Efficient Strategy Based On Centrifugal Partition Chromatography to Recover Bioactive Components From Chilean Plants And Agrofood By-Products

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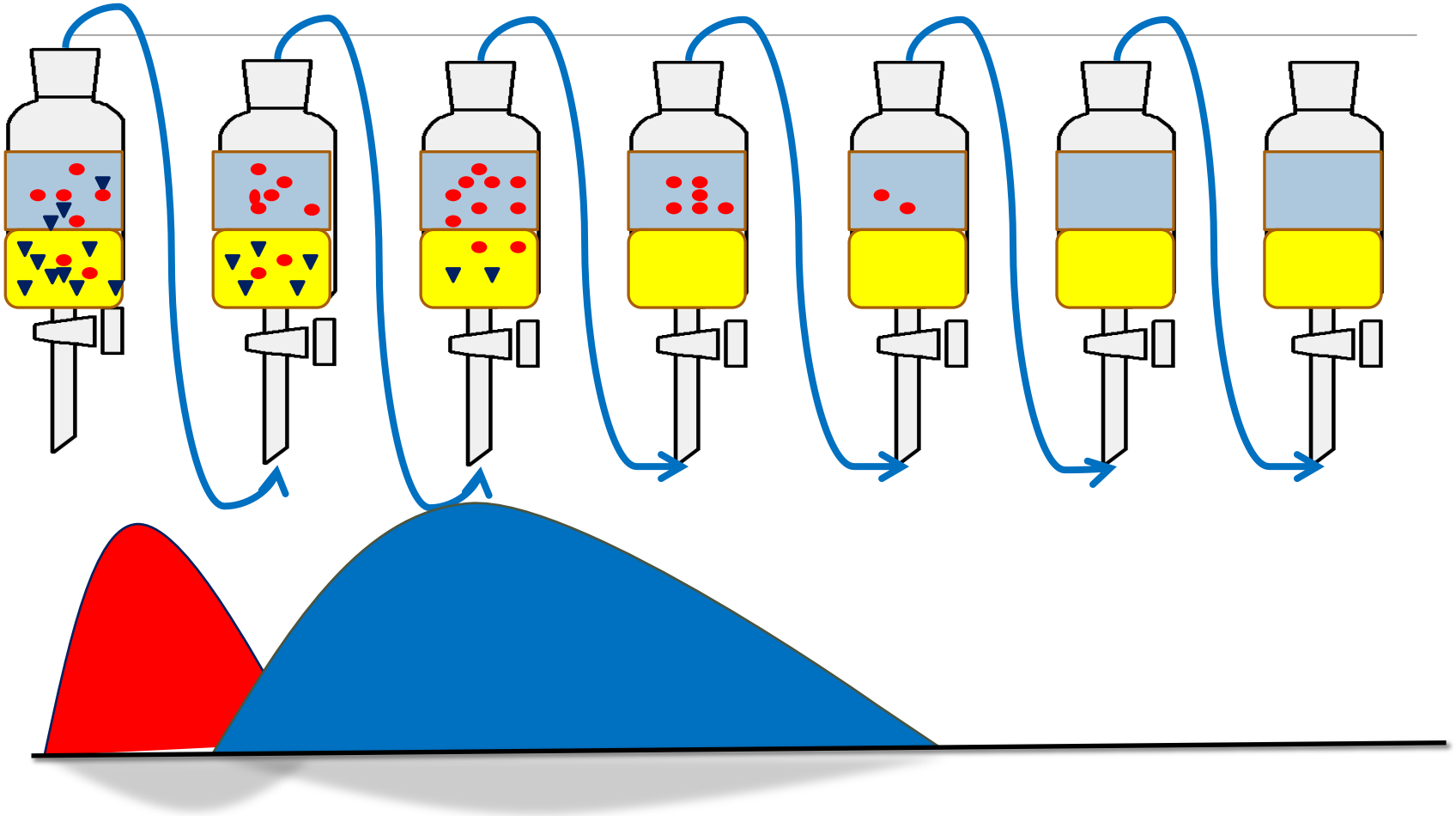
Introduction

- - Applications to polyphenols: proanthocyanidin.
- - Application to purification of semi-synthetic antimicrobial molecules.
- - Sequential CPC/TMB chromatography.

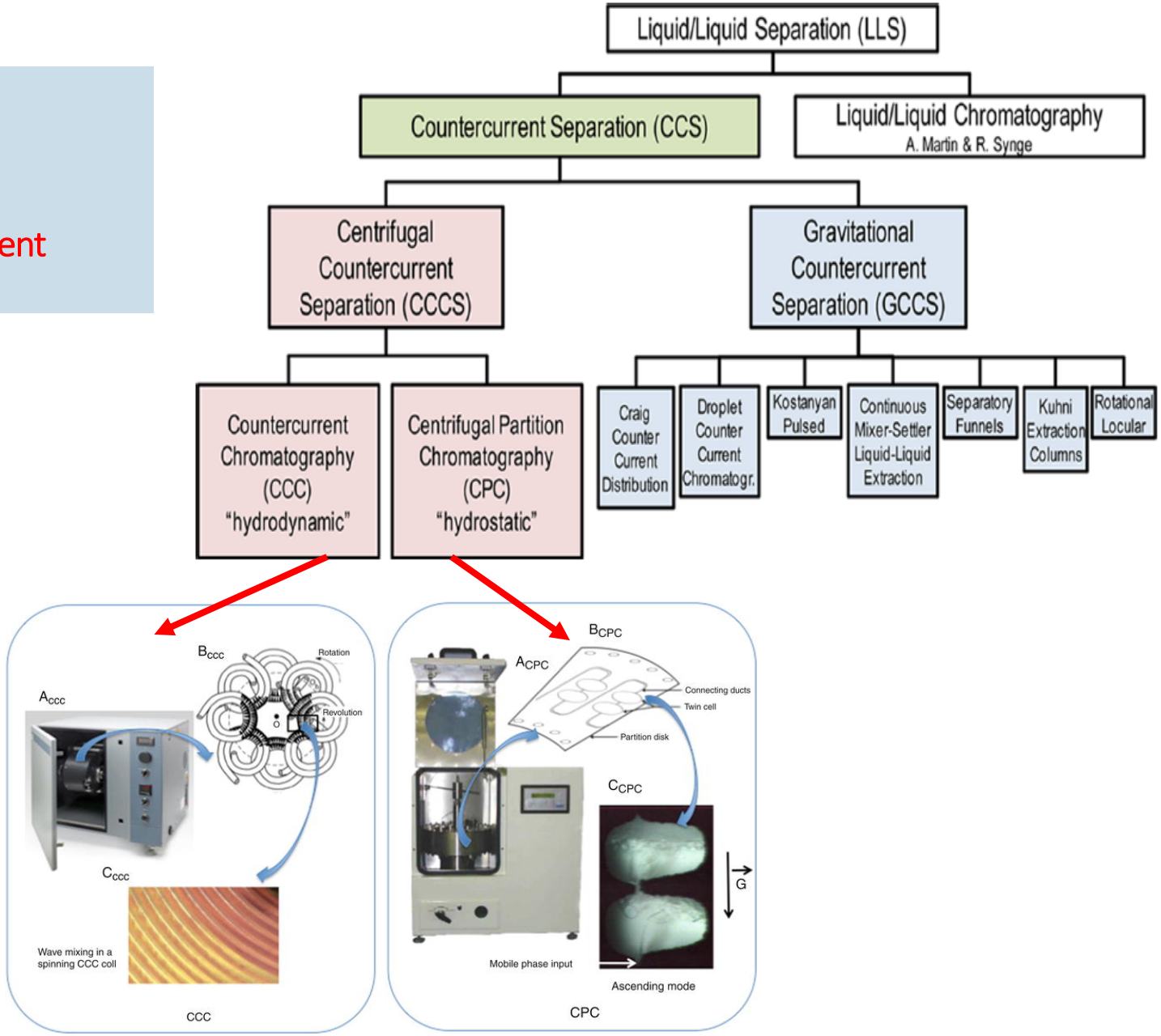
Countercurrent Chromatography is a liquid-liquid separation based on partition coefficients (K_D)



Volume

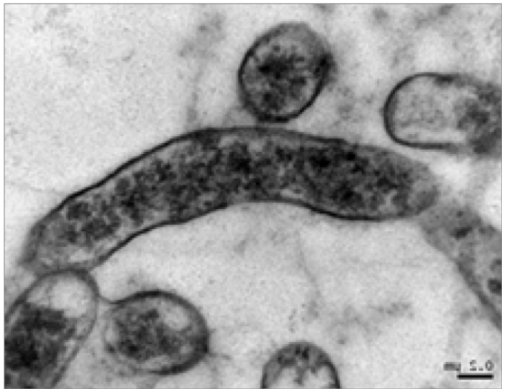
CCS

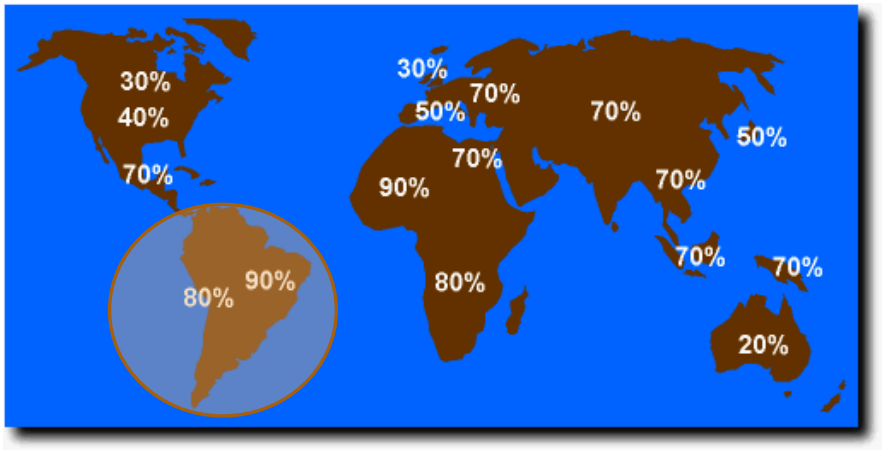
Counter-current Separations

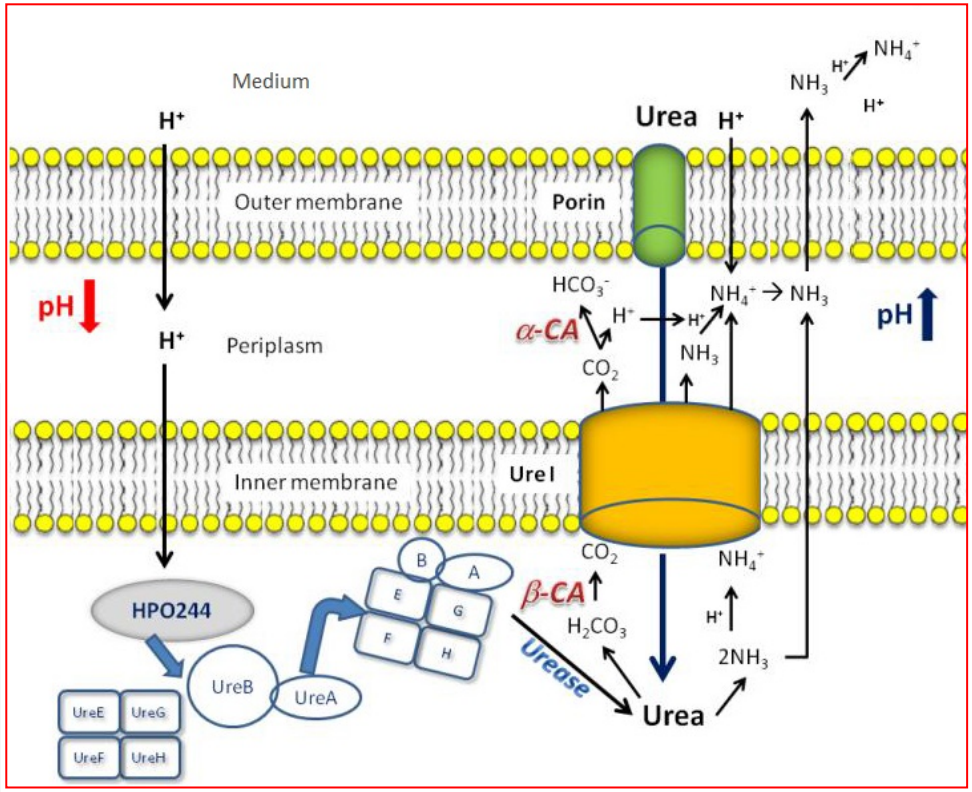


Hubert J., Plé K., Hamzaoui M., Renault JH. (2013) Polyphenol Purification by Solid Support-Free Liquid–Liquid Chromatography (CCC, CPC). In: Ramawat K., Mérillon JM. (eds) Natural Products. Springer, Berlin, Heidelberg

Anti-H. pylori natural products from Chilean Medicinal and Food Plants

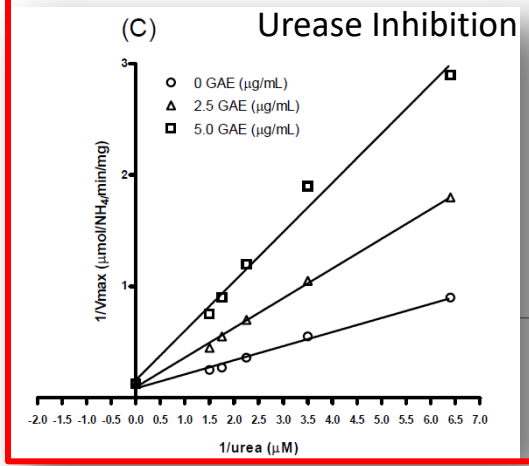


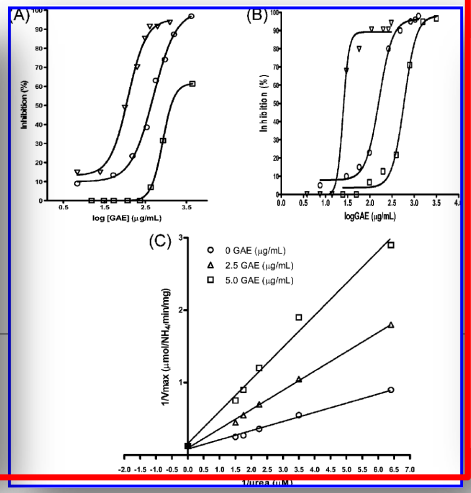




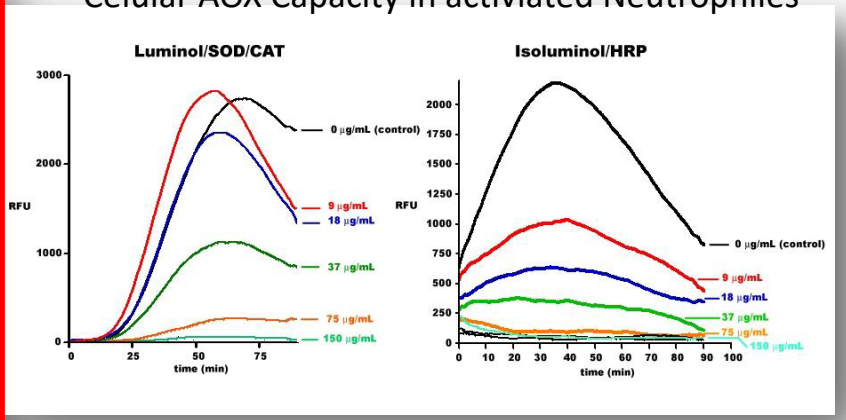
Pastene E., Carvajal R., Zúñiga, F., Alarcón, J., García A. ***Revista Soc. Farmacología*** de Chile. 2016

Urease Inhibition

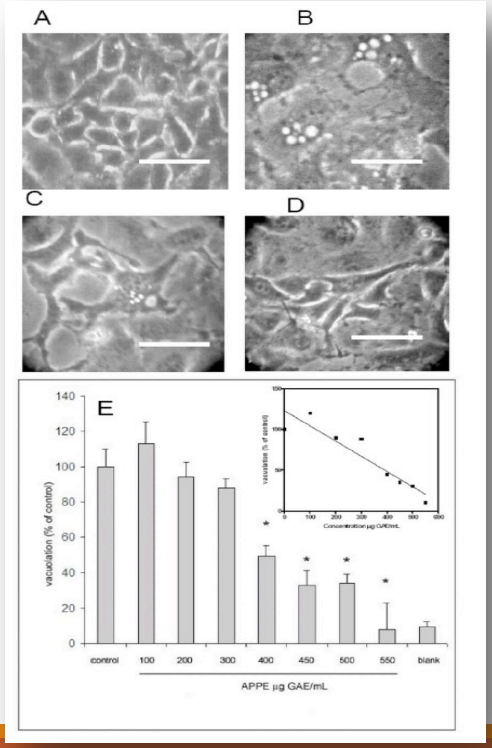




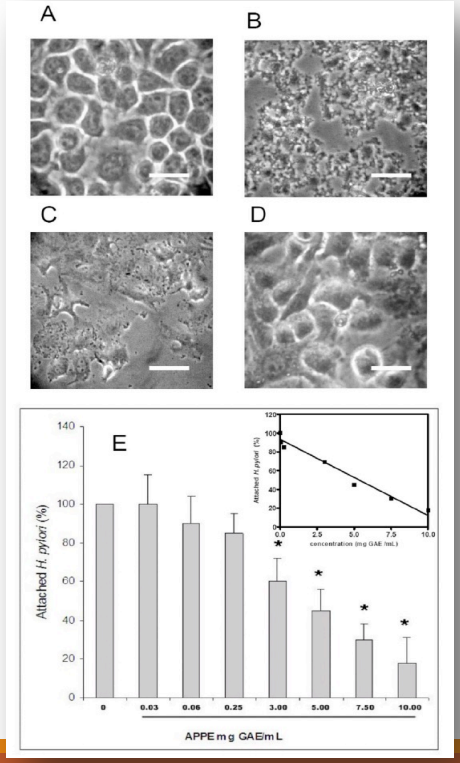
Celular AOX Capacity in activiated Neutrophiles



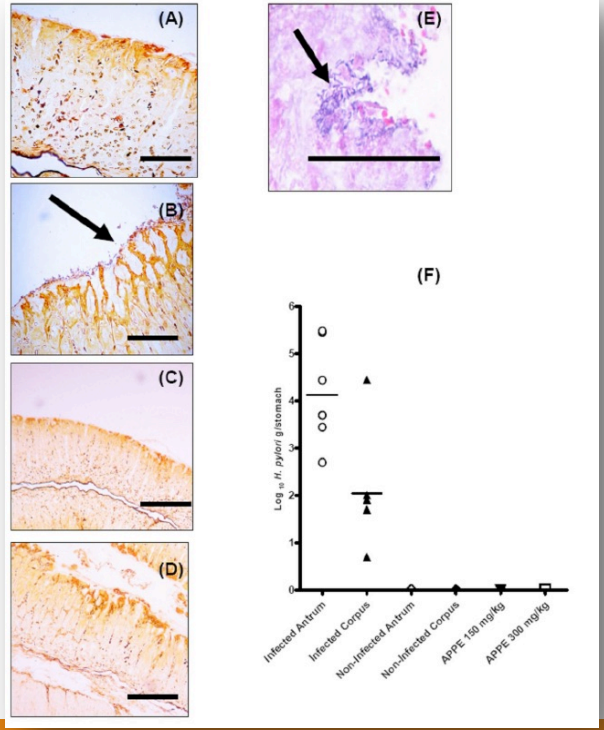
Inhibition of Vacuolation



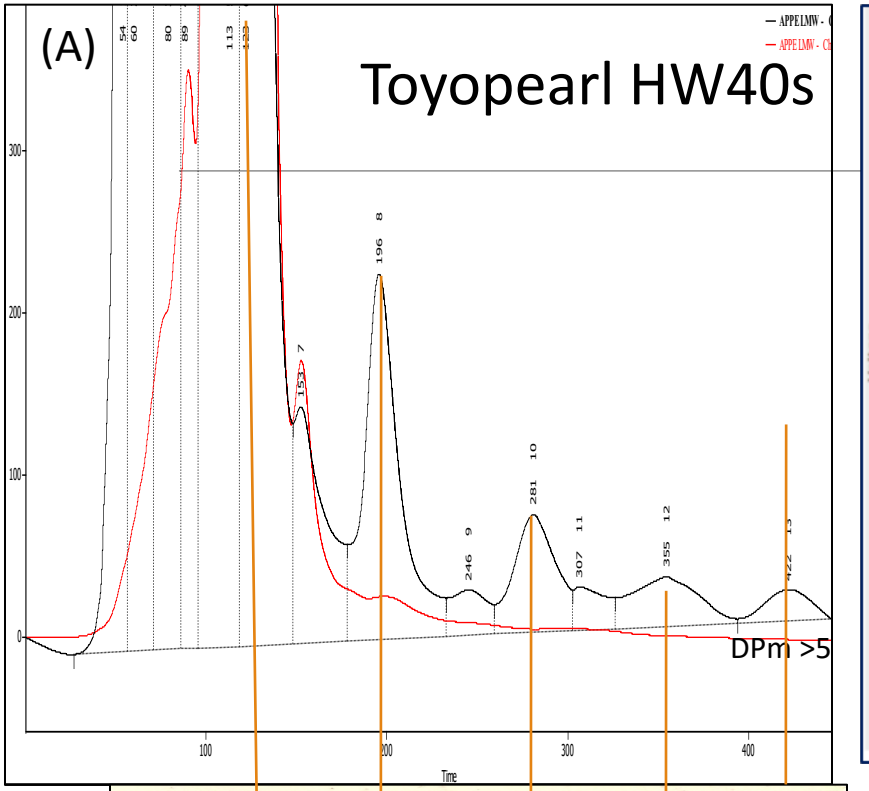
Inhibition of Adherence

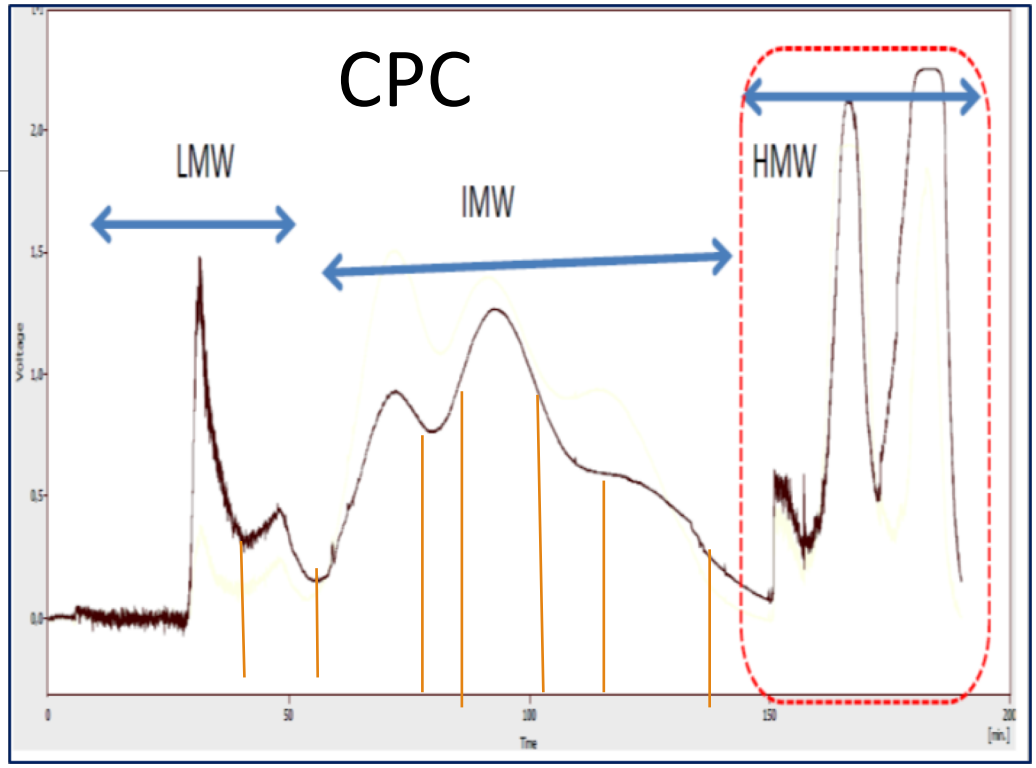


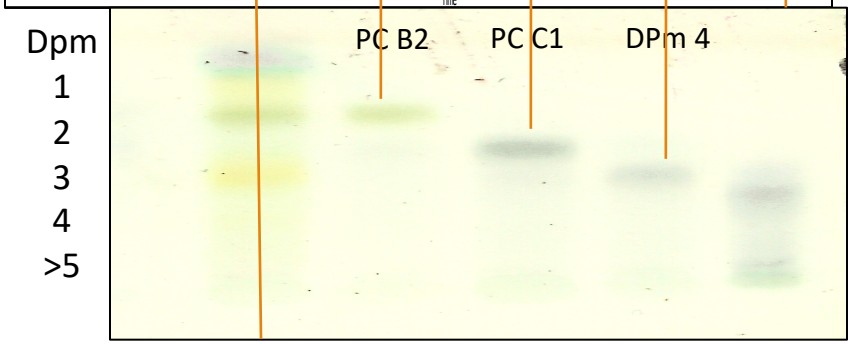
Inhibition of in vivo infection

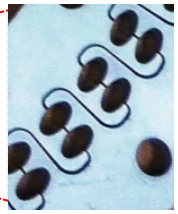


Fractionation of Apple Peel Procyanidins (APPE)







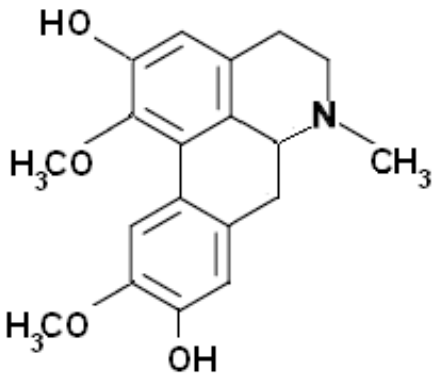


SCPC-250 (Armen)

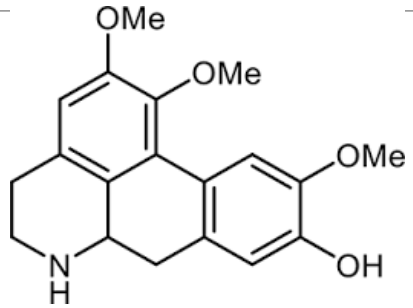



Application 1: Peumus boldus Mol.

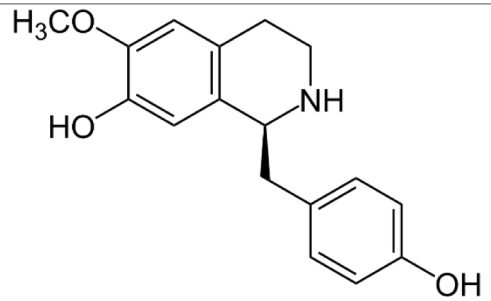
Boldo leaf Chemistry: Alkaloids



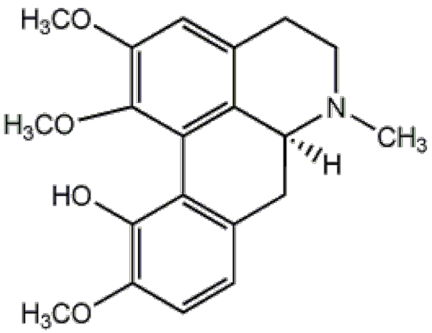
Boldine



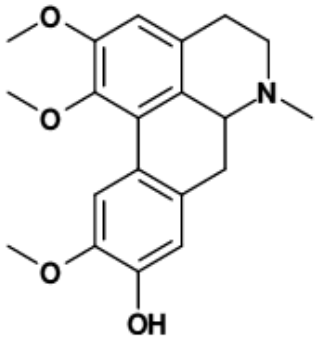
Laurotetanine



Coclaurine



Isocorydine

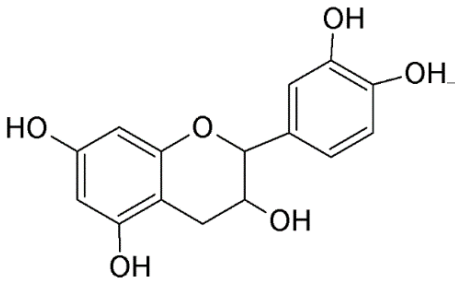


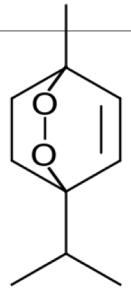
N-methyllaurotetanine

Gonzalo Fuentes-Barros, Sebastián Castro-Saavedra, Leonel Liberona, Williams Acevedo-Fuentes, Cristian Tirapegui, César Mattar, Bruce K. Cassels, Variation of the alkaloid content of Peumus boldus (boldo), Fitoterapia, Volume 127, 2018, Pages 179-185,

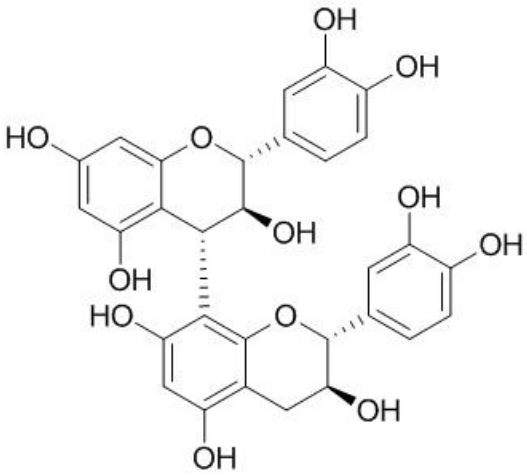
Boldo leaf Chemistry: Phenolics and Volatiles

Catechin

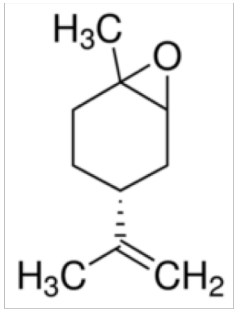




Procyanidin B3



Ascaridole



Limonene dioxide

Gotteland M., Espinoza J., Cassels B., Speisky H. Efecto del extracto de boldo en el transito intestinal oro-cecal en voluntarios sanos. Rev. Med Chil 1995, 123: 955-960.

Jiménez I., Speisky H. Biological disposition of boldine: in vitro and in vivo studies. Phytoterapy Research 2000, 14: 254-260.

Kubinova R, Machala M, Minksova K, Neca J, Suchy V Chemoprotective activity of boldine: modulation of drug-metabolizing enzymes. Pharmazie 2001, 56: 242-243.

Rodrigues E., Melo A.M., Xavier H. Toxicological evaluation of the hidro-alcohol extract of the dry leaves of Peumus boldus and boldine in rats. Phytoterapy Research 2000, 14: 99-102.

Speisky H., Cassels B. K. Boldo and boldine: an emerging case of natural drug development. Pharmacol Res. 1994, 29: 1-12.

Vila R., Valenzuela L, Bello H., Cañigueral S., Montes M., Adzet T. Composition and antimicrobial activity of the essential oil of Peumus boldus leaves. Planta Med 1999, 65: 178-179.

PHYTOTHERAPY RESEARCH
Phytother. Res. (2014)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.5176

Catechin-based Procyanidins from *Peumus boldus* Mol. Aqueous Extract Inhibit *Helicobacter pylori* Urease and Adherence to Adenocarcinoma Gastric Cells

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[1]Laboratory of Pharmacognosy, Department of Pharmacy, Faculty of Pharmacy, University of Concepción, Concepción, Chile
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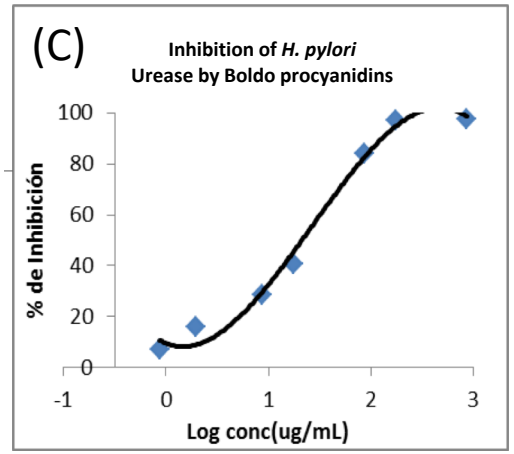
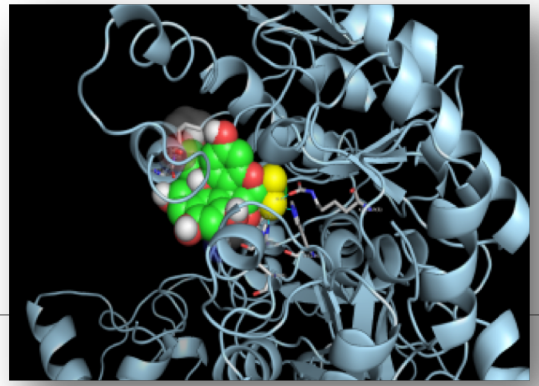
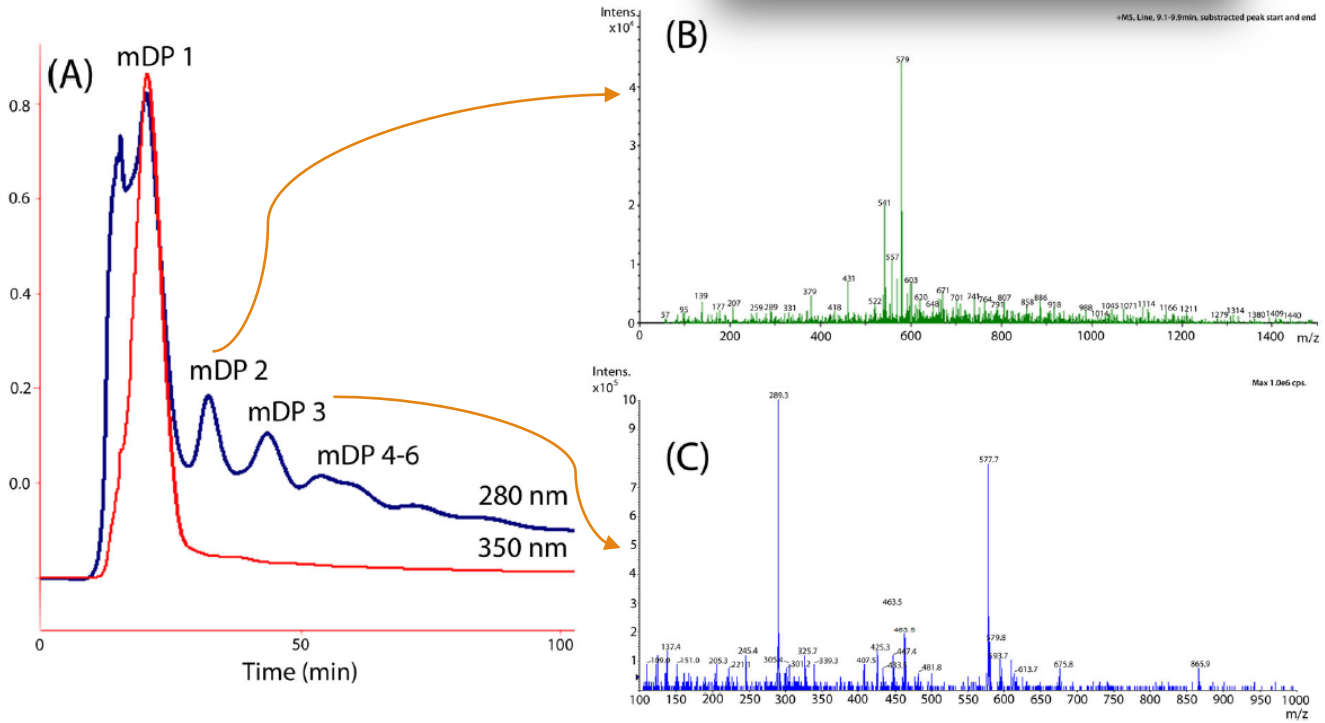


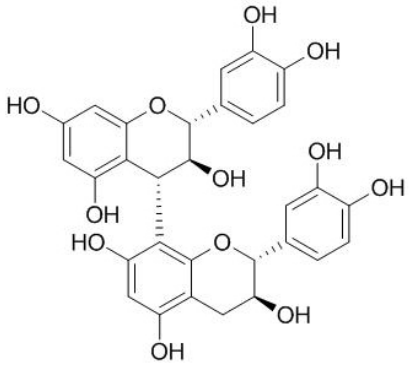
Table 1. Yield, total polyphenol content, mean degree of polymerization, minimal inhibitory concentration, and urease inhibitory activities of boldo extracts and fractions obtained by column chromatography on Sephadex LH-20

Fraction	Yield (g%)	TPC (g%)	mDP	MIC (μg/mL)	IC_{50} (μg GAE/mL)
BAE	10.1	21.90±0.10	4.10	>1500	23.4
F1	0.40 (2.00)	8.37±0.11	1.3	>1500	>400
F2	0.53 (2.65)	58.3±0.86	2.1	>1500	144.4
F3	0.21 (1.05)	48.5±0.13	3.3	>1500	58.3
F4	0.08 (0.40)	48.9±0.07	6.2	>1500	48.3
F5	0.51 (2.55)	49.9±0.37	7.8	>1500	15.9
Boldine	—	—	—	>1500	>400
Ascaridole	—	—	—	>1500	>400
Amoxicillin	—	—	—	0.08	>400
AHA	—	—	—	398	5.60

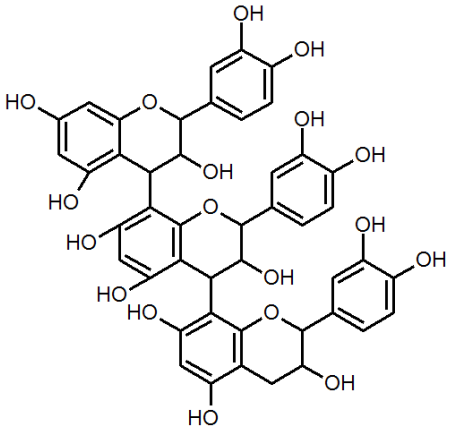
BAE, boldo aqueous extract; AHA, acetohydroxamic acid in μg/mL; MIC, minimal inhibitory concentration; TPC, total polyphenol content.







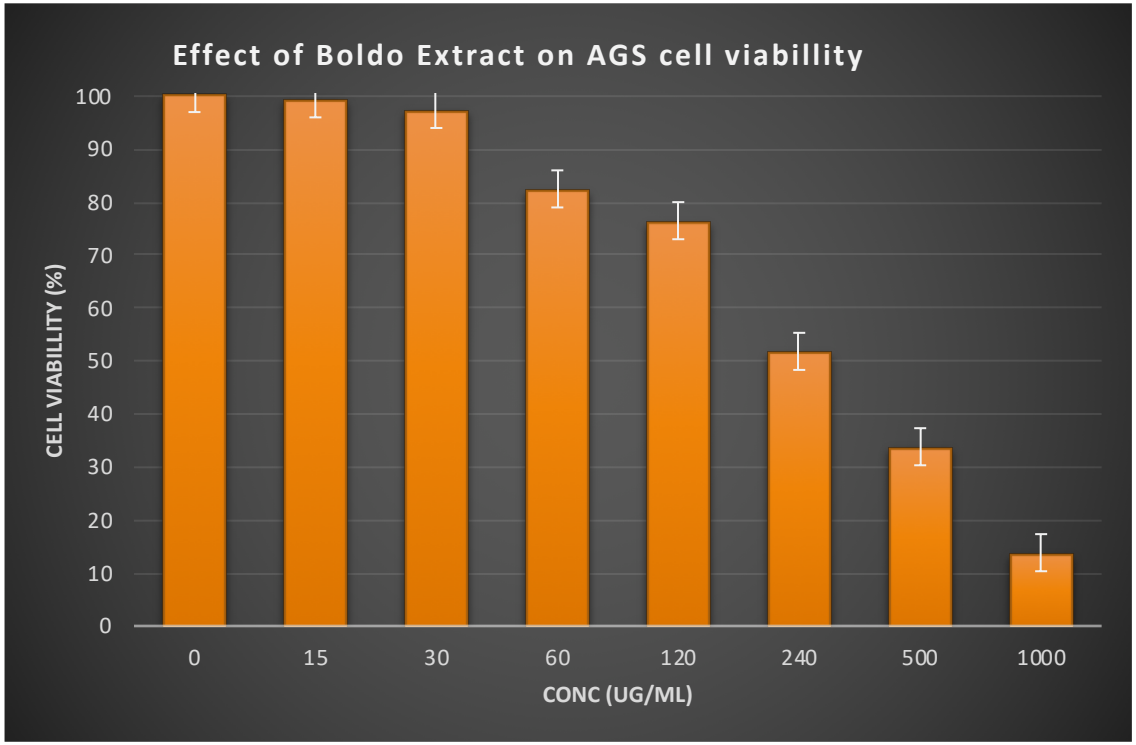
Procyanidin B3



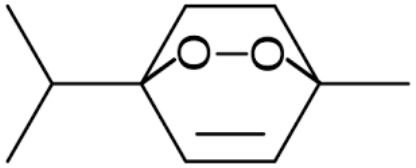
Procyanidin C2

Pastene E, Parada V, Avello M, Ruiz A, García A. Catechin-based procyanidins from Peumus boldus Mol. aqueous extract inhibit Helicobacter pylori urease and adherence to gastric adenocarcinoma gastric cells. ***Phytother. Res***. 2014: 28(11): 1637-1645.

Effect of Boldo extract on AGS cell viabillity

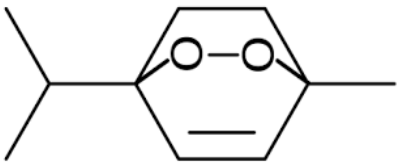


Ascaridol

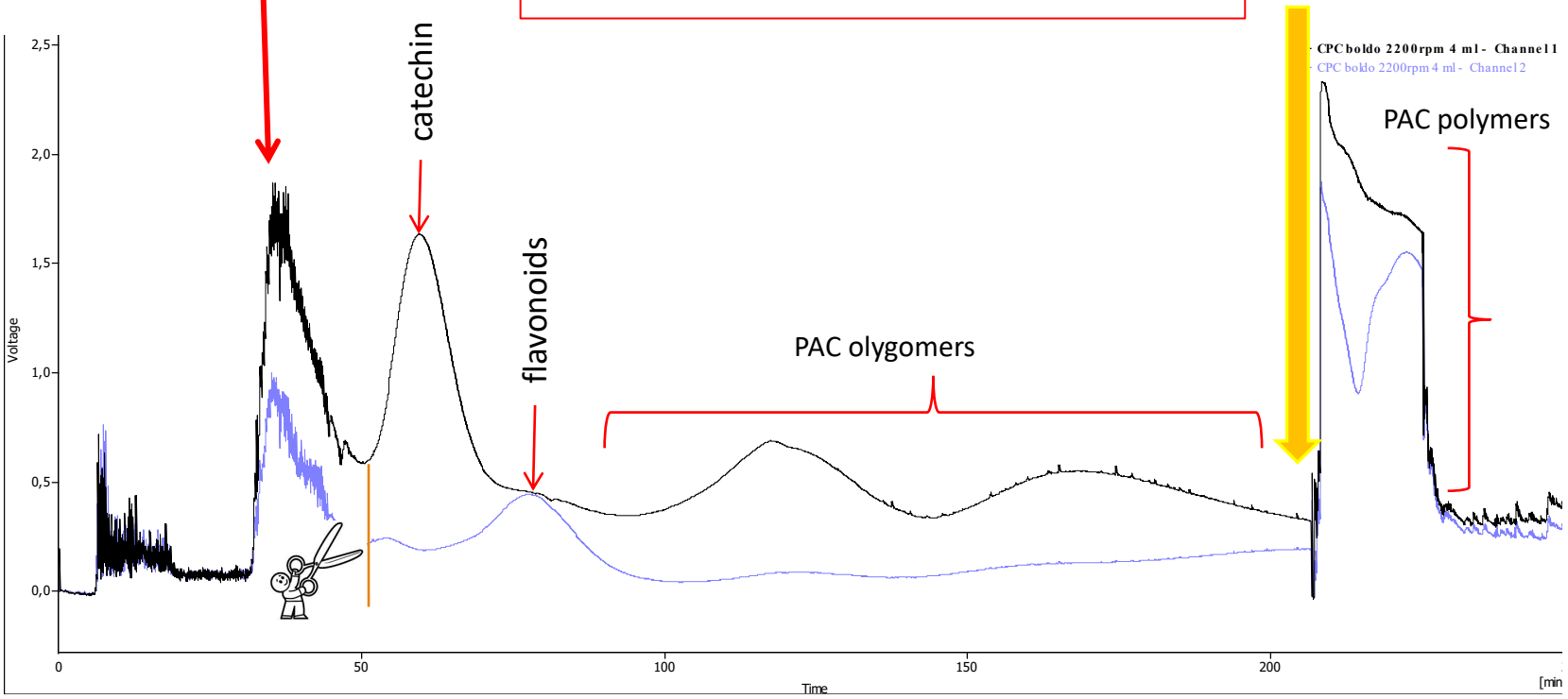


Chemical substraction of ascaridol from Peumus boldus (Boldo) using CPC

ascaridol



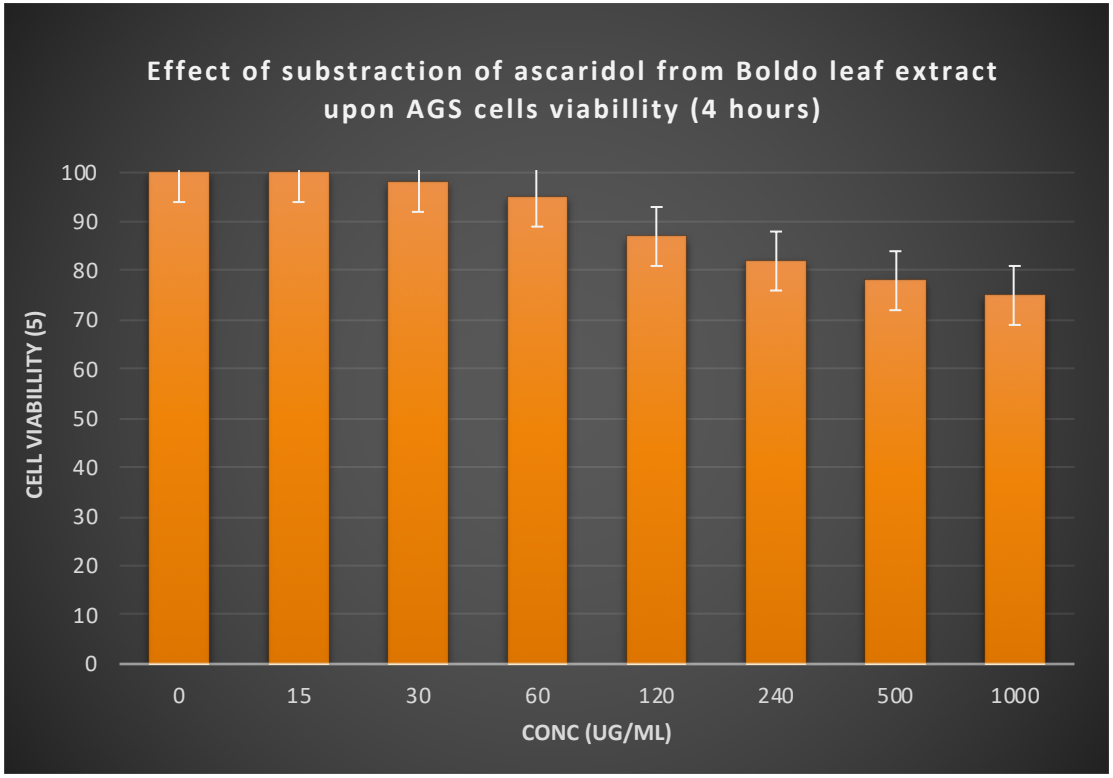
Injection: 3 g en 10 mL de50% FA/FB
Flow: 4 ml/min
Rotation: 2200 rpm (840 psi)
Detection: 280/350 nm
System: **hexano-EA-MeOH-agua (0.1:5:0.1:5, v/v/v/v)**
(Kohler and Winterhalter, 2005)
Retention: 72%



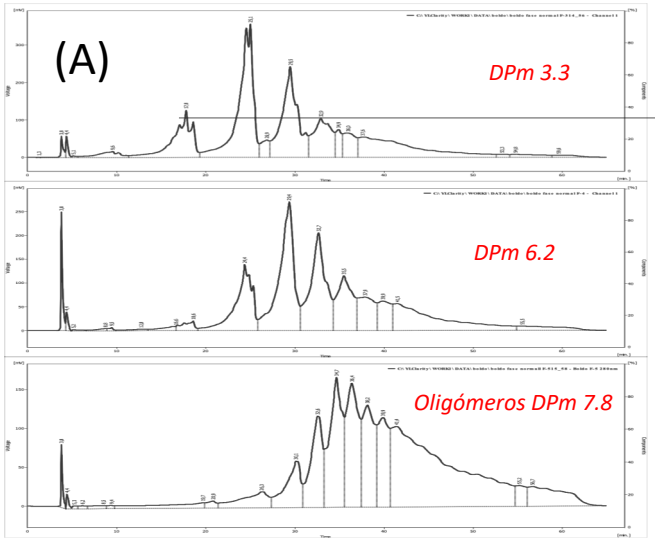
Ascendent

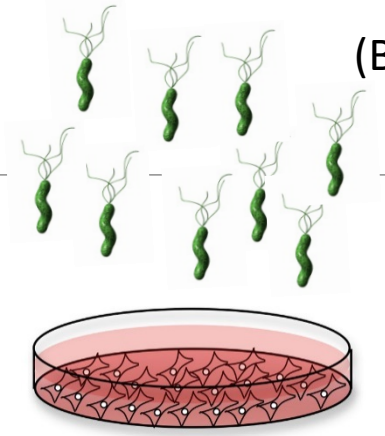
Extrusion

Effect of substraction of ascaridol from Boldo leaf extract upon cell viability



Catechin-based Proanthocyanidins from *Peumus boldus inhibit H. pilory adherence to AGS cells*





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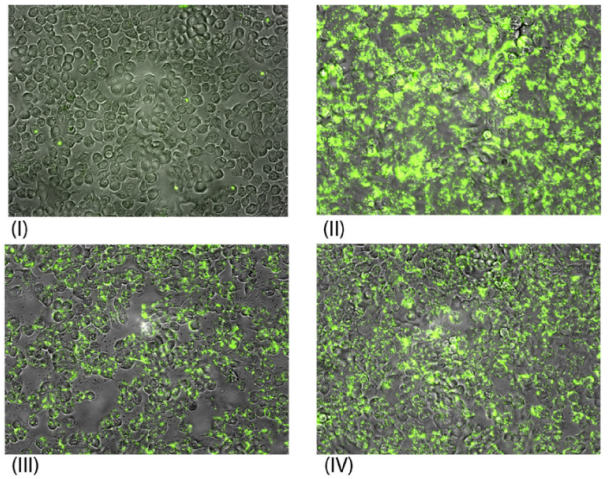
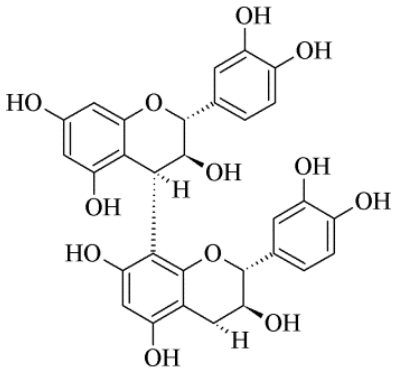
Boldo Extract

(B)



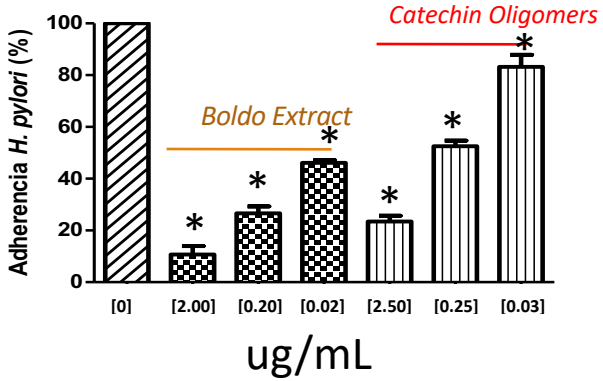
(D)



350 nm

Adhesion of *H. pylori* to AGS cells treated with Boldo extract (without ascaridol)

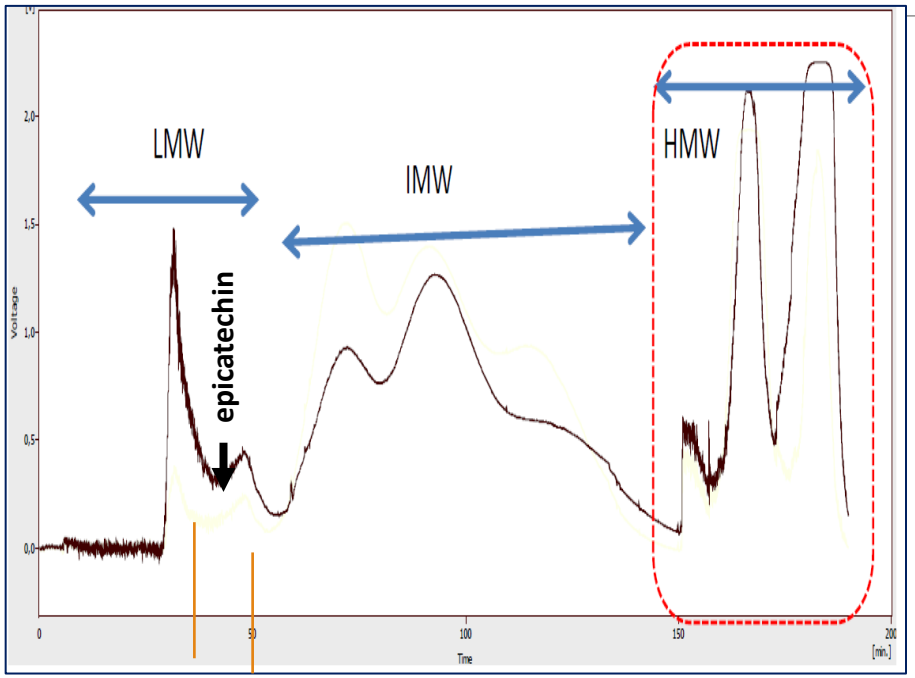
(E)

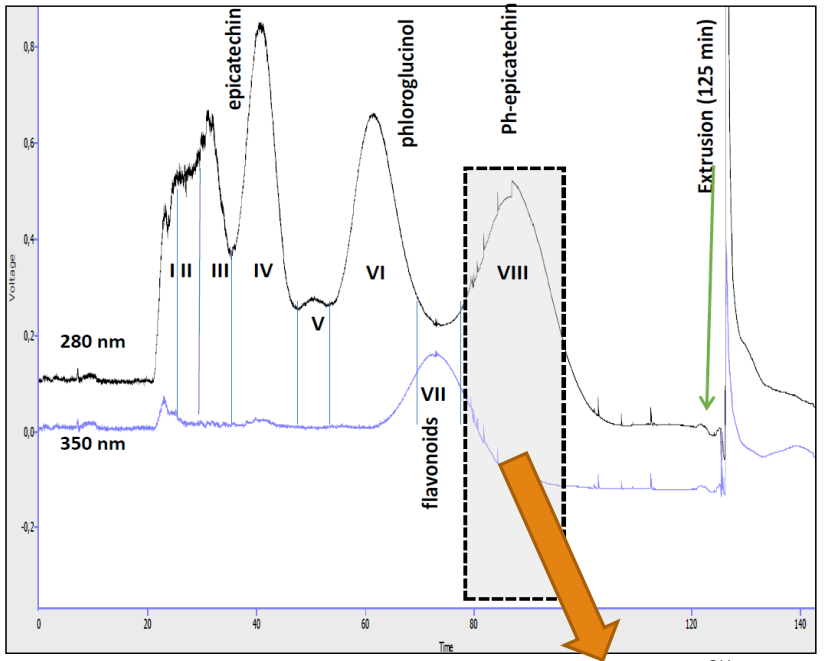


n = 5, *p<0.05

Pastene E, Parada V, Avello M, Ruiz A, García A. Catechin-based procyanidins from Peumus boldus Mol. aqueous extract inhibit Helicobacter pylori urease and adherence to gastric adenocarcinoma gastric cells. ***Phytother. Res***. 2014: 28(11): 1637-1645.

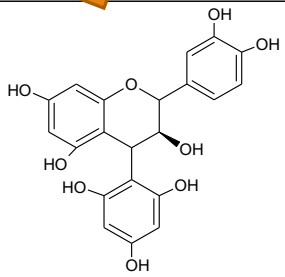
One-Step purification of epicatechin-phloroglucinol adduct by CPC (From apple peel PACs)



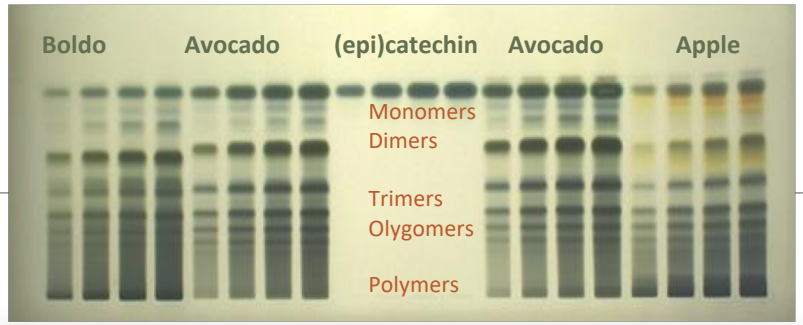


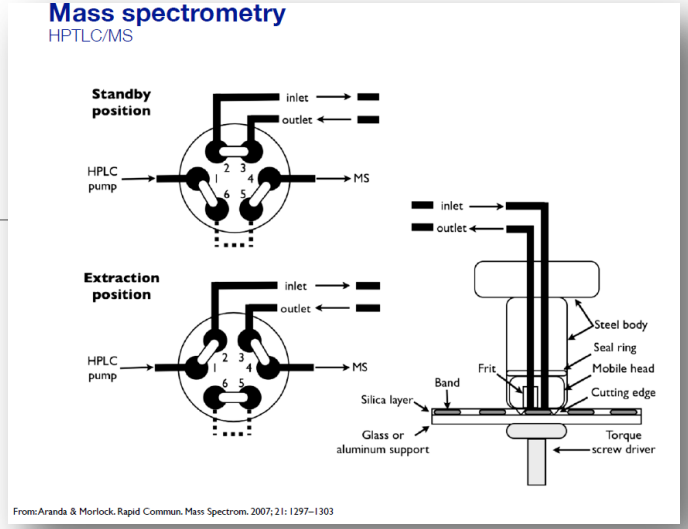
Kürbitz, C. , Heise, D. , Redmer, T. , Goumas, F. , Arlt, A. , Lemke, J. , Rimbach, G. , Kalthoff, H. and Trauzold, A. (2011). Cancer Science, 102: 728-734.

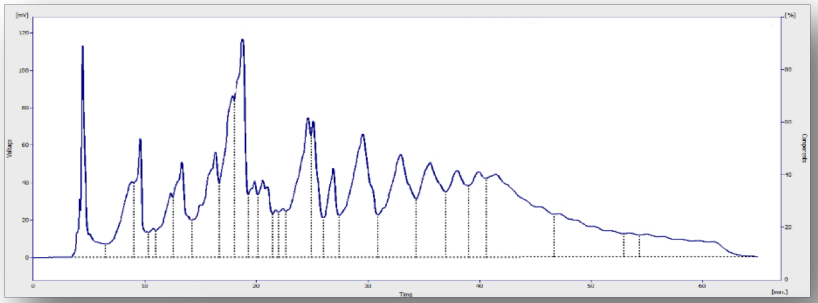
System = Arizona C
Total time = 150 min
Rotation speed = 1800
Flow rate = 8 mL/min
Pressure = 860 psi
SP Retention = 72%

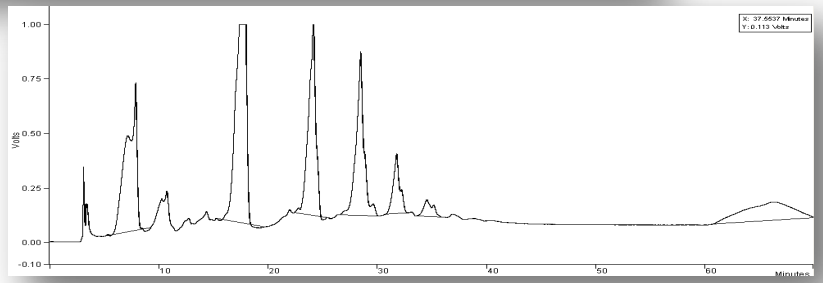


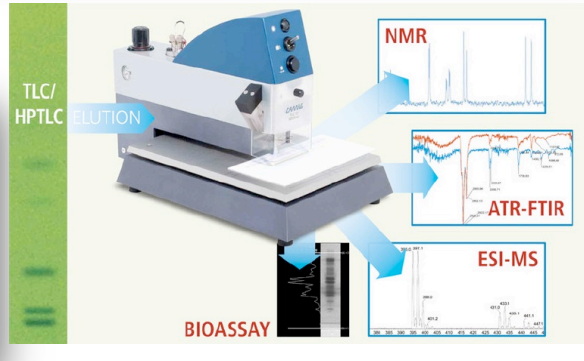
Procyanidins from Boldo leaves and Apple and Avocado Peels

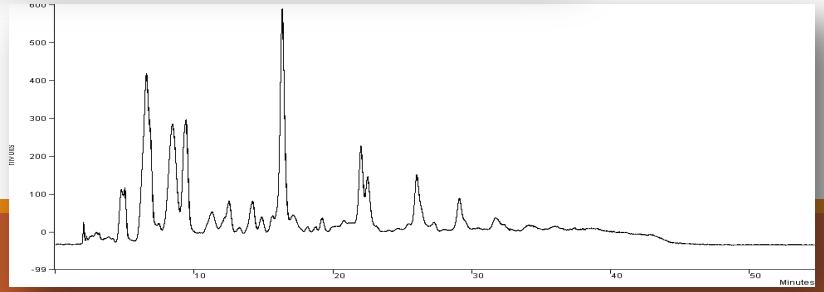












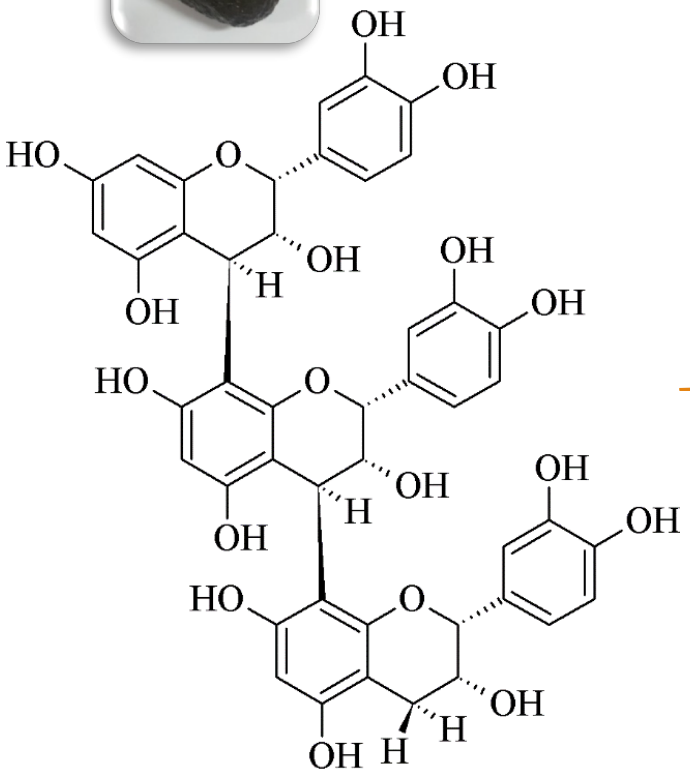
Analysis of Avocado peel and Apple peel polyphenols

Compounds	Apple peel Extract (%)[a]	Avocado peel Extract (%)[a]
Flavonoids		
Hyperoside	22.12 ± 1.23	ND
Isoquercitrin	6.58 ± 0.98	ND
Quercitrin	8.23 ± 1.02	ND
Quercetin	0.09 ± 0.01	1.23 ± 0.02
Quercetin-O-pentosides(≠ of rutin) [b]	19.21 ± 2.66	ND
Rutin	4.01 ± 0.12	1.09 ± 0.04
Apigenin	ND	12.80 ± 0.12
Kaempferol	ND	ND
Kaempferol derivatives [c]	ND	ND
Myricetin	ND	ND
Myricetin hexosides [d]	ND	ND
Isorhamnetin	ND	ND
Isoramnetin derivatives [e]	ND	ND
∑ Flavonoids	60.24 ± 1.00	15.12 ± 0.06
Flavan-3-ol monomers		
Epicatechin	6.08 ± 0.98	12.09 ± 0.38
Catechin	0.03 ± 0.01	1.92 ± 0.01
Epicatechin gallate	ND	ND
Catechin gallate	ND	ND
∑ flavan-3-ol monomers	6.11 ± 0.50	14.01 ± 0.20
∑ total procyanidins [f]	22.01 ± 2.88	29.08 ± 1.01
Dihydrochalcones		
Phloretin-2´-glucoside	10.34 ± 3.88	ND
Phloretin-2´-xyloglucoside	0.22 ± 0.11	ND
∑ dihydrochalcones	10.56 ± 2.00	ND

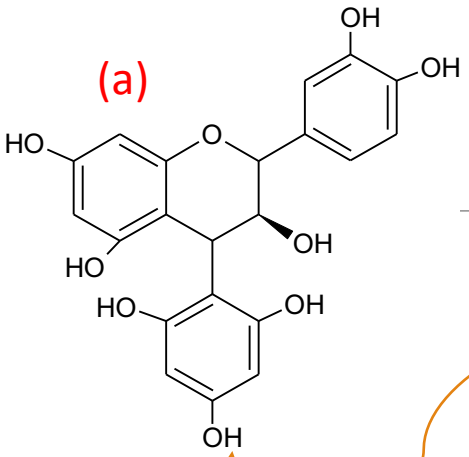
Anthocyanins		
Cyanidin -O-hexosides [g]	ND	ND
Cyanidin-O-arabinoside [g]	ND	ND
Cyanidin-3-O-glucoside [g]	ND	3.00 ± 0.03
Peonidin-O-hexosides [h]	ND	ND
Peonidin-O-arabinoside [h]	ND	ND
Malvidin -O-hexosides [i]	ND	ND
Delphinidin-O-hexosides [j]	ND	ND
Petunidin-O-hexosides [k]	ND	ND
∑ total anthocyanins	ND	3.00 ± 0.03
Phenolic acids		
Gallic acid	ND	ND
Syringic acid	ND	ND
Vanillic acid	ND	3.16 ± 0.01
Chlorogenic acid	1.02 ± 0.05	1.00 ± 0.02
Caffeic acid	0.06 ± 0.01	8.01 ± 0.01
Ellagic acid	ND	ND
Sinapic acid	ND	0.25 ± 0.02
Ferulic acid	ND	6.54 ± 0.02
Anisic acid	ND	2.28 ± 0.02
p-coumaric acid	ND	ND
Cinnamic acid	ND	ND
5-caffeoyl quinic acid	ND	ND
4-caffeoyl quinic acid	ND	ND
Protocatechuic acid	ND	0.98 ± 0.09
p-hydroxybenzoic acid	ND	9.92 ± 0.08
m- hydroxybenzoic acid	ND	6.65 ± 0.10
Caffeoyl glucoside	ND	ND
Feruloyl glucoside	ND	ND
Coumaroyl glucoside	ND	ND
∑ phenolic acids	1.08 ± 0.03	38.79 ± 0.04
Total	100 ± 1.28	100 ± 0.27

Ximena Wong, Catalina Carrasco-Pozo, Elizabeth Escobar, Paola Navarrete, François Blachier, Mireille Andriamihaja, Annaig Lan, Daniel Tomé, María José Cires, Edgar Pastene, and Martin Gotteland *Journal of Agricultural and Food Chemistry* **2016** *64* (18), 3574-3583

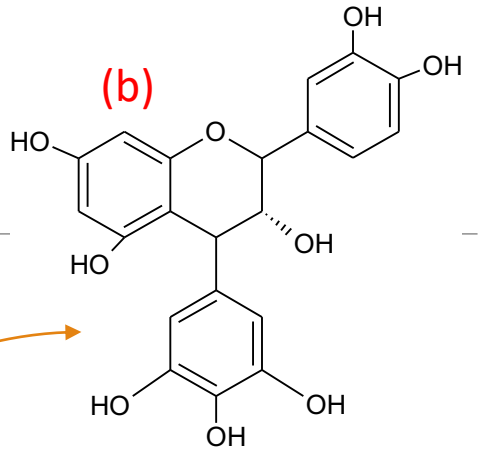
Application : Separation by CPC of flavan-3-ol adducts prepared with different nucleophiles



(a)



(b)



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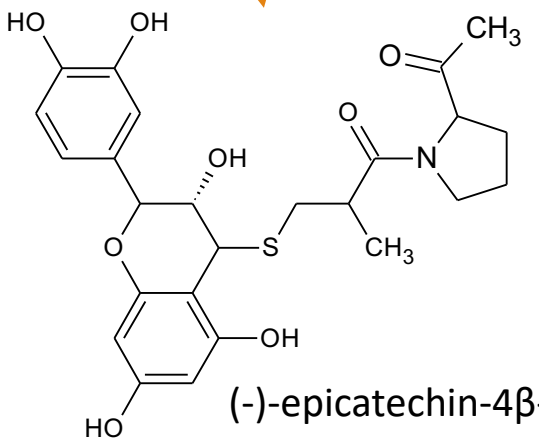
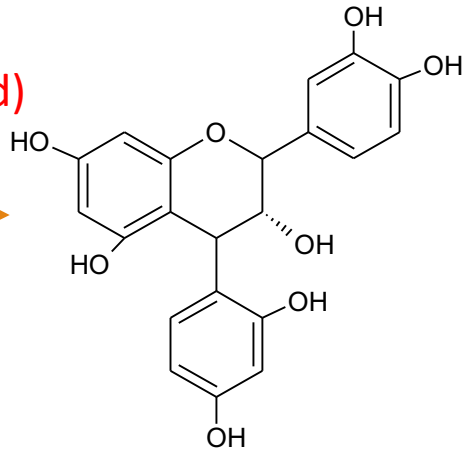


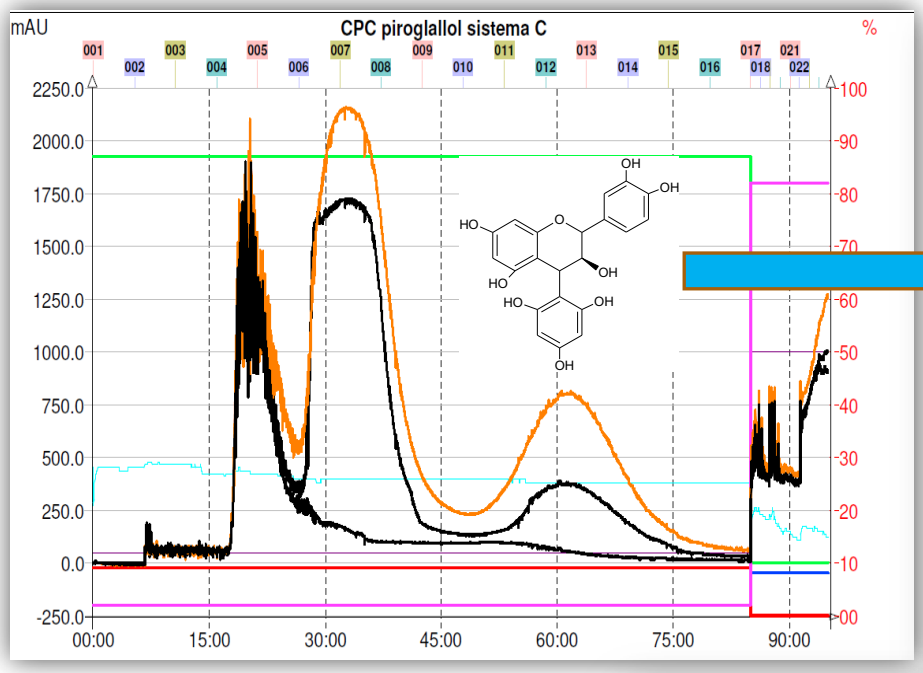
(d)



(-)-epicatechin-4β- S-captopril methyl ester

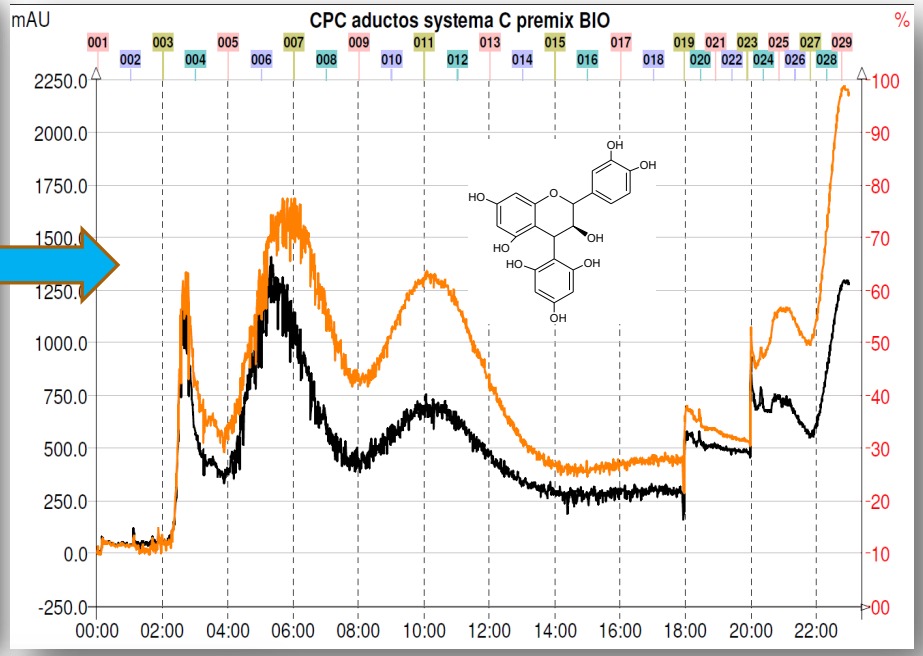
One-Step purification of epicatechin-phloroglucinol adduct by CPC (From avocado peel PACs)

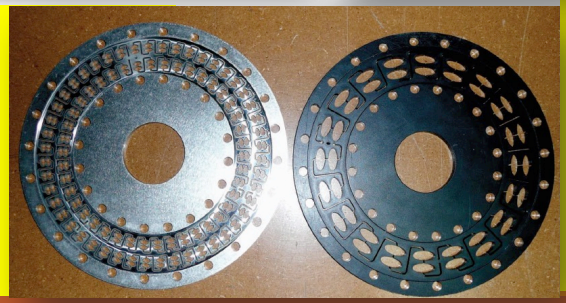
SCPC-L (250 mL)



System = Arizona C
Total time = 95 min
Rotation speed = 1800
Flow rate = 6 mL/min
Pressure = 1004 psi
SP Retention = 68%

SCPC-Bio-Extractor (250 mL)

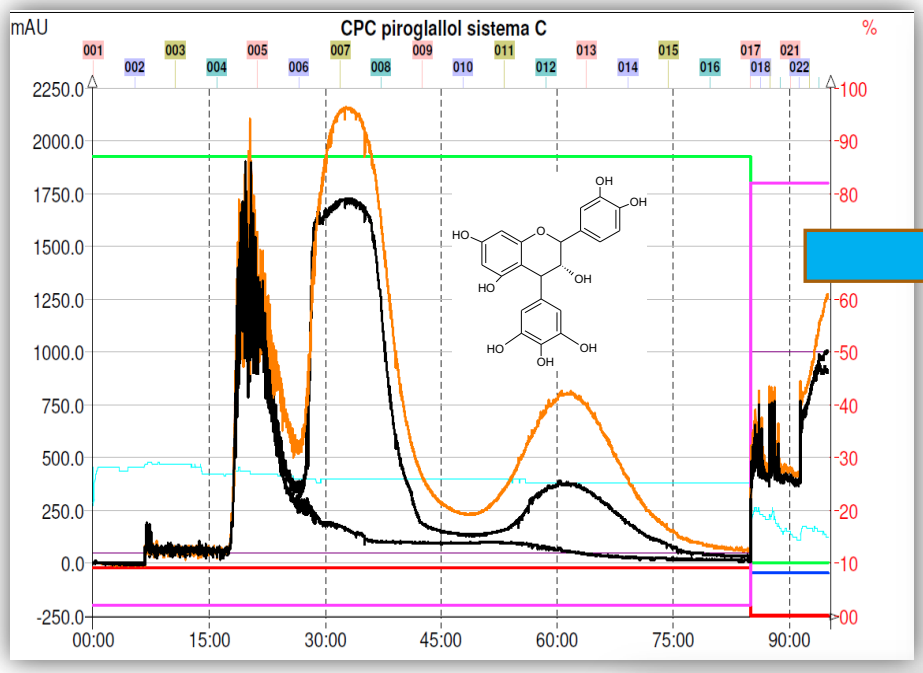




System = Arizona C
Total time = 25 min
Rotation speed = 2200
Flow rate = 15 mL/min
Pressure = 482 psi
SP Retention = 84 %

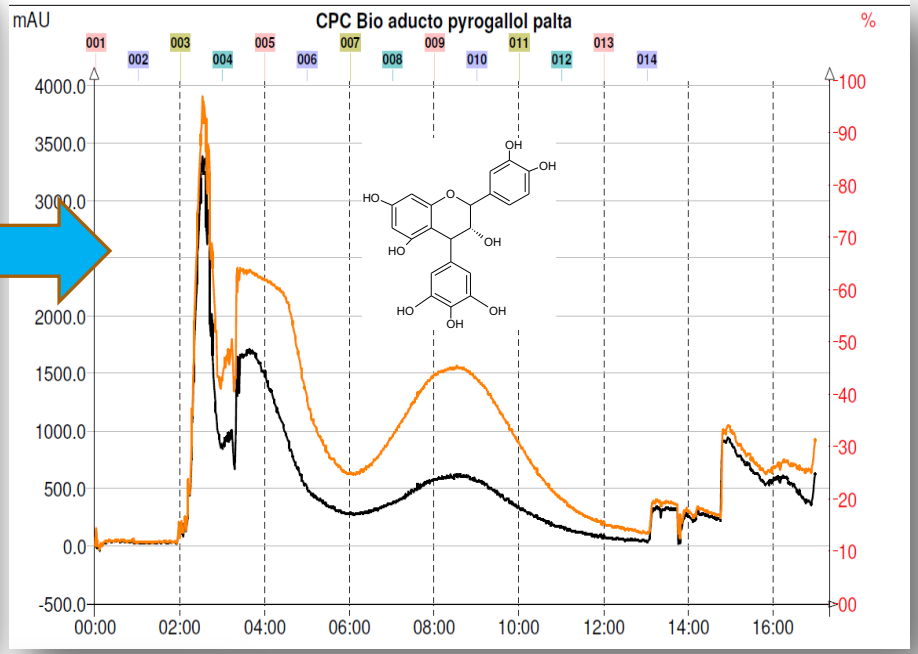
One-Step purification of epicatechin-pyrogallol adduct by CPC (From avocado peel PACs)

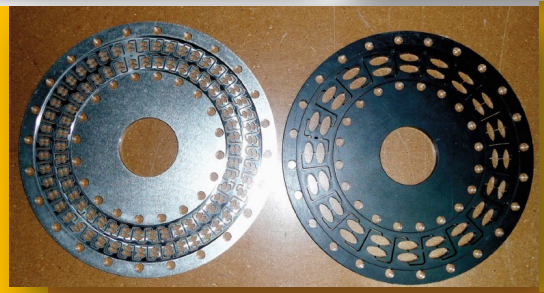
SPOT-CPC (250 mL)



System = Arizona C
Total time = 95 min
Rotation speed = 1800
Flow rate = 6 mL/min
Pressure = 1004 psi
SP Retention = 68%

SPOT-CPC-Bio Extractor

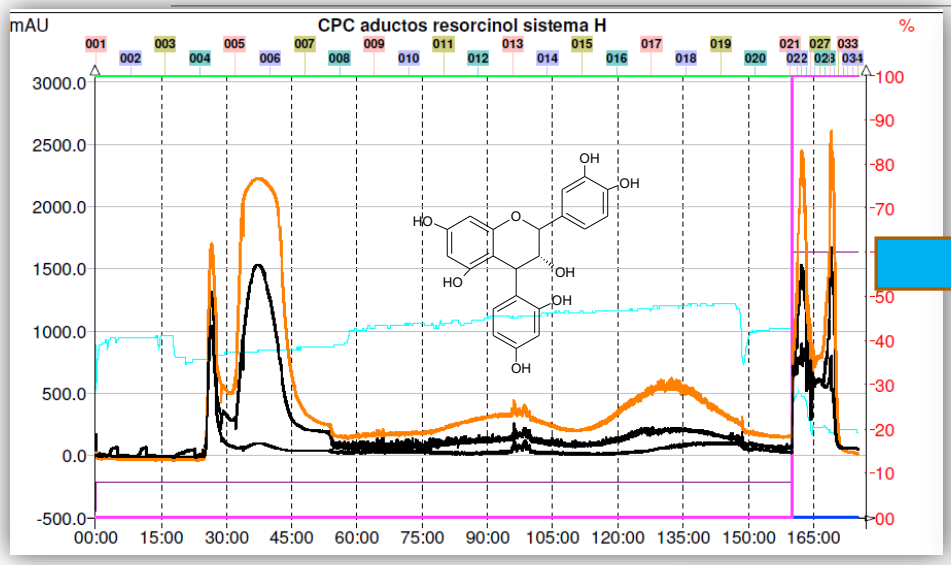




System = Arizona C
Total time = 20 min
Rotation speed = 2200
Flow rate = 15 mL/min
Pressure = 482 psi
SP Retention = 84 %

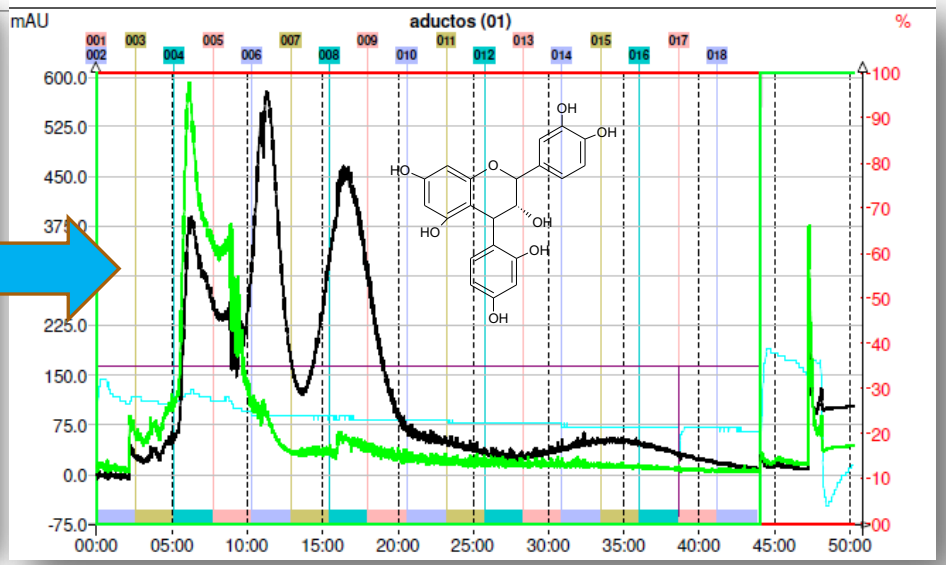
One-Step purification of epicatechin-resorcinol adduct by CPC (From avocado PACs)

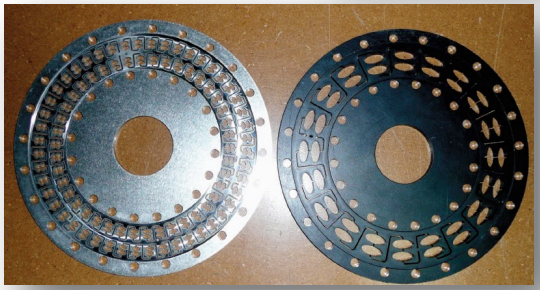
SPOT-CPC (250 mL)



System = Arizona H
Total time = 170 min
Rotation speed = 1800
Flow rate = 6 mL/min
Pressure = 889 psi
SP Retention = 70 %

SPOT-CPC-Bio Extractor





System = Arizona H
Total time = 50 min
Rotation speed = 2200
Flow rate = 35 mL/min
Pressure = 482 psi
SP Retention = 82 %

Anti-*Helicobacter pylori* activities of avocado peel PACs and adducts obtained by nucleophilic attack with resorcinol and pyrogallol

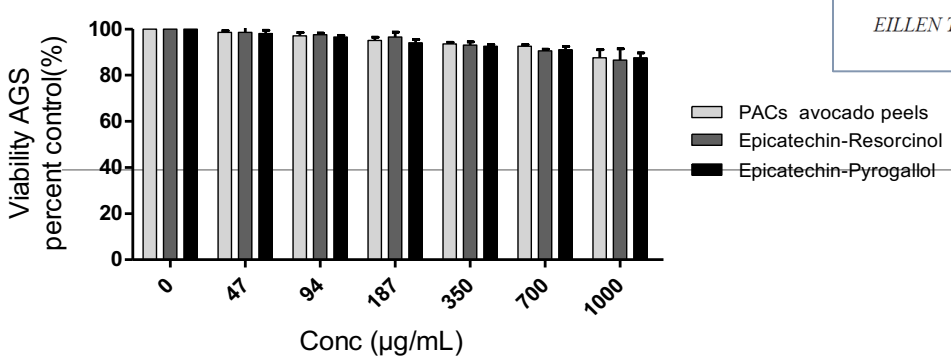
Sample*	*H. pylori* ATCC 43504 Halo (mm)	*H. pylori* ATCC 43504 MIC_{90} (µg/mL)
PACs avocado peels	R	>1500
Epicatechin-resorcinol	12	965.4
Epicatechin-pyrogallol	11	970.1
Epicatechin	R	>1500
Procyanidin C2	R	>1500
Amoxicilin[a]	62	0.04

J. Chil. Chem. Soc., 63, Nº 4 (2018)

ONE-STEP PURIFICATION OF TWO SEMI-SYNTHETIC EPICATECHIN ADDUCTS PREPARED FROM AVOCADO PEELS PROCYANIDINS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY AND EVALUATION OF THEIR ANTI-INFLAMMATORY EFFECTS ON ADENOCARCINOMA GASTRIC CELLS INFECTED WITH *Helicobacter pylori*

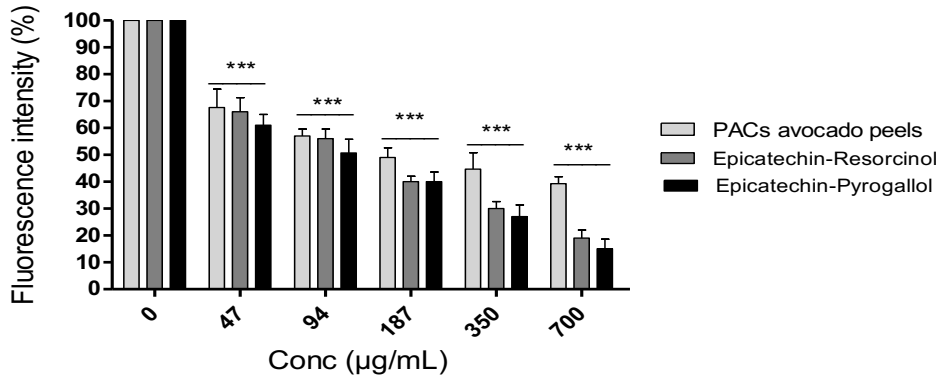
*EILLEN TORRES[1,6], APOLINARIA GARCÍA[1], MARIO ARANDA [2], VANIA SAÉZ [3], FELIPE ZÚÑIGA [4], JULIO ALARCÓN [5], MARCIA AVELLO [6] AND EDGAR PASTENE [6]**

A



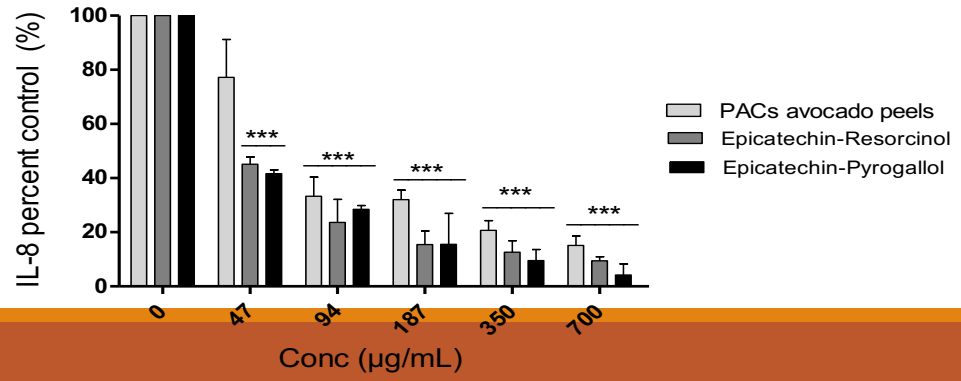
(A) Cytotoxicity of avocado PACs and adducts against AGS cell line

B



(B) Inhibitory effect of avocado PACs and adducts on bacterial adherence to AGS cells after pre-incubation followed by infection with *H. pylori* ATCC 43504

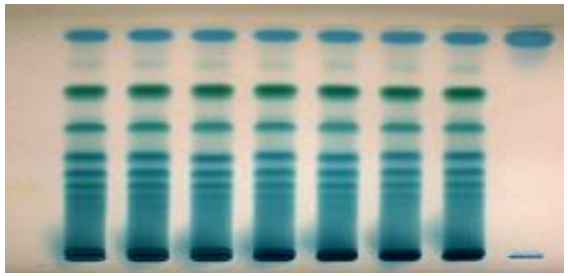
C

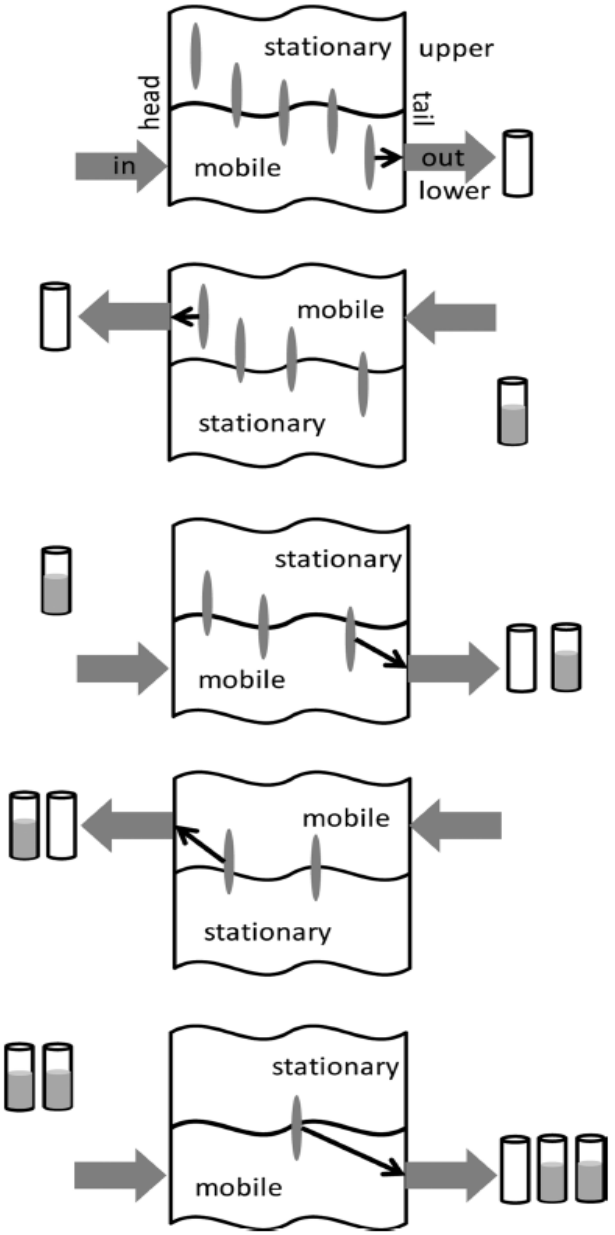


(C)Effect of avocado PACs and adducts on IL-8 release by AGS cells infected with *H. pylori* ATCC 43504

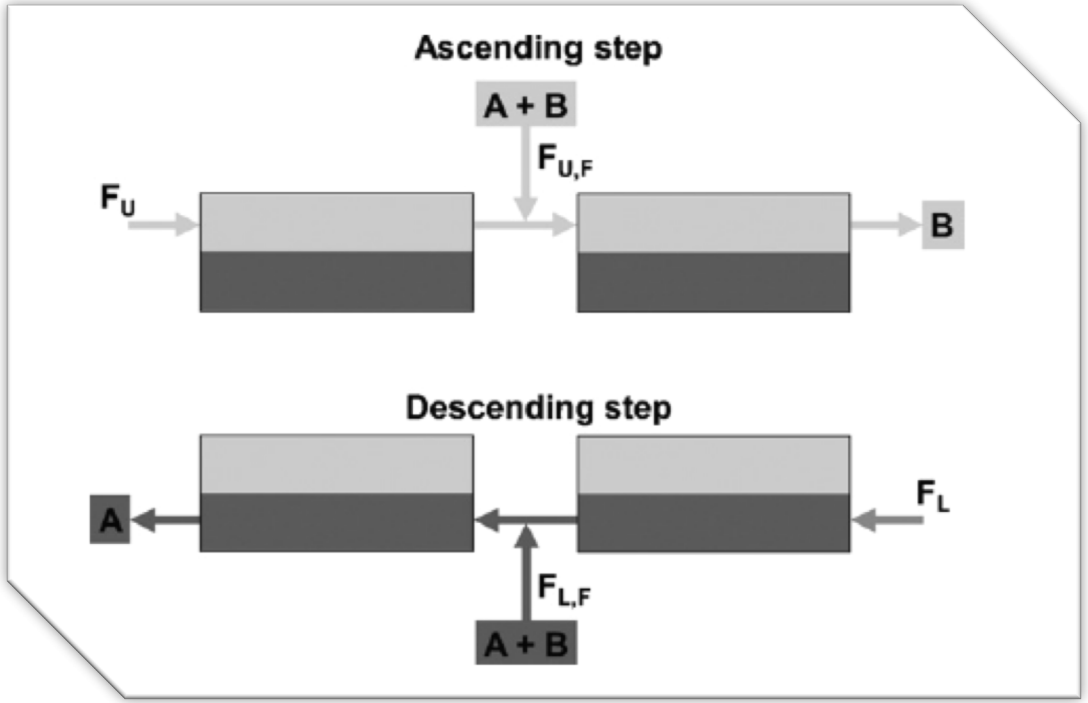
Fractionation of Proanthocyanidins from *Peumus boldus* leaves

Injection: 3 g in 10 mL of 50% UP/LP
Flow: 4 ml/min
Rotation: 2200 rpm (840 psi)
Detection: 280/350 nm
System: **hexane-EA-MeOH-W (0.1:5:0.1:5, v/v/v/v)**
(Köhler and Winterhalter, 2005)
Retention: 72%



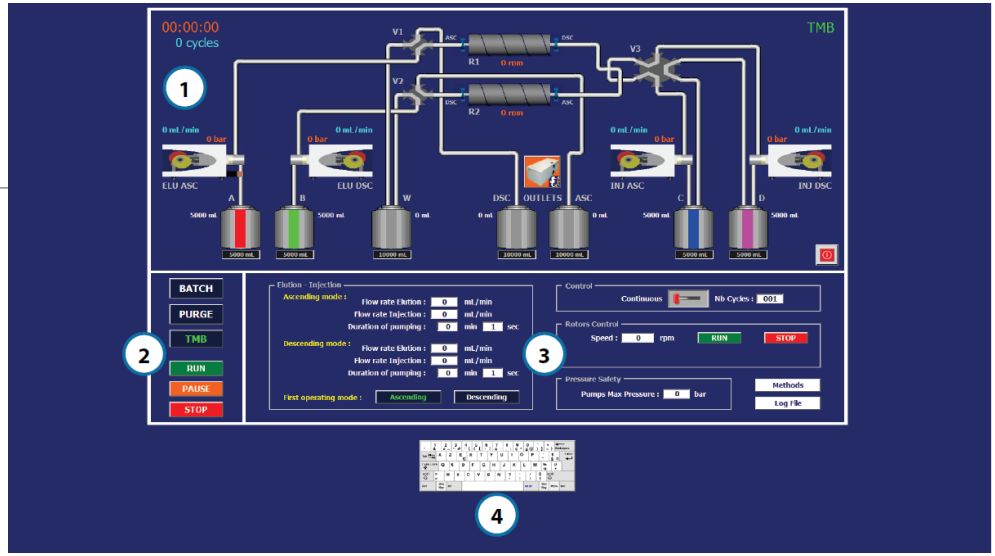


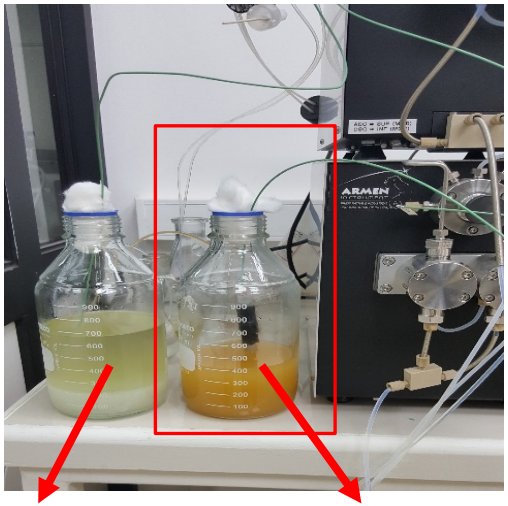
True Moving Bed Chromatography: Is an advance Methodology that combine elements from Dual-Mode CPC, Successive-CPC and Intermittent-CPC



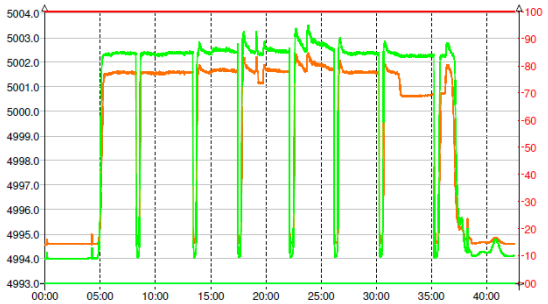
Friedsen et al., J. Nat Prod. 2015, 78: 1765-1796

Enrichment of Boldo extract using TMB system





Impurities **Enrichment**



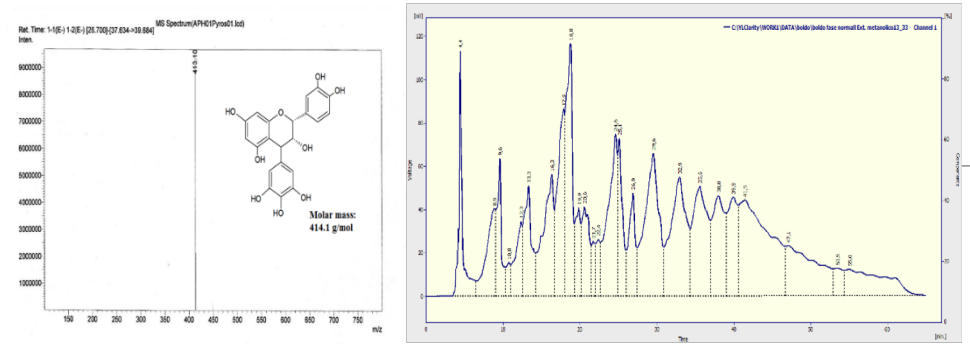
TMB-500 conditions	
Flow rate ASC	25 mL/min
Flow rate DSC	25 mL/min
Flow rate inj ASC	10 mL/min
Flow rate inj DSC	10 mL/min
Rotation speed	2400
Injection volume	Continuous
Sample	Crude extract: 12,5 mg/mL in 50/50 UP/LP

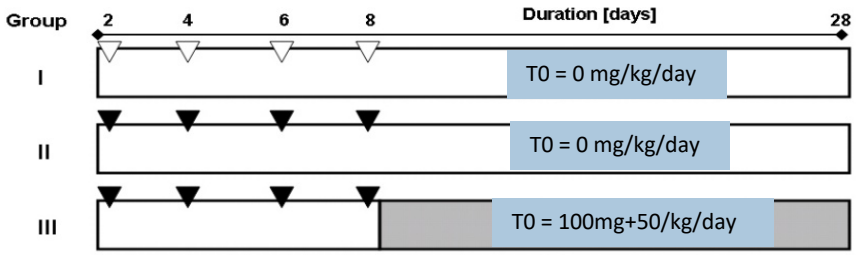
Analysis of Fraction A Impurities) by GC-MS

Compound	RI [a]	(%)	Identification [b]
a-thujene	930	0,32	RI, MS, S
1R-a -pineno	939	2,28	RI, MS, S
Camphene	952	0,10	RI, MS
b-pinene	979	0,42	RI, MS, S
b-myrcene	993	2,00	RI, MS, S
d-2-carene	1001	0,25	RI, MS
a-phellandrene	1002	0,21	RI, MS, S
Unknown	1003	1,43	---
a-Terpinene	1013	3,94	RI, MS, S
Limonene	1030	2,95	RI, MS, S
b-phellandrene	1031	4,42	RI, MS, S
Trans-b-ocymene	1036	12,87	RI, MS
1,8 cineol	1039	14,85	RI, MS, S
Cis-b-ocymene	1042	0,45	RI, MS
g-terpinene	1057	1,86	RI, MS, S
Terpinolene	1086	0,15	RI, MS, S
p-cymenene	1090	0,21	RI, MS
2-nonanone	1093	0,15	RI, MS, S
Trans-sabinene hydrate	1097	4,07	RI, MS
Dehydro-sabina ketone	1121	0,36	RI, MS
Trans-pinocarveol	1140	0,57	RI, MS
Camphor	1146	0,10	RI, MS, S
Terpinen-4-ol	1179	3,37	RI, MS, S
Cryptone	1186	0,68	RI, MS
a-terpineol	1187	2,14	RI, MS, S
Myrtenal	1193	0,31	RI, MS, S
Myrtenol	1194	0,20	RI, MS, S
Unknown	1202	0,11	---
Bornyl acetate	1265	0,64	RI, MS, S
Ascaridole	1273	24,37	RI, MS, S
b-elemene	1280	0,73	RI, MS
Safrole	1285	1,45	RI, MS
2-undecanone	1295	0,27	RI, MS
Methyleugenol	1372	0,46	RI, MS, S
b-Caryophyllene	1419	0,78	RI, MS, S
Unknown	1423	0,34	---
Unknown	1434	3,72	---
Aromadendrene	1440	0,43	RI, MS, S
a-caryophyllene	1454	0,73	RI, MS, S
Unknown	1470	2,78	--
Germacrene D	1480	0,45	RI, MS
Unknown	1512	0,67	---
d-cadinene	1524	0,24	RI, MS, S
Unknown	1550	0,39	---
8,9-dehydro-neoisolongifolene	1558	0,80	RI, MS

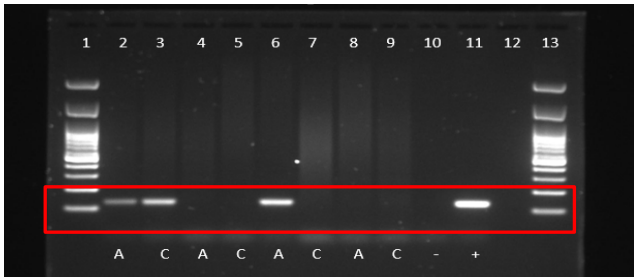
In vivo *anti-H. pylori* effect of CPC refined Boldo extract plus epicatechin-pyrogallol adduct

Pyrogallol-epicatechin adduct + TMB-enriched Boldo PACs



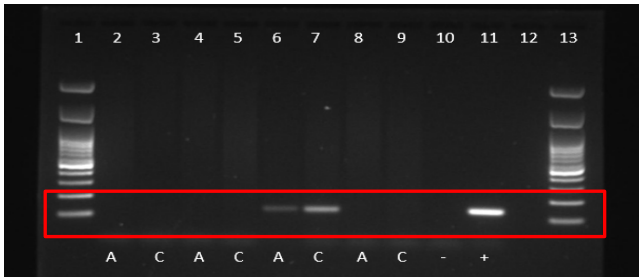


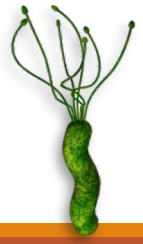
H. pylori infected Gerbils



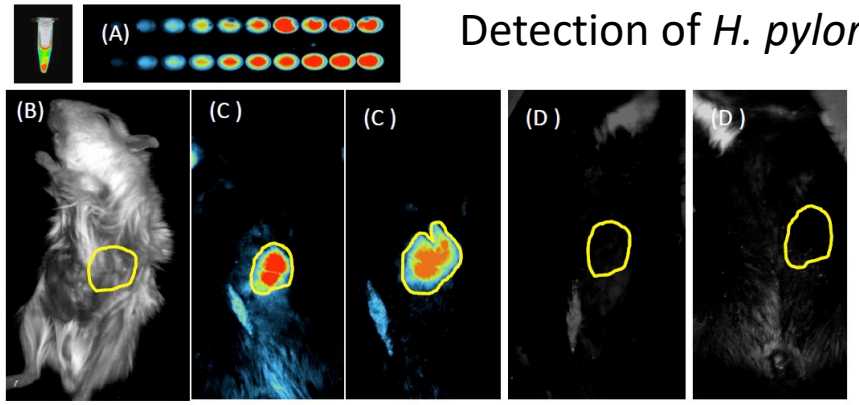
specie	Primers ARN 16S	Sequence 5'-3'	T° alineamiento (°C)	Amplicon
H. pylori	Hp1 Hp2	5' -CTGGAGAGACTAAGCCCTCC– 3' 5' -ATTACTGACGCTGATTGT GC – 3'	55	109 pb

Non-infected Gerbils





Molecular Imaging Detection of *H. pylori*



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Romina Carvajal (MgSc): I+D Antimicrobial-Antibiofilm natural and semi-synthetic molecules.

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