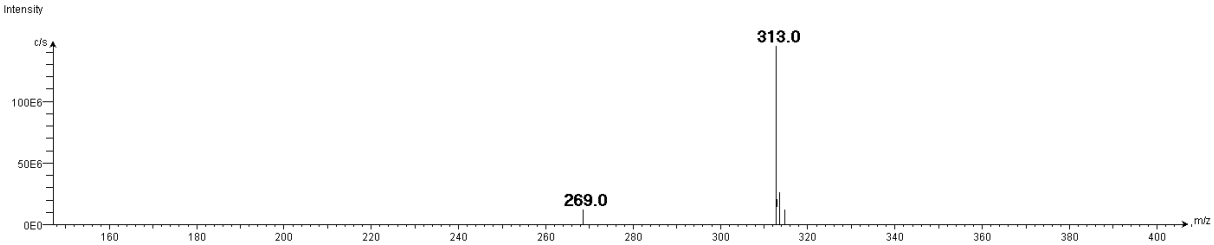


Figure S- 50 : Negative ESI mass spectrum of variolaric acid

DIBENZOFURAN AND RELATED

Pannaric acid

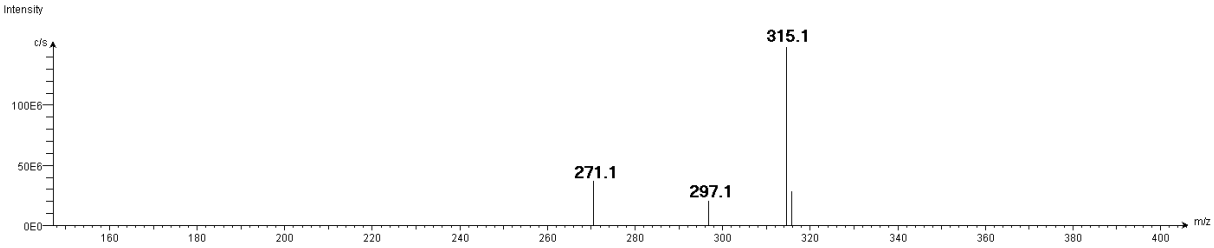


Figure S- 51 : Negative ESI mass spectrum of pannaric acid

Usnic acid

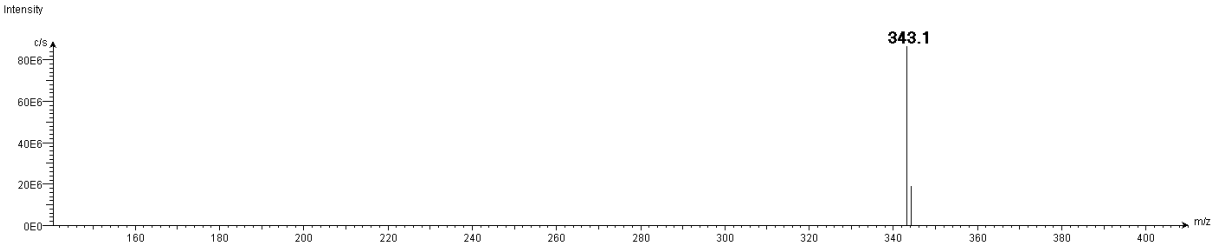


Figure S- 52 : Negative ESI mass spectrum of usnic acid

DIPHENYLETHERS

β-collatolic acid

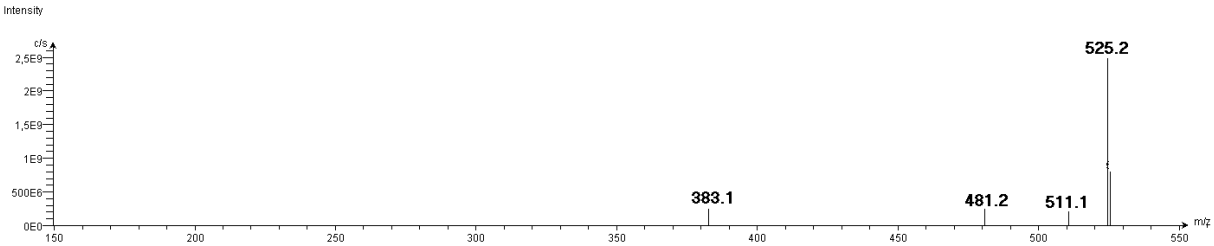


Figure S- 53 : Negative ESI mass spectrum of β-collatolic acid

Lobarin

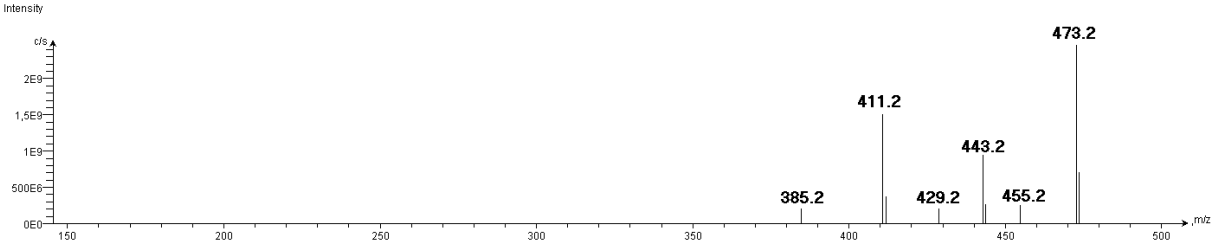


Figure S- 54 : Negative ESI mass spectrum of lobarin

Sakisacaulon A

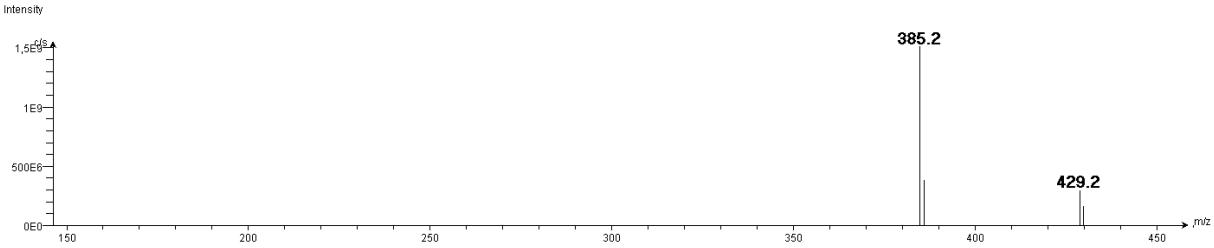


Figure S- 55 : Negative ESI mass spectrum of sakisacaulon A

QUINONES

Emodin

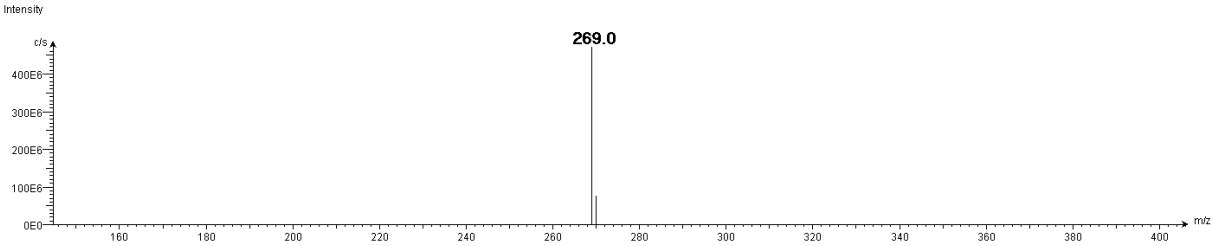


Figure S- 56 : Negative ESI mass spectrum of emodin

PULVINIC ACID DERIVATIVES

Epanorin

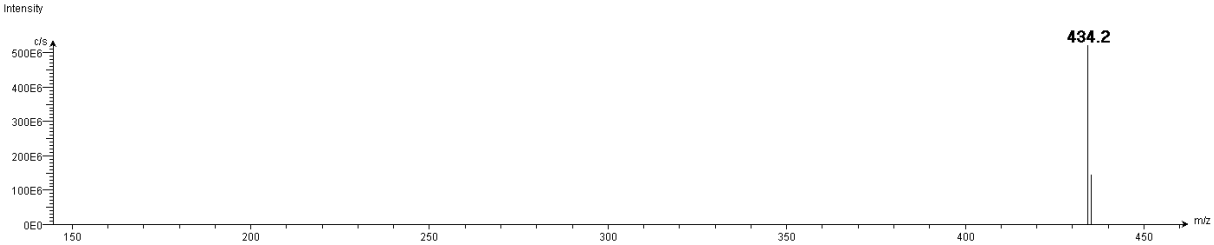


Figure S- 57 : Negative ESI mass spectrum of epanorin

Rhizocarpic acid

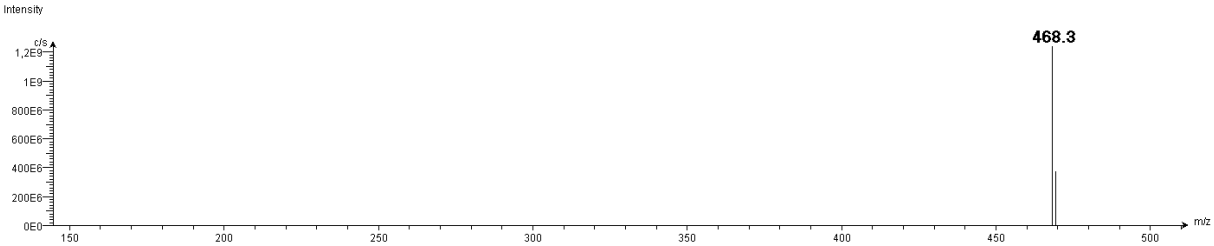


Figure S- 58 : Negative ESI mass spectrum of rhizocarpic acid

Vulpinic acid

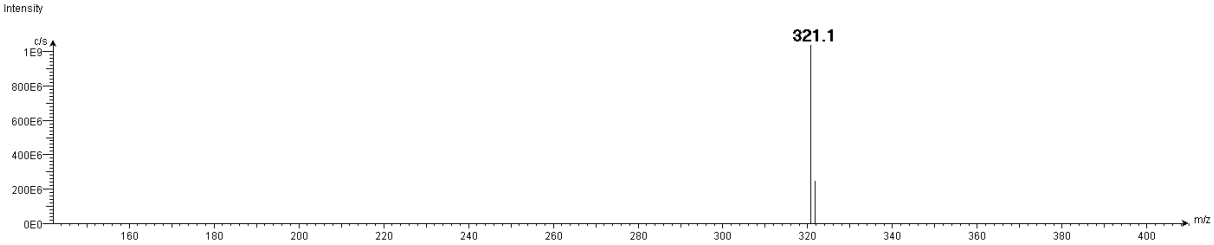


Figure S- 59 : Negative ESI mass spectrum of vulpinic acid

XANTHONES

Thiophanic acid

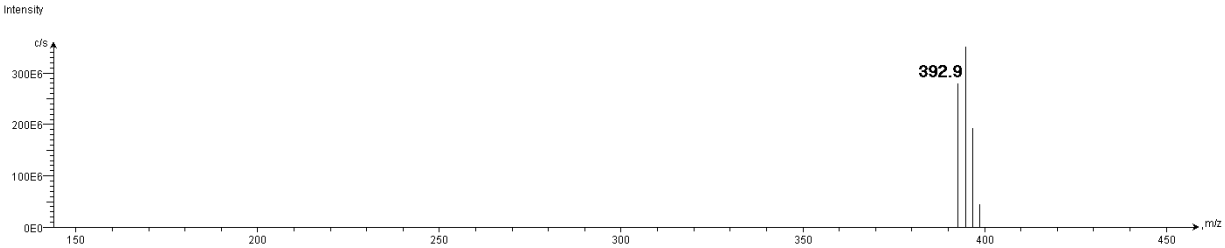


Figure S- 60 : Negative ESI mass spectrum of thiophanic acid

Secalonic acid D

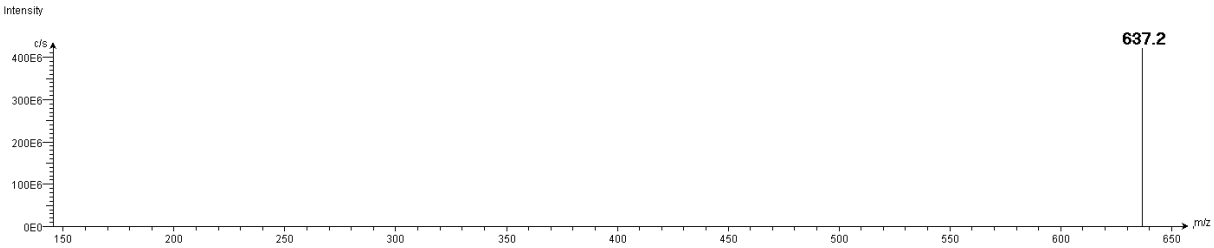


Figure S- 61 : Negative ESI mass spectrum of secalonic acid D

ALIPHATIC AND PARACONIC ACIDS

Roccellic acid

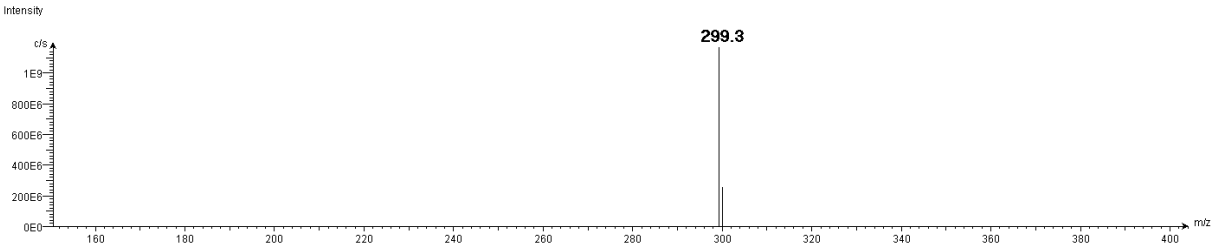


Figure S- 62 : Negative ESI mass spectrum of roccellic acid

Lichesterinic acid

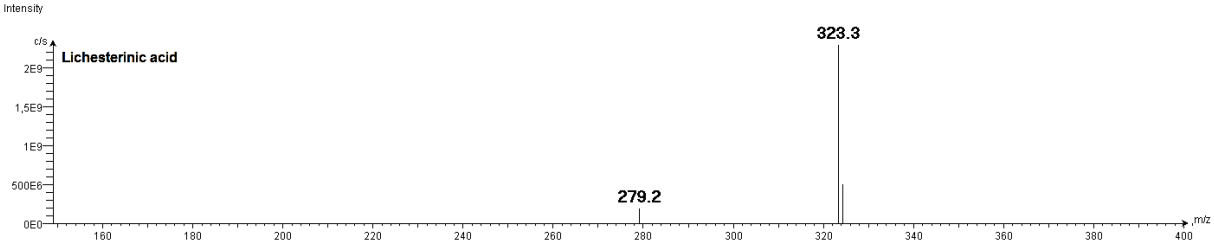


Figure S- 63 : Negative ESI mass spectrum of lichesterinic acid

MYCOSPORINE

Mycosporine serinol

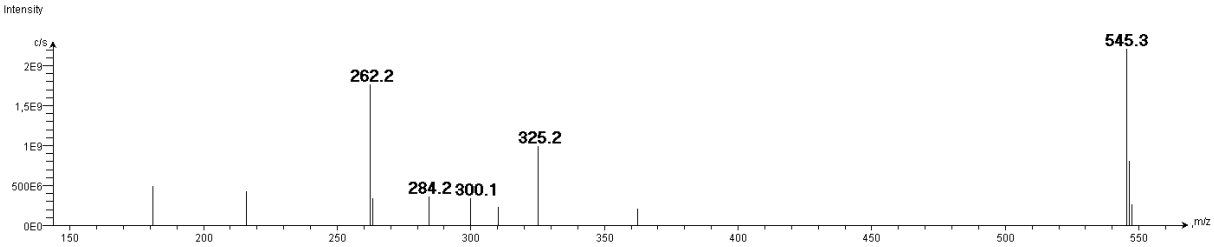


Figure S- 64 : Positive ESI mass spectrum of mycosporine serinol

EXTRACTS

Cladonia portentosa

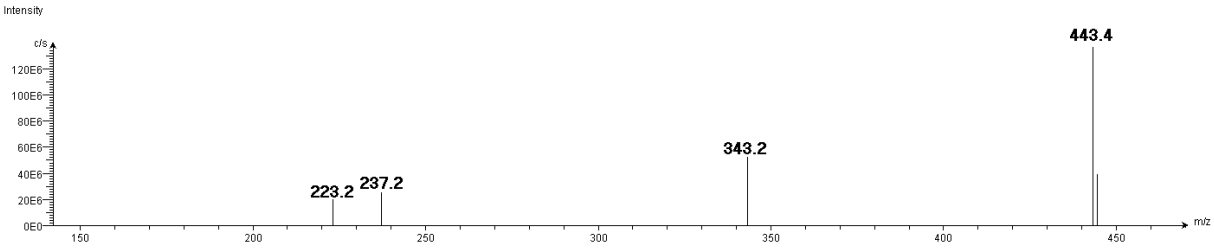


Figure S- 65 : Negative ESI mass spectrum of the acetone extract of *Cladonia portentosa*

Diploicia canescens

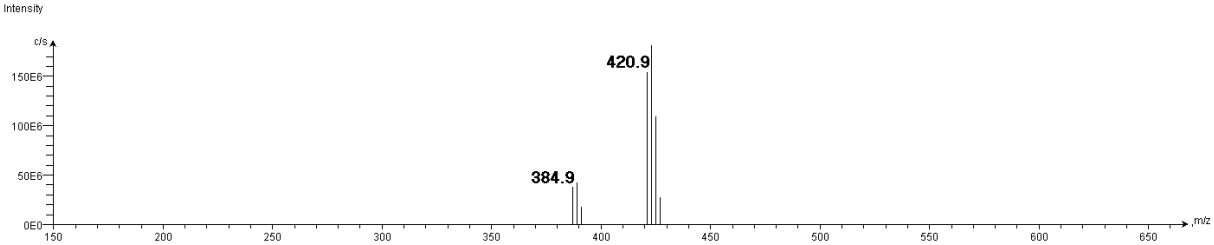


Figure S- 66 : Negative ESI mass spectrum of the acetone extract of *Diploicia canescens*

Evernia prunastri

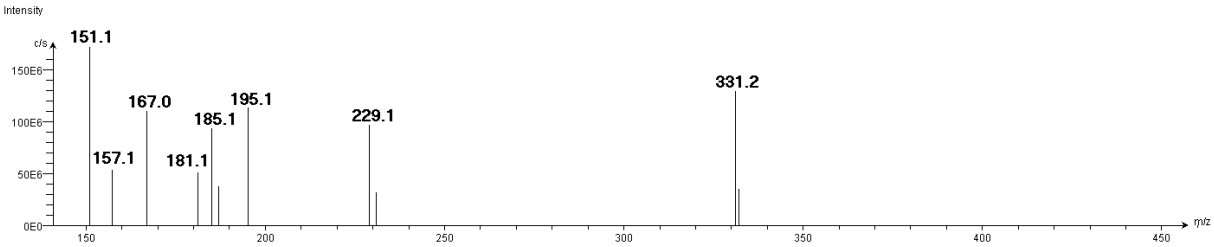


Figure S- 67 : Negative ESI mass spectrum of the acetone extract of *Evernia prunastri*

Flavocetraria nivalis

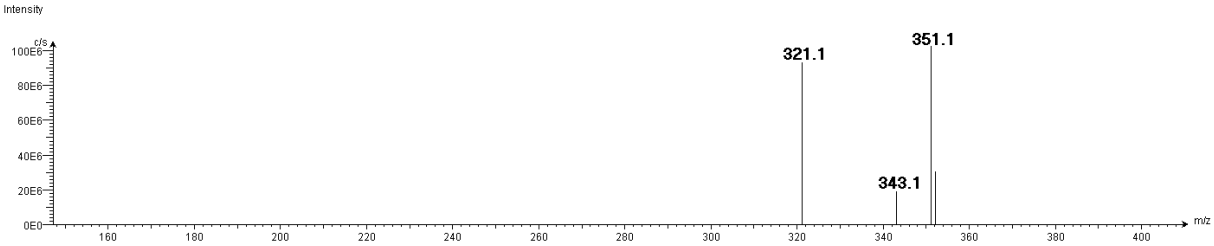


Figure S- 68 : Negative ESI mass spectrum of the acetone extract of *Flavocetraria nivalis*

Lecidella asema

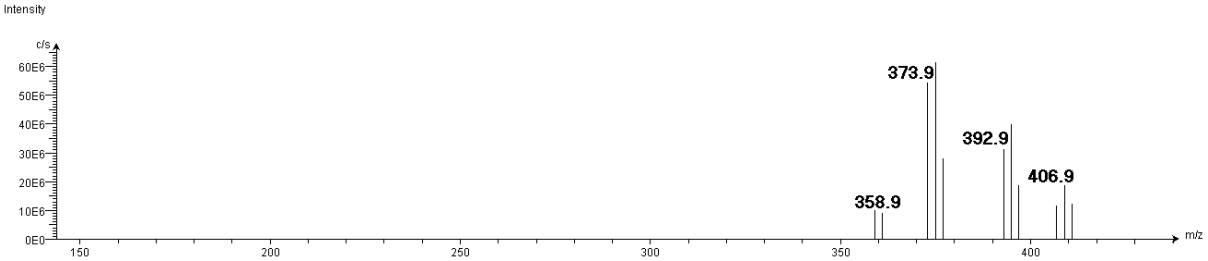


Figure S- 69 : Negative ESI mass spectrum of the acetone extract of *Lecidella asema*

Ochrolechia parella

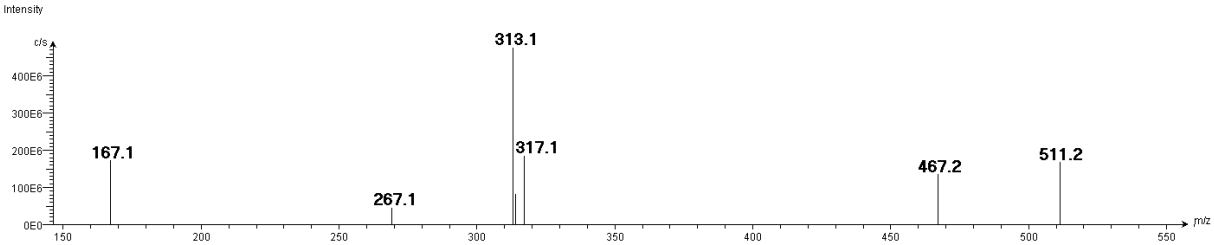


Figure S- 70 : Negative ESI mass spectrum of the acetone extract of *Ochrolechia parella*

Ophioparma ventosa

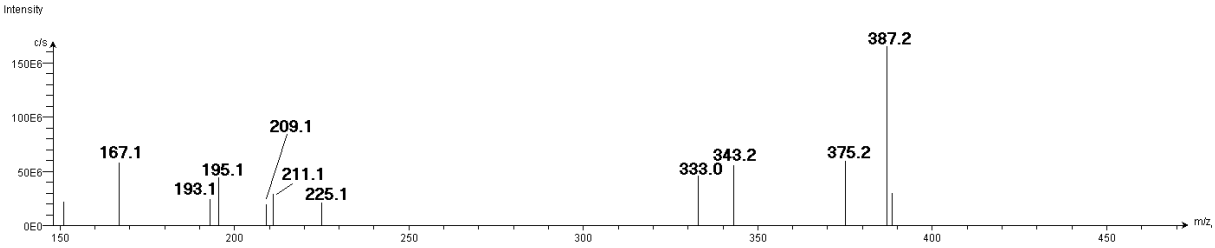


Figure S- 71 : Negative ESI mass spectrum of the acetone extract of *Ophioparma ventosa*

Pertusaria amara

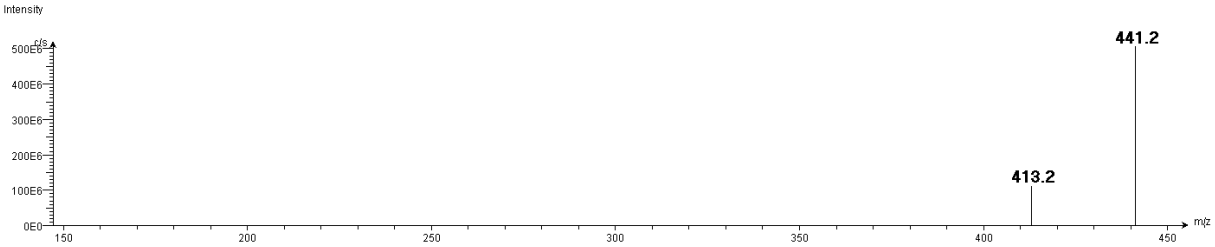


Figure S- 72 : Negative ESI mass spectrum of the acetone extract of *Pertusaria amara*

Pseudevernia furfuracea

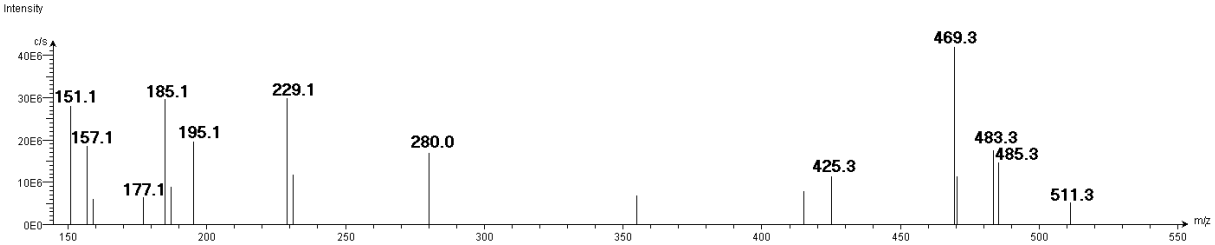


Figure S- 73 : Negative ESI mass spectrum of the acetone extract of *Pseudevernia furfuracea*

Ramalina cuspidata var *stenoclada*

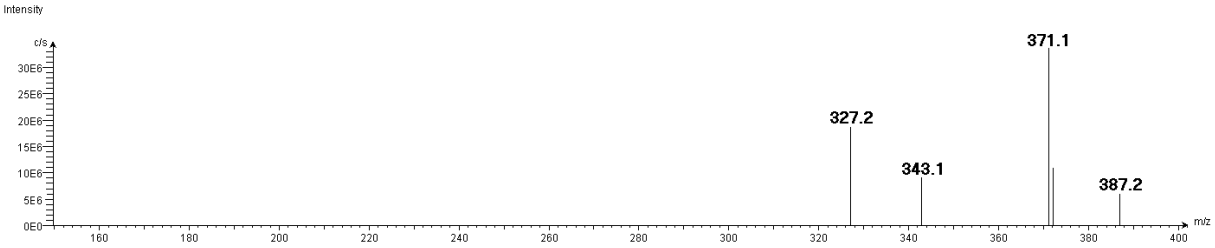


Figure S- 74 : Negative ESI mass spectrum of the acetone extract of *Ramalina cuspidata* var. *stenoclada*

Ramalina siliquosa var *crassa*

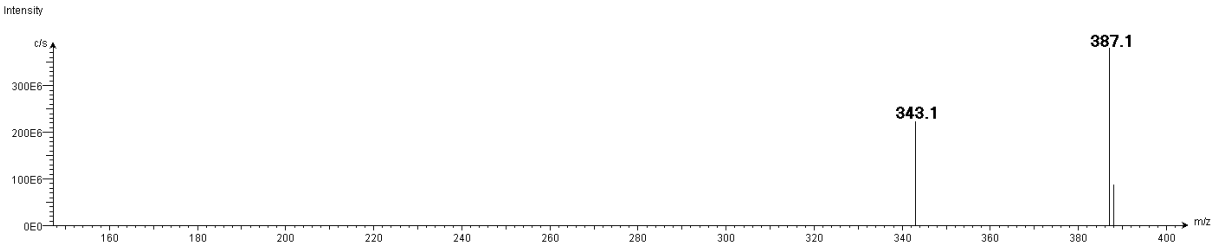


Figure S- 75 : Negative ESI mass spectrum of the acetone extract of *Ramalina siliquosa* var. *crassa*

Roccella fuciformis

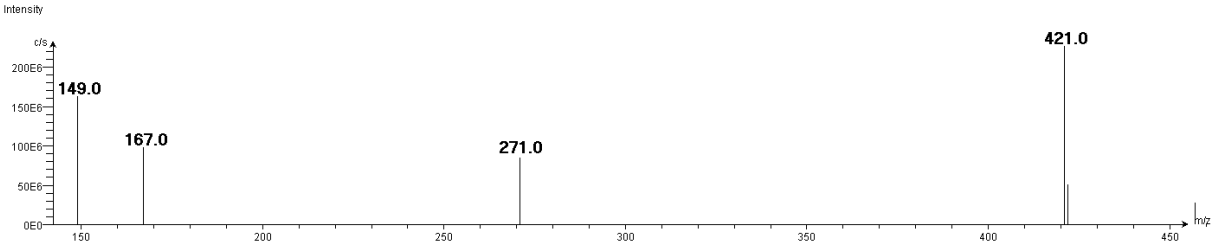


Figure S- 76 : Negative ESI mass spectrum of the acetone extract of *Roccella fuciformis*

Roccella phycopsis

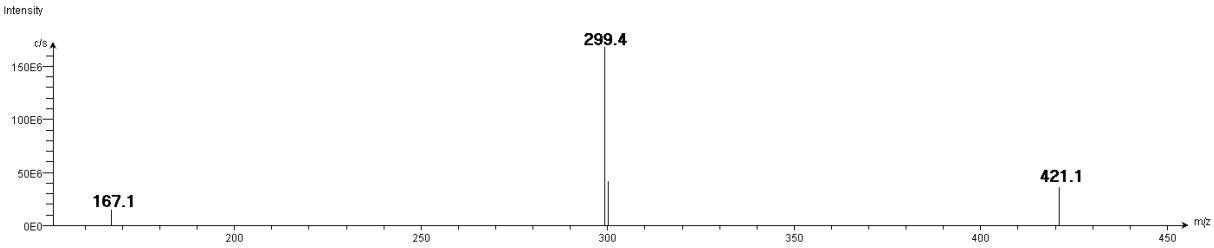


Figure S- 77 : Negative ESI mass spectrum of the acetone extract of *Roccella phycopsis*

Tephromela atra

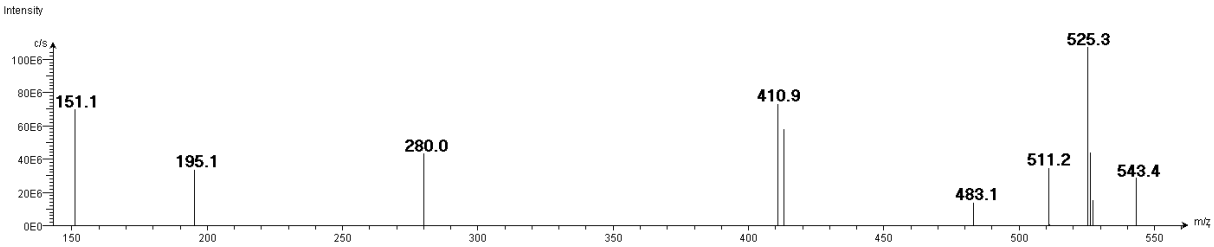


Figure S- 78 : Negative ESI mass spectrum of the acetone extract of *Tephromela atra*

Usnea filipendula

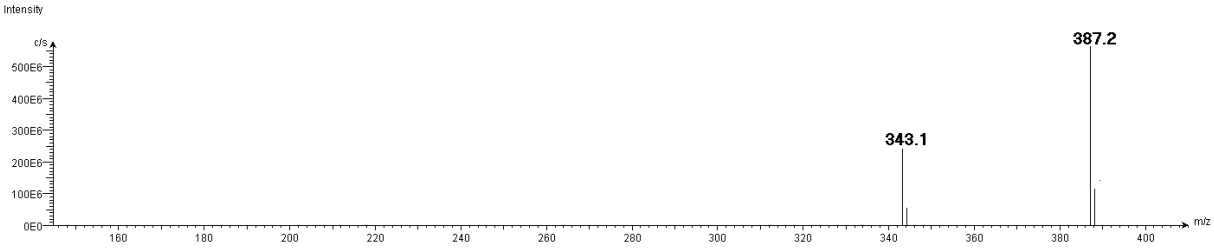


Figure S- 79 : Negative ESI mass spectrum of the acetone extract of *Usnea filipendula*

Vulpicida pinastri

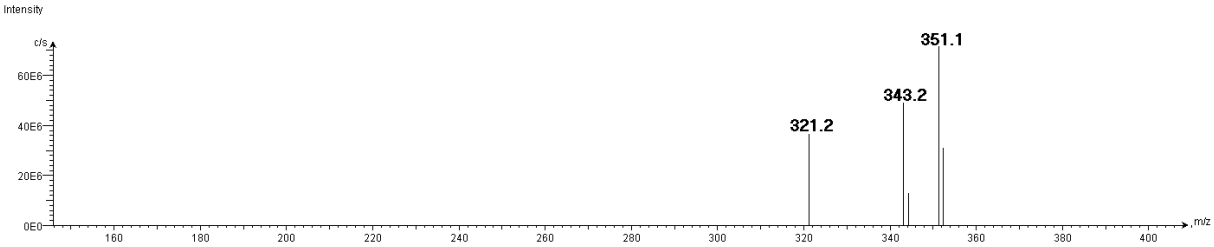


Figure S- 80 : Negative ESI mass spectrum of the acetone extract of *Vulpicida pinastri*

Xanthoria parietina

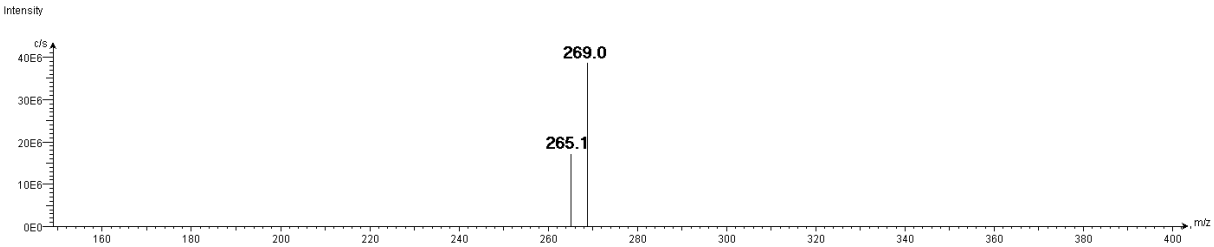


Figure S- 81 : Negative ESI mass spectrum of the acetone extract of *Xanthoria parietina*