

Bioprospection and study of endemic plants from South America

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June 9th, 2023, 1440 h



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Conocimiento y Naturaleza



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Why to study endemic plants?



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Chile's case: having a small number of species,
it has a large percentage of endemic plants,
almost 50%. More or less than 5000 Chilean
species, 2500 are endemic.

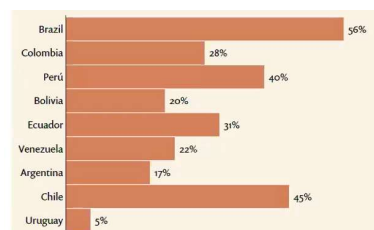
(C. Marticorena, "Composition of the vascular flora of Chile,
" Wild flora of Chile (Jürke Grau & Georg Zizka (eds.), Sonderheft 19, Palmengarten, 1992), p. 74).



This is simple: It is the second country
in the region with the most endemism



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Search by Tentative identification of compounds: What are hyphenated HPLC-PDA-MS methods?



Techniques Combining: Liquid chromatography (HPLC, UFLC or UPLC) with UV detectors (UV or diode array, DAD or PDA), and A mass spectrometer (usually a ToF, a quadrupole, An ion trap, or a hybrid such as the Q-orbitrap). Connected by an interface (APCI, ESI)



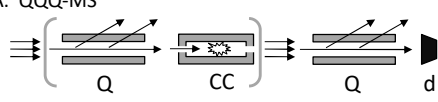
3




MORE USED COMBINATIONS

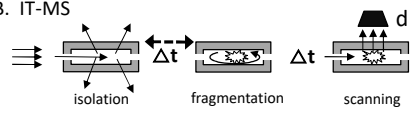


A. QQQ-MS



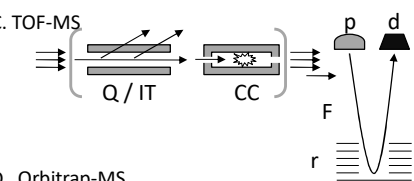
Low resolution
Slow scanning speed

B. IT-MS



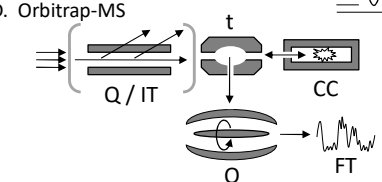
Low resolution
Fast scanning speed

C. TOF-MS



High resolution
Fast scanning

D. Orbitrap-MS



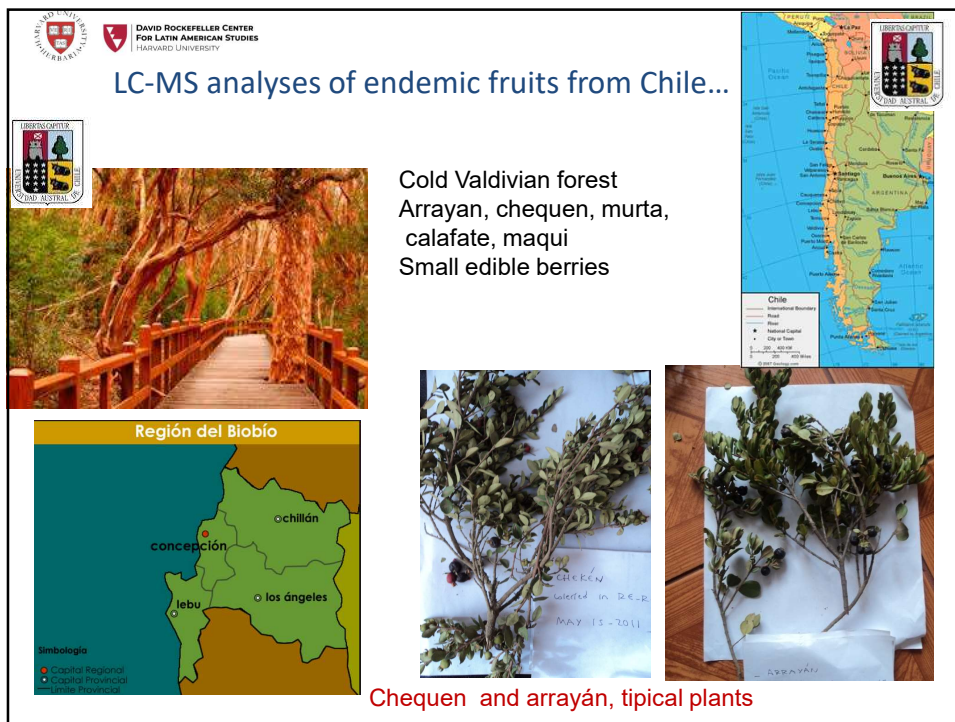
High resolution
Fast scanning

Selectivity
UHPLC
Global

Q: quadrupole; CC: collision cell; d: detector; IT: ion trap; p: pusher; (TO)F: (Time-Of-) flight; r: reflectron; t: trap; O: orbitrap; FT: Fourier transform

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LC-MS analyses of endemic fruits from Chile...



Cold Valdivian forest
Arrayán, chequen, murta,
calafate, maqui
Small edible berries

Región del Biobío

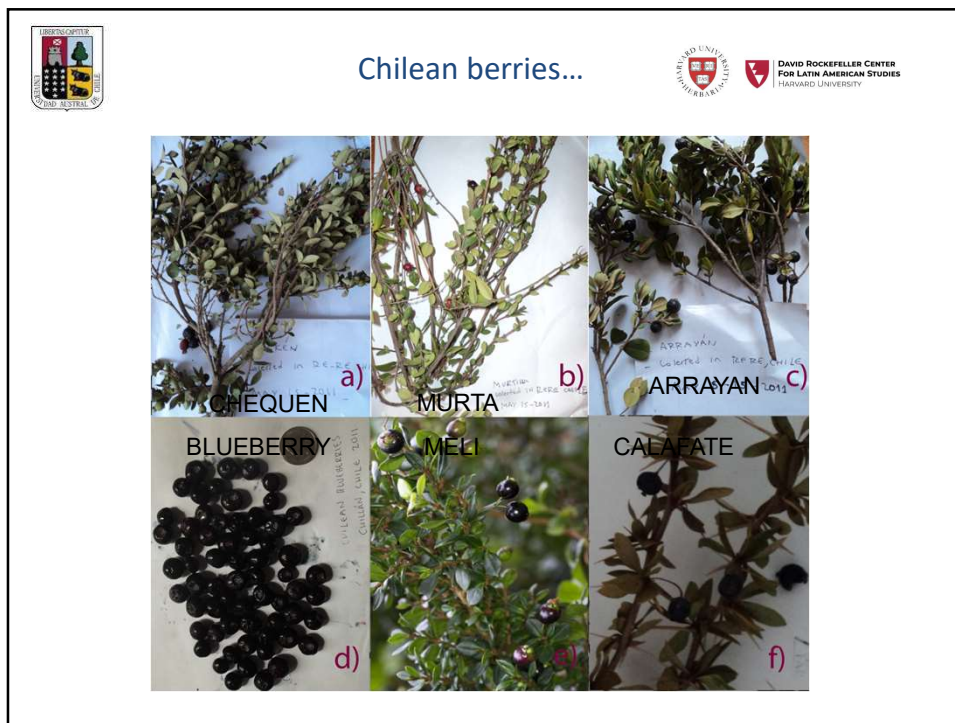
concepción, chillán, lebu, los ángeles

Simbología

Chequen and arrayán, typical plants

5

Chilean berries...



CHEQUEN, MURTA, ARRAYÁN, BLUEBERRY, MELI, CALAFATE

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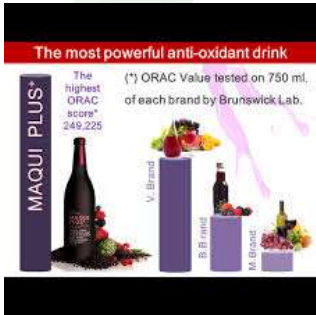
Some dietary supplements











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Fingerprints of berries



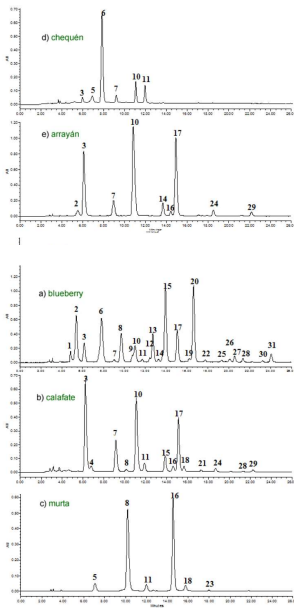

Oc1c(O)c2c(c1)oc(O)c(O)c2Oc3c(O)c(O)c(O)c(O)c3
Oc1c(O)c2c(c1)oc(O)c(O)c2Oc3c(O)c(O)c(O)c(O)c3

Peak	R ₁	R ₂	R ₃	Peak	R ₄	R ₅	R ₆	R ₇
3 ^a	OH	OH	Gal	1	OH	OCH ₃	Glu	H
4	OH	OH	Glu	2	OH	OCH ₃	Glu	H
6 ^a	OH	H	Gal	5	OH	H	Rha	H
7 ^a	OH	H	Glu	8	OH	OCH ₃	Rha	H
9	OCH ₃	OCH ₃	Rha	9	OCH ₃	OCH ₃	Rha	H
10 ^a	OH	OCH ₃	Glu	12	H	OCH ₃	Rha	H
11 ^a	OH	OCH ₃	Gal	21	OCH ₃	OCH ₃	Cou	H
13 ^a	H	OCH ₃	Gal	23	OH	H	Succ	H
14	OCH ₃	OCH ₃	Gal	24	H	OCH ₃	Glu	H
15	OH	OH	Ara	25	OH	OH	Cou-rha	Glu
16 ^a	H	OCH ₃	Glu	26	OH	OH	Ac	H
17 ^a	OCH ₃	OCH ₃	Glu	27	OH	H	Ac	H
18	H	OCH ₃	Ara	28	OH	OCH ₃	Ac	H
				29	OCH ₃	OCH ₃	Ac	H
				30	OH	OH	Cafl	H
				31	OCH ₃	OCH ₃	Ac	H

Anthocyanins mainly

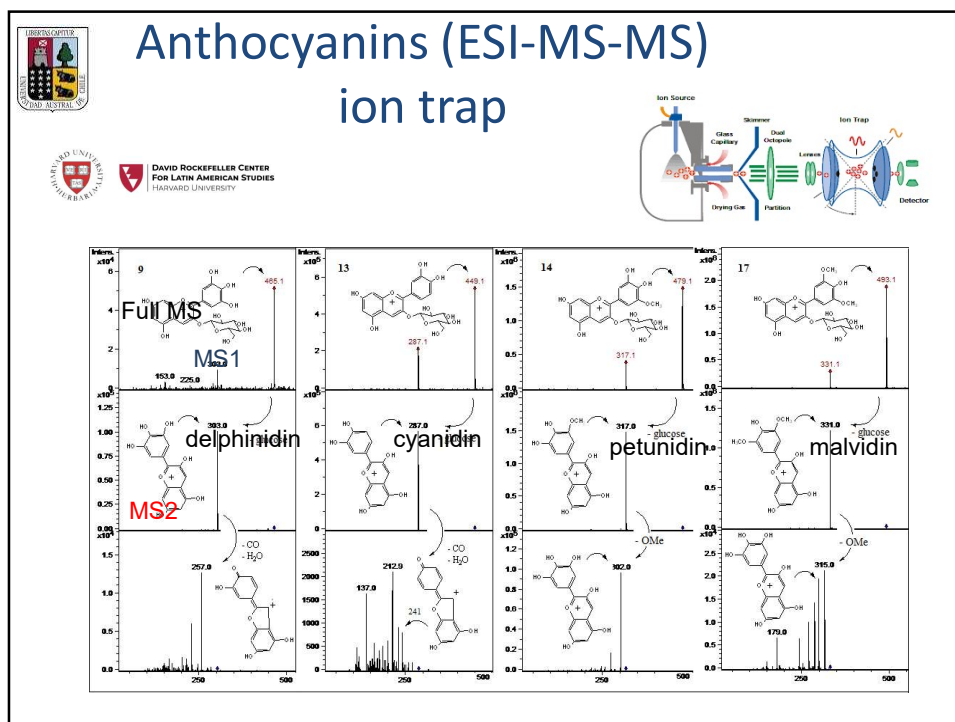
^a A  Food Chemistry
Volume 176, 1 June 2015, Pages 106–114

Analytical Methods
Anthocyanins and antioxidant capacities of six Chilean berries by HPLC–HR-ESI-ToF-MS
Javier E. Ramirez*, Ricardo Zambrano*, Beatriz Sepúlveda*, Edward J. Kennelly*, Mario J. Simiriotis*



mnose; Cou: Coumaric acid;

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Weinmannia trichosperma

Went to the fair found bark of this
Its bark is widely used in traditional
Mapuche medicine:

Treatment of chronic diarrhea.
Prevention of some types of cancer.
Promote the healing of wounds
caused by burns





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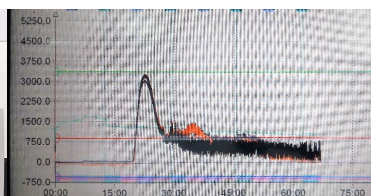
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CONCURRENCE PARTITION CHROMATOGRAPHY allow separation..

from bark of *W. trichosperma* in only one step!

Half an hour!



HEMWAT 1:9:1:9 v/v

Dio un valor de k value de $1,55 \pm 0,09$
(Thermo UHPLC-orbitrap MS)

Frontiers in Pharmacology, 2020. doi:
10.3389/fphar.2020.00780, May 2020

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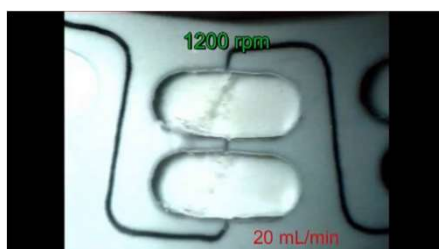


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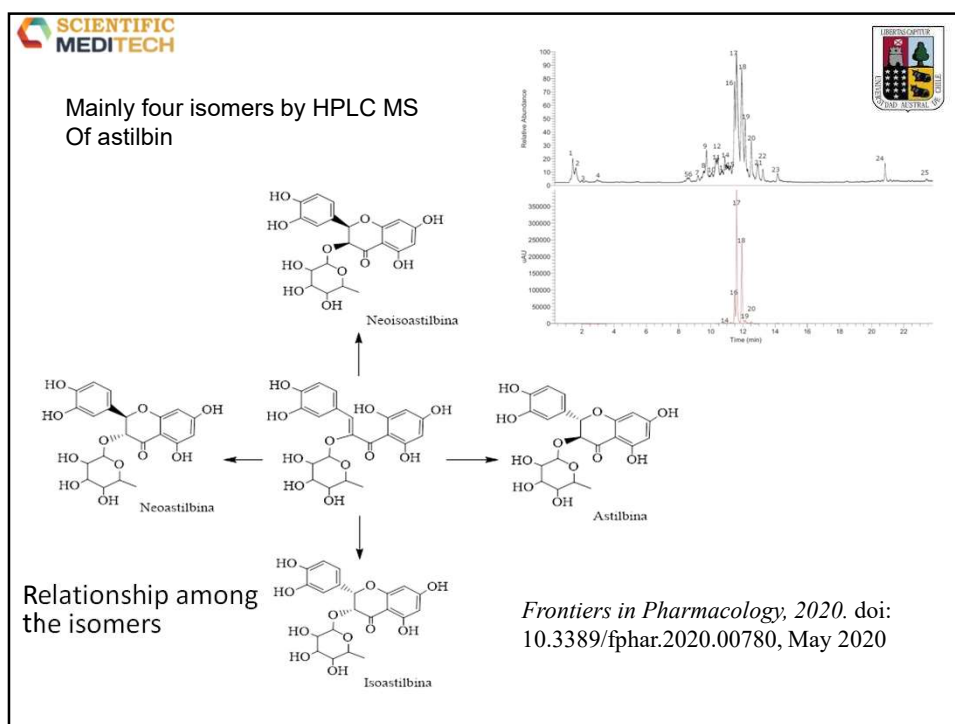
CONCURRENCE PARTITION CHROMATOGRAPHY

- CPC one step, (from 11 g of extract 270 mg pure compound).



Frontiers in Pharmacology, 2020. doi:
10.3389/fphar.2020.00780, May 2020

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Table 3. Total phenolics, flavonoids, antioxidant, 5-hLOX and inhibitory potencies of choline esterase inhibitors for infusion and tincture of *W. trichosperma* and main isolated isoastilbin.

Assay	Infusion of <i>W. trichosperma</i>	Tincture of <i>W. trichosperma</i>	Gallic acid	Isoastilbin	Galanthamine
TPC	442.1 ± 21.1 ^a	438.3 ± 22.2 ^a	-	-	-
TFC	15.54 ± 0.11 ^b	15.75 ± 0.11 ^b	-	-	-
DPH IC ₅₀	20.58 ± 2.46 ^c	20.83 ± 2.18 ^c	0.4622 ± 0.0015 ^k	33.1 ± 2.1 ^d	33.1 ± 2.1 ^d
ABTS IC ₅₀	3.070 ± 0.095 ^c	3.055 ± 0.082 ^c	0.09855 ± 0.00026 ^k	2.525 ± 0.2611 ^d	2.525 ± 0.2611 ^d
FRAP	2.700 ± 0.554 ^e	2.650 ± 0.532 ^e	-	0.1494 ± 0.0166 ^f	0.1494 ± 0.0166 ^f
ORAC	1270 ± 36.5 ^e	1248 ± 32.3 ^e	-	1901 ± 33.5 ^e	-
Acetylcholine esterase inhibition IC ₅₀	3.13 ± 0.03 ^h (33.8 % at 1 mg mL ⁻¹)	3.08 ± 0.02 ^h (33.5 % at 1 mg mL ⁻¹)	-	4.68 ± 0.03 ^h (51.70 % at 2.2 μM)	0.26 ± 0.02 ^h (69.42 % at 3.48 μM)
Butyrylcholine esterase inhibition IC ₅₀	2.94 ± 0.08 ^h (33.12 % at 1 mg mL ⁻¹)	2.76 ± 0.08 ^h (33.12 % at 1 mg mL ⁻¹)	-	8.51 ± 0.03 ^h (50.10 % at 2.2 μM)	3.82 ± 0.02 ^h
5-hLOX inhibition (%)	82.86 ⁱ (at 0.05 mg mL ⁻¹)	82.71 ⁱ (at 0.05 mg mL ⁻¹)	-	34.29 (at 10 μM) 80.71 (at 40 μM)	-

All values were expressed as means ± SD (n = 3).

^a expressed in mg of gallic acid equivalents per gram of dry plant.

^b expressed in mg of quercetin equivalents per gram of dry plant. ^c expressed in μg of extract per mL.

^d expressed in μg of isoastilbin per milliliter. ^e expressed in mmol Trolox equivalent per gram of dry plant.

^f expressed in mmol trolox equivalent per mg of isoastilbin. ^g expressed in μg of gallic acid per mL.

^h expressed in μM Trolox equivalents per 100 grams of dry plant.

ⁱ IC₅₀ in μg per mL. ^j Concentration Stock Solution equal to 1.52 mg per mL⁻¹.



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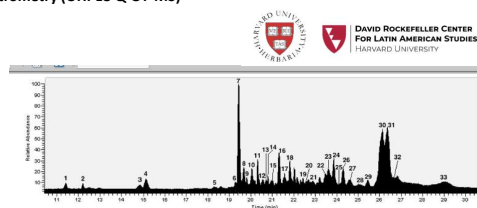
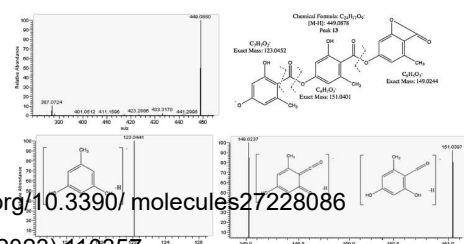
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Chemistry and bioactivity of Lichens from Antarctica grant INACH RT 16 17



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- New depside from lichen *Everniopsis trulla* by metabolomics UHPLC–DAD–MS analysis
- CHROMATOGRAPHIA, 2017 Metabolomic Analysis of the Lichen *Everniopsis trulla* Using Ultra High Performance Liquid Chromatography–Quadrupole–Orbitrap Mass Spectrometry (UHPLC–Q–OT–MS)

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

Molecules 2022, 27, 8086. <https://doi.org/10.3390/molecules27228086>

Chemico-Biological Interactions 372 (2023) 110357

Fig. 2. Chemical structure of new compound (structure identified by 13C/13C-ESI-MS-MS and in agreement with fragmentation pattern).

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Promising for Alzheimer

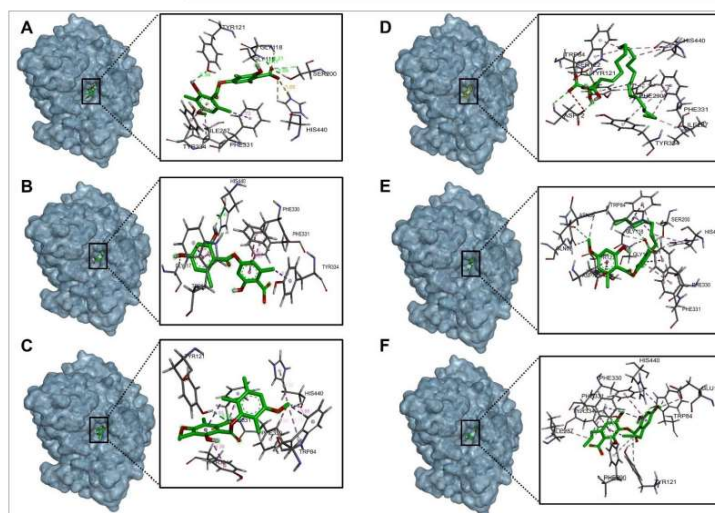


Figure 3. Binding mode and predicted intermolecular interactions of selected compounds from *Lecania brialmontii*, *Pseudophebe pubescens* and *Sphaerophorus globosus* extracts and residues of the *Torpedo californica* acetylcholinesterase (TcAChE) catalytic site; (A) Barbatic acid at the catalytic site; (B) Lecanoric acid at the catalytic site; (C) Brialmontin 2 at the catalytic site; (D) Tetrahydroxytricosanoic acid at the catalytic site; (E) Sphaerophorin at the catalytic site; (F) Sekikaic acid at the catalytic site.

Molecules 2022, 27, 8086. <https://doi.org/10.3390/molecules27228086>

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Baccharis grisebachii



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Table 2. Gastroprotective effect of *B. grisebachii* LD on ethanol induced gastric lesions in rats.

<i>B. grisebachii</i> Treatment (mg/kg)	Gastroprotective Effect	
	Ulcer Index	% Lesion Reduction
LD (250)	4.4 ± 0.2	9.6
LD (500)	2.1 ± 0.5 ***	56.0
LD (750)	0.5 ± 0.2 ***	93.2
Omeprazole (50)	3.2 ± 0.2 **	34.2
Control EtOH	4.8 ± 0.1	0

Gastroprotective effect shown as mean Ulcer Index ± error standard of mean (SEM) and percent lesion reduction compared with untreated controls. Omeprazole was used as reference drug. Asterisks denote significant differences from the control: *** $p < 0.001$ and ** $p < 0.01$ (ANOVA and posterior comparison by Tukey-Kramer).

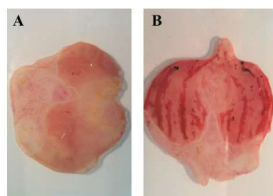


Figure 1. Effects of BLD at 750 mg/kg (A) on the macroscopic aspect of stomach in ethanol-induced gastric lesions in rats (B).



Endemic species *Baccharis grisebachii* Hieron
(Asteraceae, name 'quilchamali') used as digestive
gastroprotective, wound healing
in Argentina.

Molecules, 2019, 442415.

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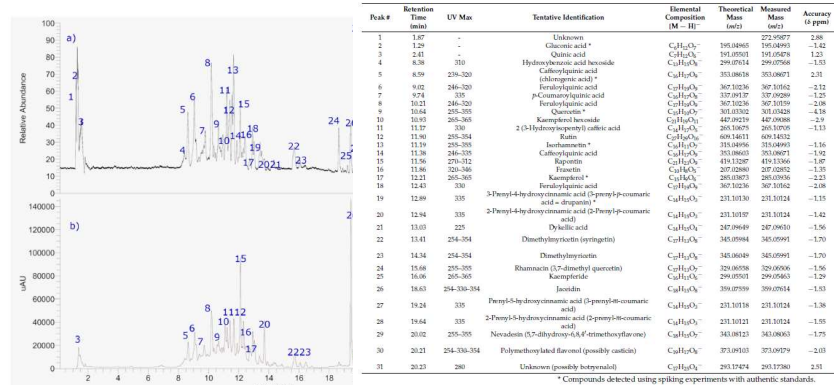
Metabolites of *Baccharis grisebachii*

Figure 3. The HPLC-MS Fingerprints of *Baccharis grisebachii* lyophilized decoction: (a) The total Ion Current (TIC) chromatogram and (b) the UV-vis chromatogram at 280 nm.



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Molecules, 2019, 442415.

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SANKY *Corryocactus brevistylus* (Schumann) Britton & Rose

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FIGURE 1 | *Corryocactus brevistylus* fruits.

fruit, consumed in Bolivia, Chile and Perú, quite acidic, for the preparation of juices, you can combine it only with water or take advantage of its nutrients by mixing it with other fruits, such as grapes, strawberries, or blueberries

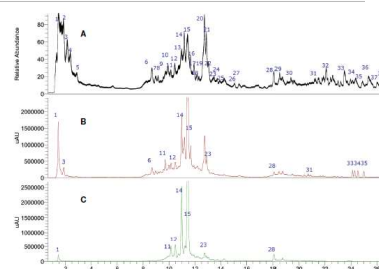


FIGURE 2 | Ultrahigh-pressure liquid chromatography (UHPLC) chromatogram of (A) total ion current (B) UV at 254 nm and (C) 330 nm of *Corryocactus brevistylus* edible fruit.



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Frontiers in Pharmacology, 2020, 11, 417

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It has flavonoids vitamin C, phenolic acids

TABLE 1 | Identification of compounds in *Coriaria brevis* fruits by UHPLC-ESI-MS/MS.

Peaks	UV Max (nm)	Tentative Identification	[M-H] ⁻	RT (min)	Theoretical Mass (m/z)	Measured Mass (m/z)	Accuracy (ppm)	MS ⁿ ions (m/z)
1	—	Malic acid	C ₄ H ₅ O ₅ ⁻	1.46	133.0132	133.0136	2.1	115.0030
2	—	(S)-citric acid	C ₆ H ₇ O ₇ ⁻	1.82	191.0197	191.0190	3.7	173.0080; 111.0078
3	—	Hydroxyglutaric acid	C ₆ H ₇ O ₇ ⁻	2.10	147.0288	147.0291	2.0	129.0184; 111.0078
4	—	Hydroxyglutaric acid isomer	C ₆ H ₇ O ₇ ⁻	2.39	147.0288	147.0291	2.0	129.0184; 111.0076
5	—	Homo (S)-citric acid	C ₇ H ₉ O ₈ ⁻	2.86	205.0342	205.0349	3.4	187.0245; 161.0451; 111.0078
6	280, 211	Hydroxybenzoic acid	C ₇ H ₅ O ₃ ⁻	8.68	137.0244	137.0237	5.1	93.1205
7	—	Dihydroxybenzoic acid	C ₇ H ₅ O ₄ ⁻	8.96	175.0612	175.0604	4.6	123.0440
8	211	Ascorbic acid	C ₆ H ₇ O ₆ ⁻	9.14	175.0248	175.0243	2.9	115.0071; 87.00942
9	213, 287, 326	Caffeoyl-O-hexoside	C ₁₈ H ₁₇ O ₆ ⁻	9.33	341.0873	341.0878	1.5	179.0549; 163.0395
10	217, 288	Benzoyl aspartic acid derivative	C ₁₁ H ₉ O ₅ N ⁻	9.74	236.0553	236.0561	3.4	192.0660
11	217, 290, 323	Benzoyl aspartic acid derivative	C ₁₁ H ₉ O ₅ N ⁻	9.88	236.0553	236.0562	3.3	192.0660
12	221, 255, 342	Caffeoyl (S)-citric acid	C ₂₀ H ₁₉ O ₁₀ ⁻	10.14	353.0514	353.0513	0.3	191.0191; 173.0085
13	221, 266, 331	Caffeoyl (S)-citric acid	C ₂₀ H ₁₉ O ₁₀ ⁻	10.42	353.0514	353.0513	0.3	154.9978; 111.0078
14	221, 268, 318	Coumaroyl (S)-citric acid	C ₁₉ H ₁₇ O ₈ ⁻	10.68	337.0565	337.0564	0.4	191.0191; 173.0085
15	254, 354	Rutin ^a	C ₂₇ H ₃₂ O ₁₆ ⁻	10.93	609.1461	609.1451	1.6	111.0078
16	225, 288, 327	Coumaroyl (S)-citric acid	C ₁₉ H ₁₇ O ₈ ⁻	11.16	337.0565	337.0563	0.6	301.0345
17	255, 350	Isorhamnetin-O-rutinoside or Isorhamnetin-O-neohesperidose	C ₂₈ H ₃₀ O ₁₆ ⁻	11.42	623.1617	623.1607	1.5	179.0343; 151.0388
18	228, 290	Tartronic acid	C ₄ H ₅ O ₅ ⁻	11.67	303.0510	303.0509	0.5	173.0086; 154.9977
19	227, 288, 327	Feruloyl (S)-citric acid	C ₂₀ H ₁₉ O ₁₀ ⁻	11.90	367.0670	367.0671	0.3	111.0078
20	223, 290, 321	Benzoyl aspartic acid derivative	C ₁₂ H ₉ O ₅ N ⁻	12.15	262.0553	262.0552	3.5	315.0509; 301.0353
21	224	Aspic acid	C ₆ H ₇ O ₄ ⁻	12.30	187.0664	187.0672	4.2	163.0394; 125.0235
22	225, 312	Methylcoumaroyl(S)-citric acid	C ₁₉ H ₁₇ O ₈ ⁻	12.72	351.0722	351.0720	0.5	193.0502; 179.0344; 163.0395
23	226, 311	Methylcoumaroyl (S)-citric acid	C ₁₉ H ₁₇ O ₈ ⁻	12.89	351.0722	351.0719	0.4	111.0079
24	225, 314	Unknown (possibly isomer of B1 or B1, C1)	C ₁₉ H ₁₇ O ₈ ⁻	13.01	381.0827	381.0826	0.3	163.0394; 119.0494
25	223, 283	Unknown (possibly isomer of B1 or B1, C1)	C ₁₉ H ₁₇ O ₈ ⁻	13.42	493.1493	493.1500	1.4	163.0394; 133.01353
26	223, 285, 328	Unknown (possibly isomer of B1 or B1, C1)	C ₁₉ H ₁₇ O ₈ ⁻	13.71	327.0863	327.0874	2.3	179.0502; 179.9986; 177.0193
27	195, 223, 283	Unknown (possibly isomer of B1 or B1, C1)	C ₁₉ H ₁₇ O ₈ ⁻	13.42	493.1493	493.1501	1.4	151.0308; 107.0129
28	254, 361	Quercetin ^a	C ₁₅ H ₁₀ O ₇ ⁻	14.27	301.0314	301.0315	0.3	301.0353; 192.0423; 179.0502
29	254, 354	Isorhamnetin ^a	C ₁₉ H ₁₇ O ₈ ⁻	15.05	315.0510	315.0511	0.4	179.9986; 177.0193; 151.0305; 107.0129

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Reduction of rat stomach injuries

TABLE 3 | Gastroprotective activity of CEX on HCl/ETOH-induced gastric lesions in mice.

Compound	n	Lesion index (mm)	% Lesion reduction	Dose (mg/kg)
CEX-10	7	36.6 ± 4.7*	18**	10
CEX-25	7	29.7 ± 3.3*	34**	25
CEX-50	7	16.7 ± 3.9*	63	50
CEX-100	7	12.0 ± 3.4*	73	100
Lansoprazole	7	15.7 ± 2.9*	67	30
Control	7	45.3 ± 5.3**	—	—

The results are expressed as mean ± SD. *P < 0.01 compared with the control and **P < 0.01 compared with lansoprazole (ANOVA followed by Dunnett's test). n = number of mice.

TABLE 4 | Effect of crude ethanolic extract (CEX) on the appearance of gastric lesions induced by HCl/ETOH (p.o.) in Indomethacin- (IND), N-ethylmaleimide- (NEM), and N-nitro-L-arginine methyl ester- (L-NAME) pretreated mice.

Treatment	Dose (mg/kg)	Lesion index (mm)
Control	—	41.6 ± 4.9
IND	10	36.0 ± 3.8
NEM	10	38.6 ± 4.4
L-NAME	70	40.7 ± 5.1
CEX	50	16.7 ± 3.5*
IND + CEX	10 + 50	35.9 ± 2.8
NEM + CEX	10 + 50	37.7 ± 5.3
L-NAME + CEX	70 + 50	18.0 ± 3.1*
Carbenoxolone	100	14.6 ± 4.2*

Results are expressed as mean ± SD. n = 7. Analysis of variance followed by Dunnett's test. *P < 0.01 compared with the respective control. IND, Indomethacin; NEM, N-ethylmaleimide; L-NAME, N-nitro-L-arginine methyl ester.

Profilactic use for stomach illnesses

Frontiers in Pharmacology, 2020, 11, 417

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Tumbo or curuba...
Passiflora tripartita

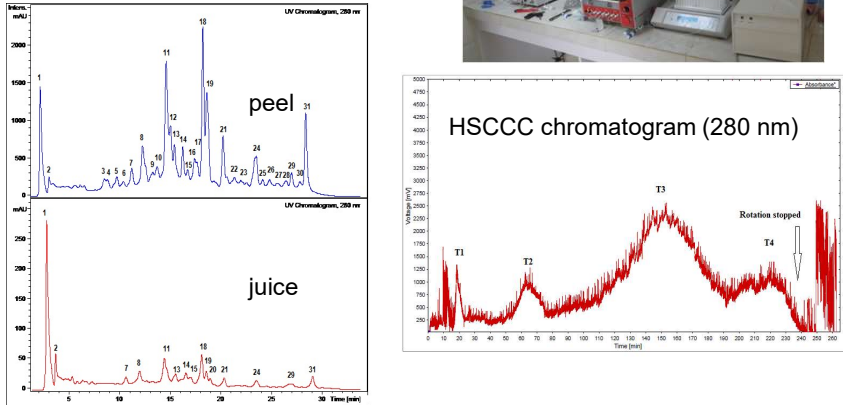


A climbing fruit from the Andes
Used a lot for sour flavored juice
and by sedative properties of the leaves

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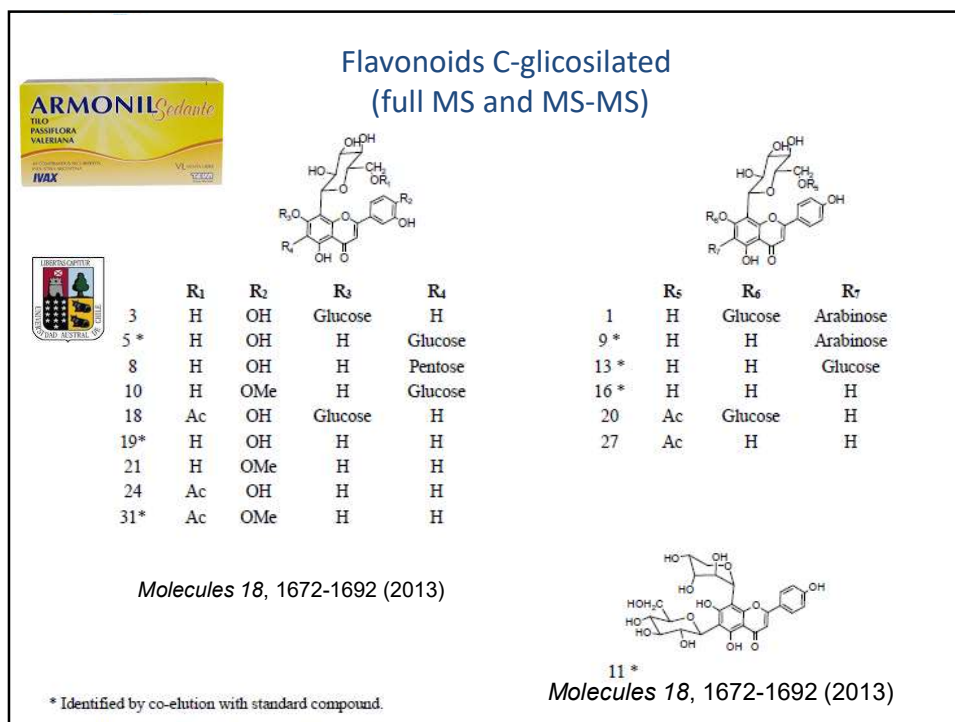
Upper HPLC trace:
peels, Lower pulp (280 nm)



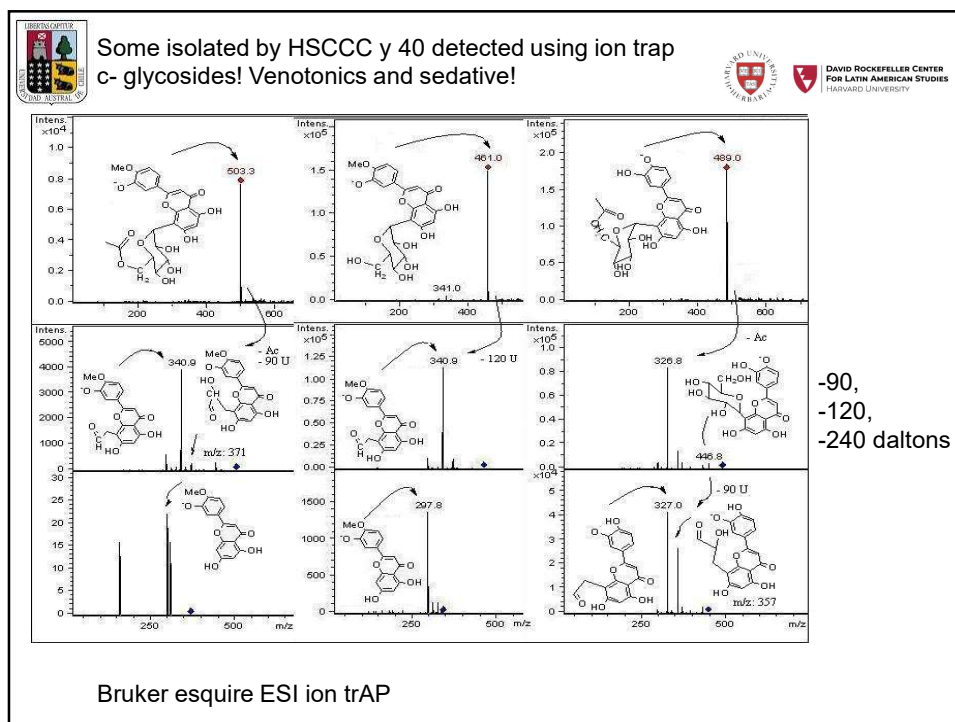
Five compounds were isolated by HSCCC, including 11: schaftoside, 13: vicianin II, 16: vitexin, 19: orientin and 31: acetyl-methyl-orientin

Molecules 18, 1672-1692 (2013)

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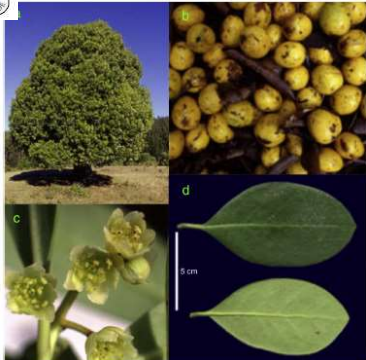


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Queule, unique species

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Gomortega keule (170.1 g)
Lyophilized (Labconco Freezone 4.5 L)

Freeze dried material (39.41 g)
Extracted with MeOH (100 mL x 3 times)
XAD-7-column (500 g)
Rinsed with water (1 L)
eluted with MeOH (1 L)
MeOH extract (9.8 g)
Defatted with n-hexane (500 mL)
Hexane extract (7.35 g)

To Sephadex LH-20 (200 g)
Water-MeOH 1:0% steps
(From 1.39 g MeOH extract)

Fractions A-P submitted to DPPH assay (micrograms)

48.64 ± 5.04 52.57 ± 3.28 67.79 ± 4.79 73.46 ± 3.30

Fraction C (260 mg) Fraction D (145 mg) Fraction J (302 mg) Fraction K (260 mg)

Prep C-18 HPLC solvent A Prep C-18 HPLC solvent A Prep C-18 HPLC solvent B Prep C-18 HPLC solvent B

Compounds 6 (10.2 mg) and 8 (9.62 mg) Compound 16 (1.24 mg) Compounds 12 (0.85 mg) and 20 (1.44 mg) Compound 22 (2.33 mg), 24 (0.36 mg) and 26 (1.61 mg)

The gomortegaceae (Gomortegaceae) are a family of angiosperms of the order laurales
It has a single genus Gomortega and a single species Gomortega keule
Old species, Dinosaurs helped to spread seeds in the past!

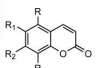
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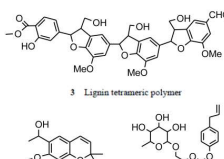
fingerprints

Coumarins, flavonoids, fatty acids...

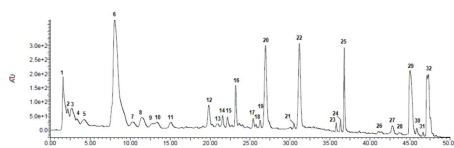
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3 Lignin tetrameric polymer



4 Orthochromene A 15 Luteinolic acid



Interesting compounds!

coumarins

Flavonoids, phenolic acids

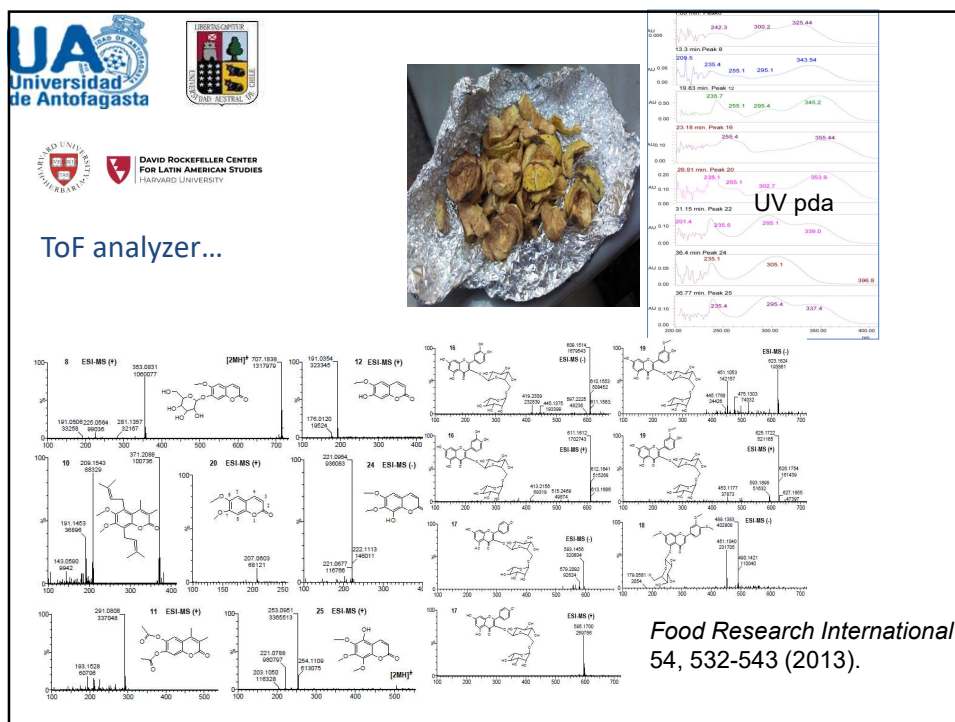
Oxilipins, dietary fatty acids!

30 10(S),11(R)-dihydroxy-15(Z)-octadecanoic acid

31

32 R12=OH
R13=H

28



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Black mount

Heliotropium taltalense from ATACAMA

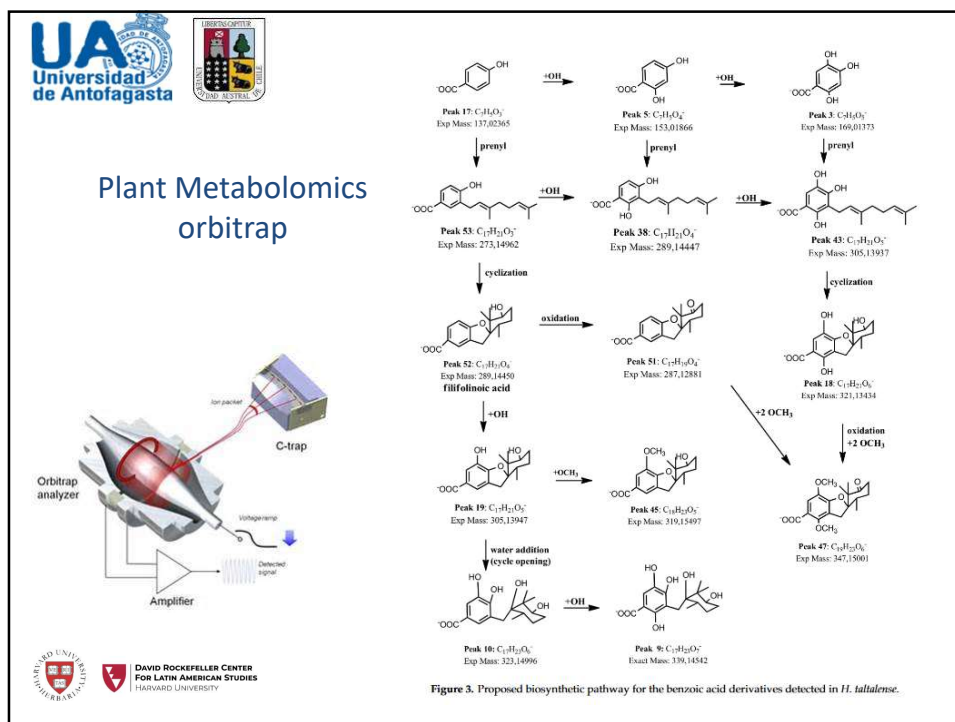




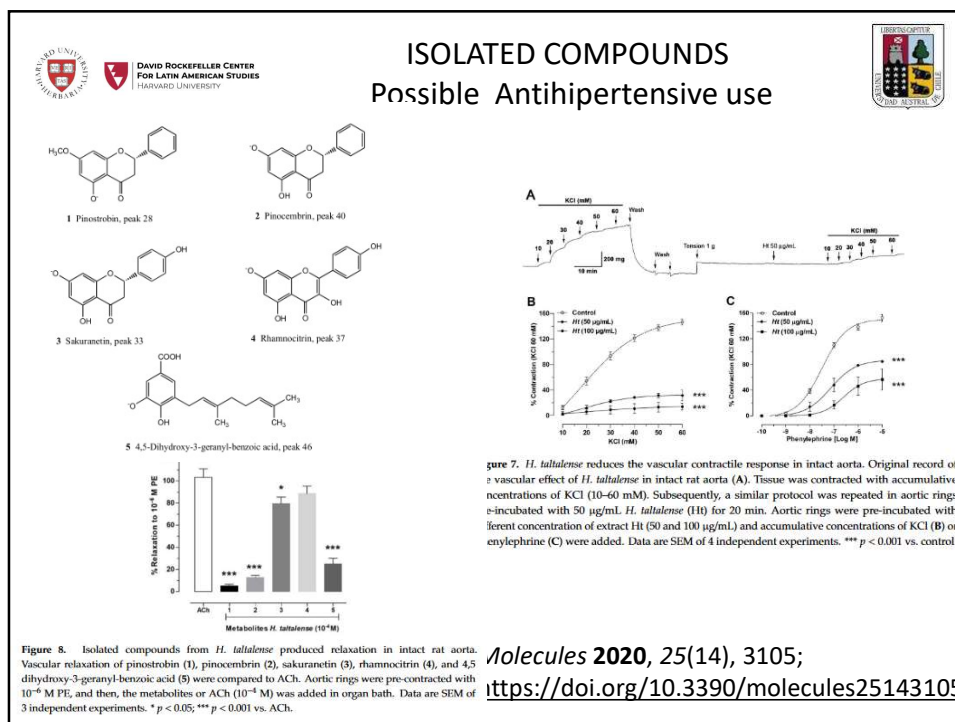


There were no studies!

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Molecules **2020**, *25*(14), 3105;
<https://doi.org/10.3390/molecules25143105>

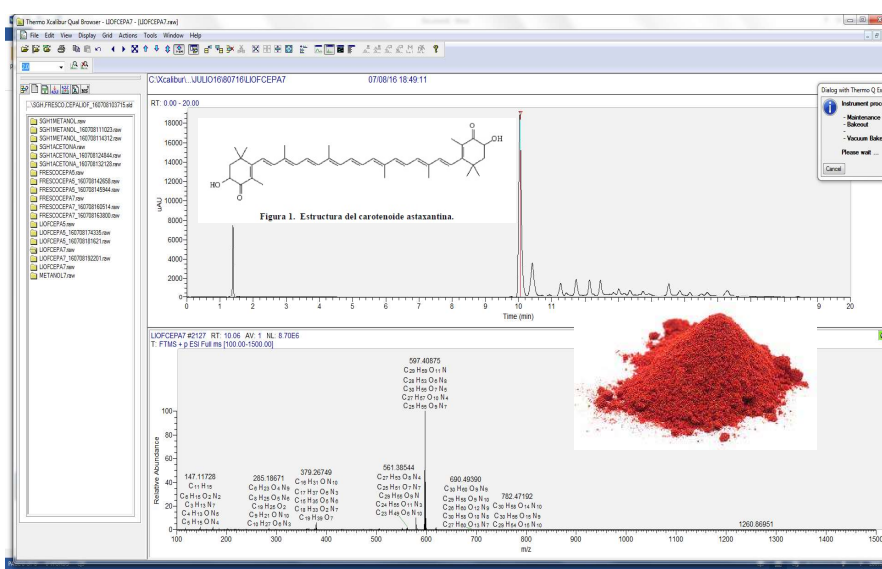
Figure 1 consists of two panels. Panel A shows three Erlenmeyer flasks containing orange liquid, labeled 'S6A7', 'S6A7 D825', and 'S6A7 2A-30'. Panel B shows a gel electrophoresis image with a green arrow pointing to a specific band.

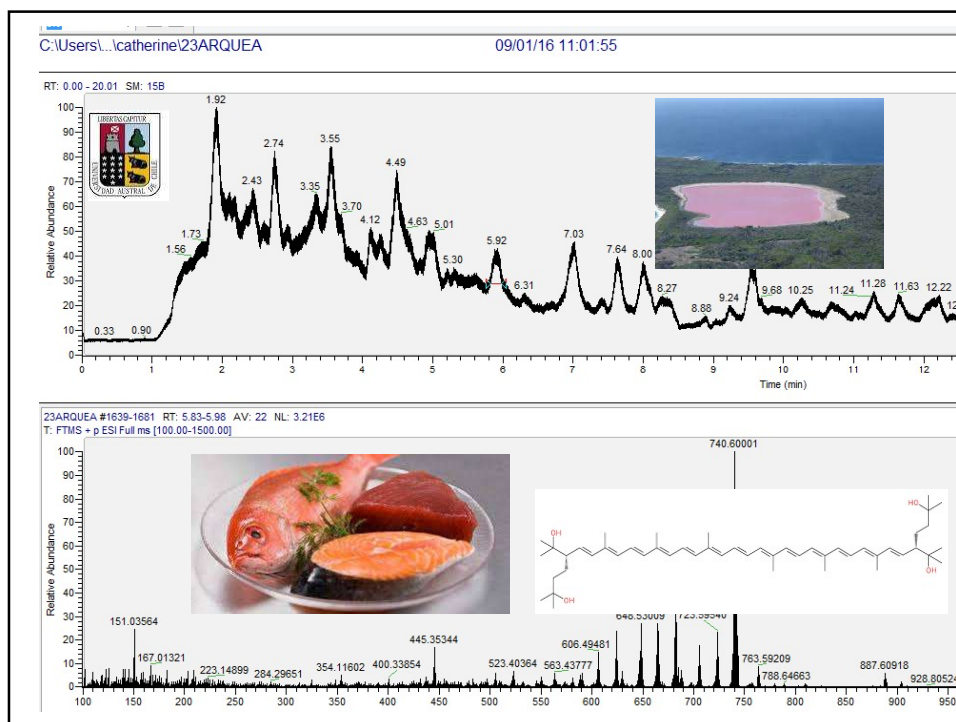
Fig.1 . (A) growth of *Haloterrigena* sp. SGH1 at 15-30% NaCl. (B) thin layer chromatography of total carotenoids. Arrow shows migration of bacterioruberin.



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También disponible en las principales farmacias del país

Bacterioruberin. This C-50 carotenoid contains 13 conjugated double carbon bonds, four hydroxyl groups, and a molecular mass of 740.6 Da.

Bacterioruberin is an effective free-radical scavenger and singlet oxygen quencher and it has been involved in survival-enhanced strategies in haloarchaea such as modulation of membrane fluidity, membrane protection and permeability to oxygen and other molecules.

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Conclusions



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FLORA From CHILE ARE SPECIALLY INTERESTING!

Research can generate growth and economic resources in Chile
and in the world!

Researchers are always Invited to
collaborate with UHPLC HR-MS!
mario.simirgiotis@uach.cl



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Thank you so much

Moon Valley, Chile

Mercado fluvial Valdivia

Lagunas de sal, El tatio

Acknowledgements:

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