Analysis by LC-ESI/LTQOrbitrap/MS/MS of the polar lipid fraction of *Portulaca oleracea* L.

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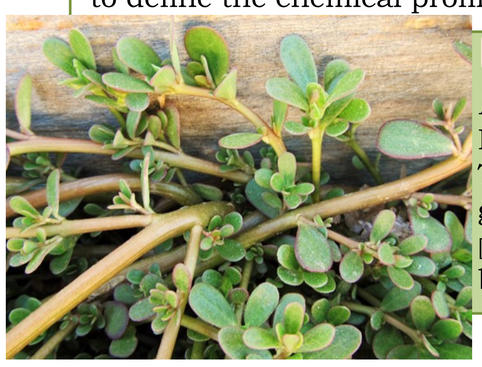
Nowadays an increasing interest in novel foods able to exert health benefits, to prevent ageing, neurodegenerative and cardiovascular diseases, can be observed. Most of these effects have been related to ω-3 content of foodstuffs usually of animal origin. Recently the attention is shifted towards innovative plant sources, whose primary metabolism produces in specialized cell organelles lipids exerting biological activities. The revaluation of food plants, appreciated in the past for their health benefits and typically used in traditional cuisine, is thereby receiving growing attention. In this frame the present work is addressed to the investigation of *Portulaca* edible parts to define the chemical profile of multiclass polar lipids, known for their bioactivity [1].



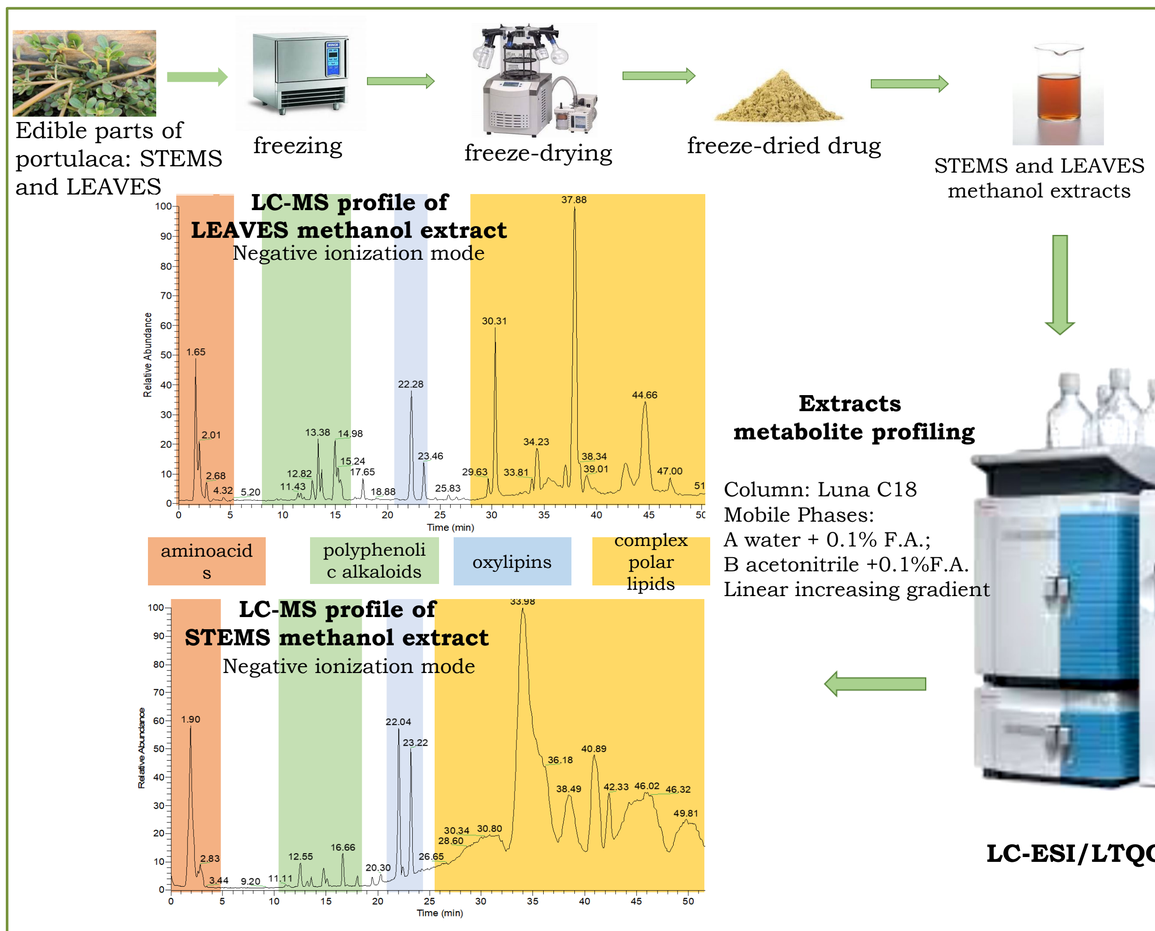
Portulaca oleracea L.

Portulaca oleracea L. (portulaca), belonging to *Portulacaceae* family, is a popular edible grass growing in mild climate countries such as Mediterranean area. It was commonly used in the local cuisines but after its consideration as "weed", this custom has gone down. In Traditional Medicine, the decoction of the aerial parts of portulaca was used to relieve diarrhea, for the treatment of diabetes [2] gonorrhea and gout [3]. Recently the promising activity of portulaca in the prevention of neurodegenerative diseases has been reported [4,5]. Nowaday this plant has gained new interest as food because of its high content of polyunsaturated fatty acids showing health benefits. Since it is commonly widespread, it could represent a low cost resource for nutraceutical formulations.

EXPERIMENTAL PLAN

The applied analytical workflow was aimed at defining the portulaca chemical profile with particular attention to the characterization of polar lipids by LC-ESI/HRMS/MS^n analysis. Powdered portulaca leaves and stems, manually separated, were defatted with petroleum ether and chloroform and subsequentially submitted to extraction with methanol at room temperature. In order to allow the detection of the several classes of polar lipids, two different analytical steps were followed.

Metabolite fingerprint of methanol extract of leaves and stems



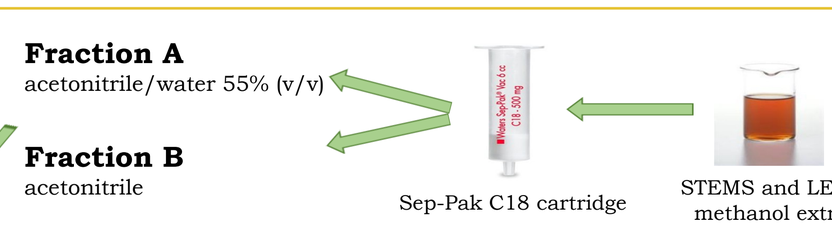
Extracts metabolite profiling

Column: Luna C18
Mobile Phases:
A water + 0.1% F.A.;
B acetonitrile +0.1%F.A.
Linear increasing gradient

LC-ESI/LTQOrbitrap/MS/MS^n analysis

The preliminary LC-ESI/HRMS/MS^n analysis of both stems and leaves methanol extracts showed the occurrence of different metabolite classes well-separated in different areas of the chromatogram (coloured legend), hihlighting multiclass polar lipids as main compounds.

Multiclass polar lipids from portulaca leaves and stems

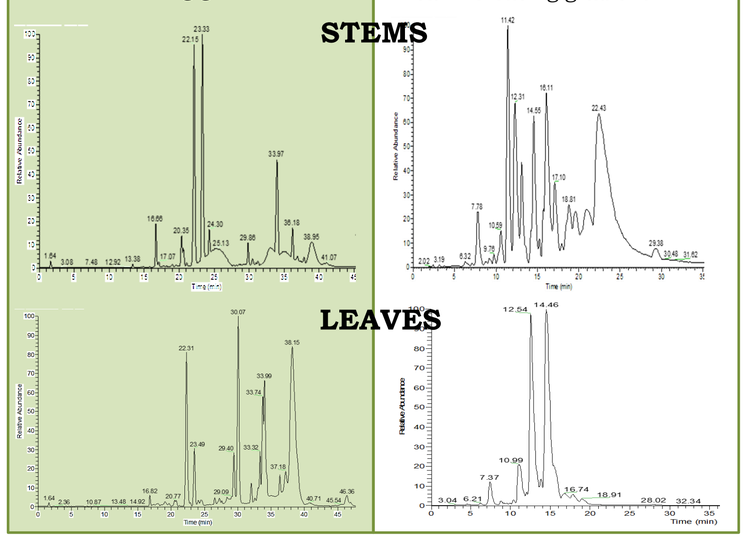


Multiclass polar lipids profiling

LC-MS profiles of lipid enriched fractions
Negative ionization mode

Fraction A
Column: Luna C18
Mobile Phases:
A water + 0.1% F.A.
B acetonitrile + 0.1%F.A.
Linear increasing gradient

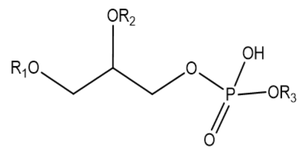
Fraction B
Column: Phenomenex C4
Mobile Phases:
A water + 0.1% F.A.
B acetonitrile + 0.1%F.A.
Linear increasing gradient



In order to obtain a comprehensive and easier structural elucidation of multiclass polar lipids from both MeOH extracts, these were submitted to a fractionation by Sep-Pak C18 cartridge, affording two lipid enriched fractions (A and B). According to their polarity, these latter were submitted to LC-ESI/HRMS/MS^n analysis, by using a RP C-18 or a RP C4 column, able to reduce the strong adsorption of complex lipid metabolites and to allow their early elution and detection. By this way, a large range of polar lipids belonging to oxylipin, phospholipid, sphingolipid and glycolipid classes were putatively identified.

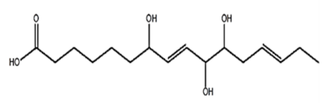
Multiclass Polar Lipids

PHOSPHOLIPIDS

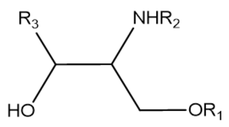


R_1 and R_2 = fatty acyl chain or hydrogen
R_3 = - **choline** in **l-PC** and **PC**,
- **ethanolamine** in **l-PE** and **PE**,
- **inositol** in **l-PI** and **PI**,
- ***N*-acylethanolamine** in **NA-GPE**

OXYLIPINS

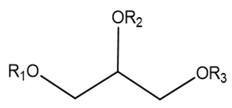


SPHINGOLIPIDS



R_1 = - **hydrogen** in Long-Chain Bases (**LCBs**) and ceramide,
- **phosphate** in Long-Chain Base Phosphate (**LCB-P**),
- **Glucose or galactose** in cerebroside
R_2 = - **hydrogen** in **LCB** and in **LCB-P**,
- **fatty acyl chain** in ceramide and **cerebroside** (**CERB**)
R_3 = backbone of the aliphatic amino alcohol composing the long chain base

GLYCOLIPIDS



R_1 and R_2 = - fatty acyl chain or hydrogen in **DGMG**,
- fatty acyl chain in **SQDG**
R_3 = - **digalactose** in **DGDG**,
- **sulfonated glucose** in **SQDG**
R_1 R_2 R_3 = one fatty acyl chain and two hydrogen in **MAG**

Main polar lipids from portulaca stems

Rt	[M-H]$^-$	Molecular formula	ppm	MS/MS	Compound
3.19	675.35706	$C_{33}H_{56}O_{14}$	-2.15	652, 521, 415, 397, 356	DGMG (18:3)
4,06	559,3112	$C_{27}H_{46}O_9$	-2.67	277, 253	MGMG (18:3; 16:1)
4,16	481,2554	$C_{22}H_{43}O_9P$	-1.45	438, 317, 255, 164, 138	PG (16:0)
4,11	555,2822	$C_{25}H_{48}O_{11}S$	-0.93	537, 347,297, 255, 225	SQMG (16:0)
4,41	483,2712	$C_{22}H_{45}O_9P$	-1.11	465, 353, 255, 227	PG (16:0)
7.78	951.56598	$C_{51}H_{84}O_{16}$	-1.66	673, 415, 397, 293, 277	ox-DGDG (18:3; ox18:3)
8,25	949,5497	$C_{52}H_{82}O_{16}$	-2.29	671, 689, 657, 509, 397, 379, 323, 291, 277	ox-DGDG (18,4 ox ;18:3)
9.76	1097.62292	$C_{57}H_{94}O_{20}$	-2.32	837, 819, 559, 541, 397	TGDG
11,42	935,5712	$C_{51}H_{84}O_{15}$	-1.52	675, 657, 397, 379, 277	DGDG (18:3; 18:3)
12,31	714,5499	$C_{40}H_{77}O_9N$	-2.22	552, 534, 271	Cerebroside (d18:1, h16:0)
12,93	716,5656	$C_{40}H_{79}O_9N$	-2.09	554, 536, 299, 272, 225	Cerebroside
13,09	913,5862	$C_{49}H_{86}O_{15}$	-2.32	675, 657, 635, 397, 379, 277	DGDG (16:0; 18:3)
14,55	773.51917	$C_{45}H_{74}O_{10}$	-1.48	513, 343, 277, 235	MGDG (18:3; 18:3)
14,91	786,6072	$C_{44}H_{85}O_{10}N$	-2.20	624, 606, 382, 327	Cerebroside (t18:1, h20:0)
15,28	941,6171	$C_{51}H_{90}O_{15}$	-2.49	675, 663, 397, 305, 283, 277	DGDG (18:3; 18:0)
15,70	789,5131	$C_{45}H_{74}O_{11}$	-2.06	529, 527, 509, 279, 277, 249	MGlcDG (18:2; 18:3)
15,80	789,5134	$C_{45}H_{74}O_{11}$	-1.73	771, 527, 509	MGlcDG (18:2)
16,06	793,5106	$C_{41}H_{76}O_{12}S$	-2.95	537, 299, 255, 225 MS3 su 537	SQDG (16:0; 16:0)
16,58	861,5469	$C_{45}H_{83}O_{13}P$	-2.18	793, 581, 577,419, 283, 279, 259, 241	PI (18:2; 18:0)
16,58	843,5269	$C_{45}H_{80}O_{12}S$	-2.08	565, 559, 283, 277	SQDG (18:0; 18:3)
16,68	552,4979	$C_{34}H_{67}O_4N$	-1.40	534, 326, 314, 312, 298, 296, 271, 270, 255, 253, 239, 225	Ceramide (d18:1, h16:0)
16,84	765,5132	$C_{43}H_{74}O_{11}$	-1.96	747, 527, 509,505, 487, 277,255	MGlcDG (18:3; 16:0)
16,94	751,5344	$C_{43}H_{76}O_{10}$	-1.48	751, 540, 513, 495, 277, 255	MGDG (16:0; 18:3)
17,05	819,5262	$C_{43}H_{80}O_{12}S$	-3.04	563, 537, 281, 255, 243	SQDG (16:0; 18:1)
17,41	791,5291	$C_{45}H_{76}O_{11}$	-1.61	529, 511, 279, 249	MGlcDG (18:2; 18:2)
17,82	777,5500	$C_{45}H_{78}O_{10}$	-1.38	777, 515, 495, 445,281, 279,	MGDG(18:2; 18:1)
18,24	845,5422	$C_{45}H_{82}O_{12}S$	-2.46	565, 561, 283, 279	SQDG(18:0; 18:2)
18,50	719.4845	$C_{38}H_{73}O_{10}P$	-1.76	483, 481, 465, 463, 391, 255, 253	PG (16:0; 16:1)
18,96	767,5287	$C_{43}H_{76}O_{11}$	-2.14	529, 511, 505, 487, 279, 255, 249	MGlcDG (18:2; 16:0)
19,22	753,5508	$C_{43}H_{78}O_{10}$	-0.46	753,515, 491, 279, 255	MGDG (18:2; 16:0)
19,22	693,4482	$C_{39}H_{67}O_8P$	-1.16	433, 431, 415, 413, 279, 277	PA (18:3; 18:2)
19,59	821,5423	$C_{43}H_{82}O_{12}S$	-2.46	565, 537,283, 255	SQDG (18:0; 16:0)
19,64	721,4997	$C_{38}H_{75}O_{10}P$	-2.39	483, 465, 391, 255	PG (16:0; 16:0)
19,74	821,5420	$C_{43}H_{82}O_{12}S$	-2.84	565, 537, 283, 255, 243	SQDG (18:0; 16:0)
19,79	793,5444	$C_{45}H_{78}O_{11}$	-2.03	537, 529, 511, 279,255	MGlcDG(18:1; 16:0)
20,41	743,5288	$C_{41}H_{76}O_{11}$	-2.12	505, 487, 255	MGlcDG (16:0)
20,47	669,4478	$C_{37}H_{67}O_8P$	-1.75	431, 413, 409, 391, 277, 255	PA (16:0; 18:3)
20,47	695.46332	$C_{39}H_{69}O_8P$	-1.89	433, 415, 279	PA (18:2; 18:2)
20,93	847, 5581	$C_{45}H_{84}O_{12}S$	-2.20	565, 563, 283, 281	SQDG (18:0; 18:1)
21,50	769,5444	$C_{43}H_{78}O_{11}$	-2.09	531, 513, 505, 281,255	MGlcDG (18:1; 16:0)
21,45	769,5443	$C_{43}H_{78}O_{11}$	-2.09	751, 531, 505, 281, 255	MGlcDG (18:1; 16:0)
22,90	795,5601	$C_{45}H_{80}O_{11}$	-2.05	777, 533, 529, 515, 283, 279	MGlcDG (18:2;18:0)
22,69	671,4636	$C_{37}H_{69}O_8P$	-1.59	433, 415, 409, 391, 279, 255	PA (16:0; 18:2)
24,92	697,4788	$C_{39}H_{71}O_8P$	-2.18	435, 433, 417, 415, 281, 279	PA(18:2; 18:1)
25,85	683,4632	$C_{38}H_{69}O_8P$	-2.09	446, 423, 405, 277, 255	PA (18:3; 16:0)

Data obtained from LC-ESI/HRMS/MS^n analysis of fraction B

Legend:

DGMG=DiGalactosylMonoacylGlycerol; MGMG=MonoGalactosylMonoacylGlycerol;
DGDG=DiGalactosylDiacylGlycerol; TGDG=TriGalactosylDiacylGlycerol;
MGlcDG=MonoGlucuronosylDiacylGlycerol; PG=PhosphatidylGlycerol;
SQMG=SulfoQuinovosylMonoacylGlycerol; PI=PhosfatidylInositol; PA= Phosphatidic Acid

Conclusions

The results obtained by the described analytical approach highlight, for the first time, the occurrence in *P. oleracea* L. of several polar lipids, supporting a revalued use of the vegetable in human nutrition as a food rich in different classes of bioactive and healthy lipids with beneficial effects [1]. Among the several detected classes, notable is the report of an unusual glycolipid class, previously reported in bacteria, fungi and in some species of algae, but whose occurrence in higher plants has been established only in recent times [6]. Finally, the abundance of bioactive lipids suggests the use of the *P. oleracea* as functional food. Moreover, the high occurrence of cerebrosides in ripe stems suggests for them alternative uses, e.g. as new matrices in cosmetic formulations.

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