POTENTIAL BIOLOGICAL ACTIVITY OF CROATIAN TRADITIONAL APPLE CULTIVAR BY-PRODUCTS

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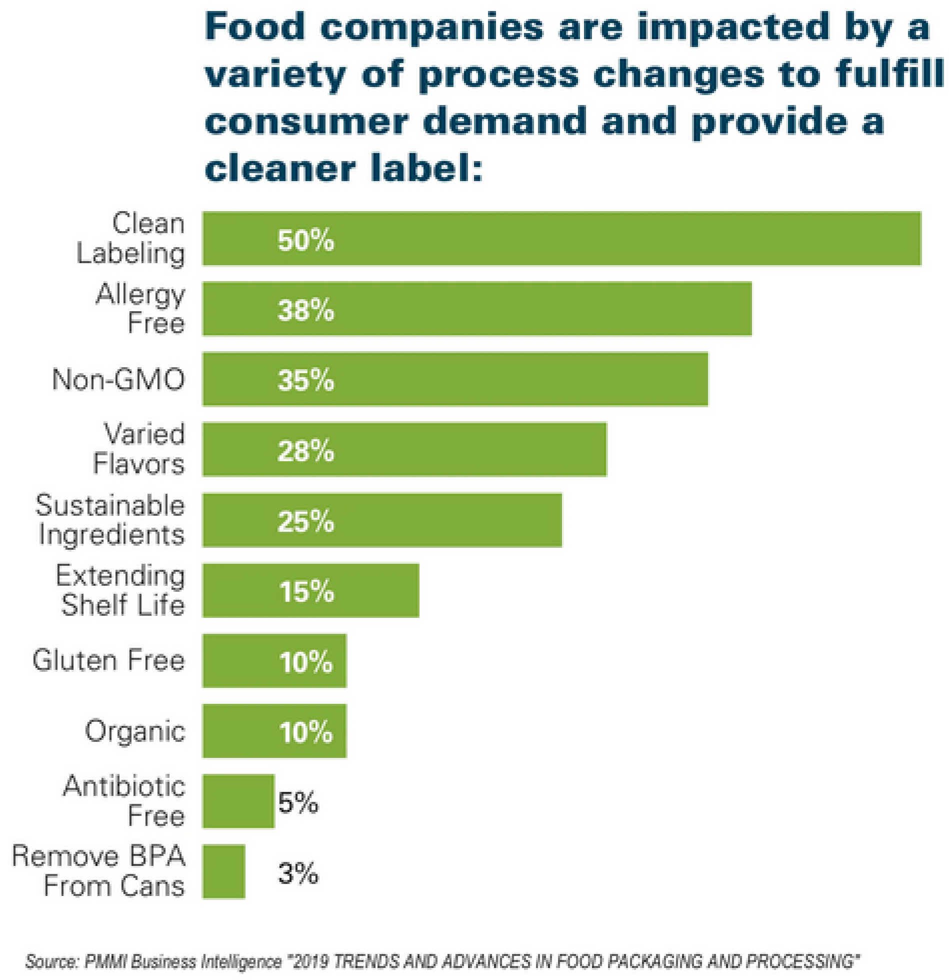
Naziv projekta: Mogućnost iskorištavanja tradicionalnih sorti jabuka za proizvodnju jabuka i soka od jabuka sa smanjenim udjelom patulina

Voditelj projekta:	**doc. dr. sc. Ante Lončarić**
Izvor financiranja:	Hrvatska zaklada za znanost
Vrijednost projekta:	1.935.745,67 HRK
Trajanje projekta:	1.3.2021. - 28.2.2026.

Clean label

Nowadays, there is an increasing consumer demand for food products with clean labels (Nikmaram et al., 2018). Overall, a clean label product is free of chemically-synthesized and other substances which, despite their origin, are not perceived by consumers as beneficial for their health.



Nikmaram, N., Budaraju, S., Barba, F. J., Lorenzo, J. M., Cox, R. B., Mallikarjunan, K., et al. (2018). Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. Meat Science, 145, 245–255. https://doi.org/10.1016/j.meatsci.2018.06.031.

Polyphenols

These nutraceutical compounds can be used in/as;

- food,
- preservatives,
- colourants
- functional foods
- pharmaceutical and cosmetic products
- antimicrobial and
- fungicides


Food Research International 134 (2020) 109214

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ELSEVIER

Antimicrobial polyphenol-rich extracts: Applications and limitations in the food industry

Magdalena A. Olszewska[a,*], Astrid Gędas[a], Manuel Simões[b,*]

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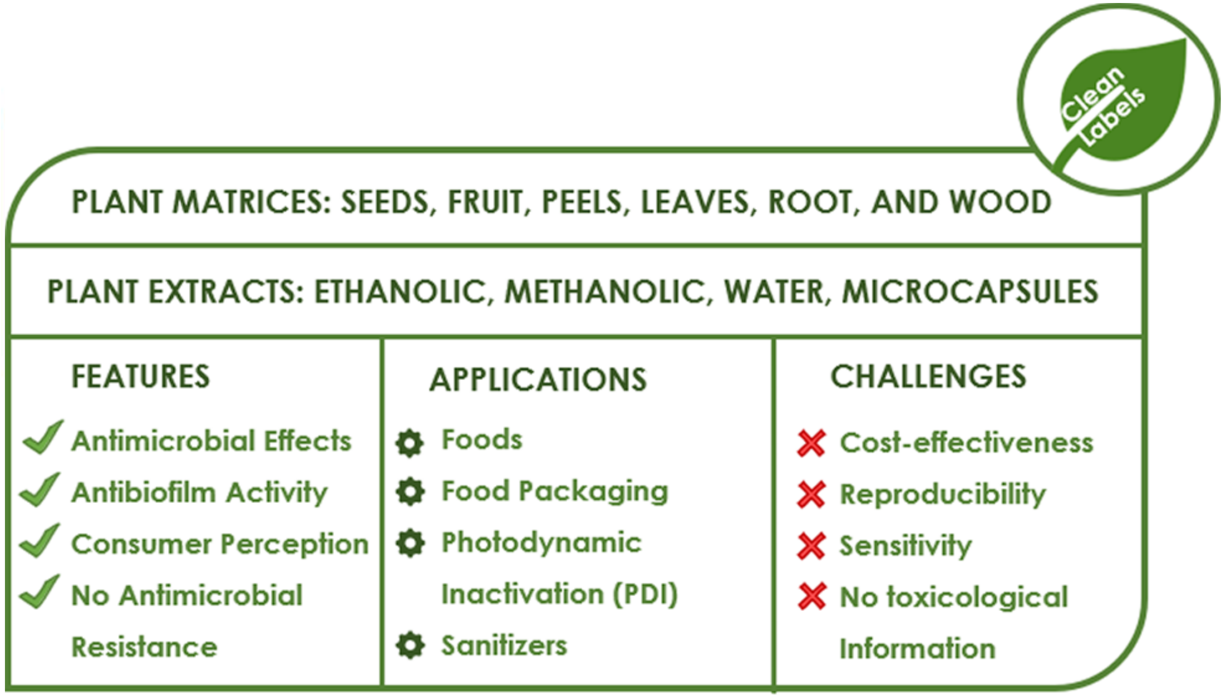
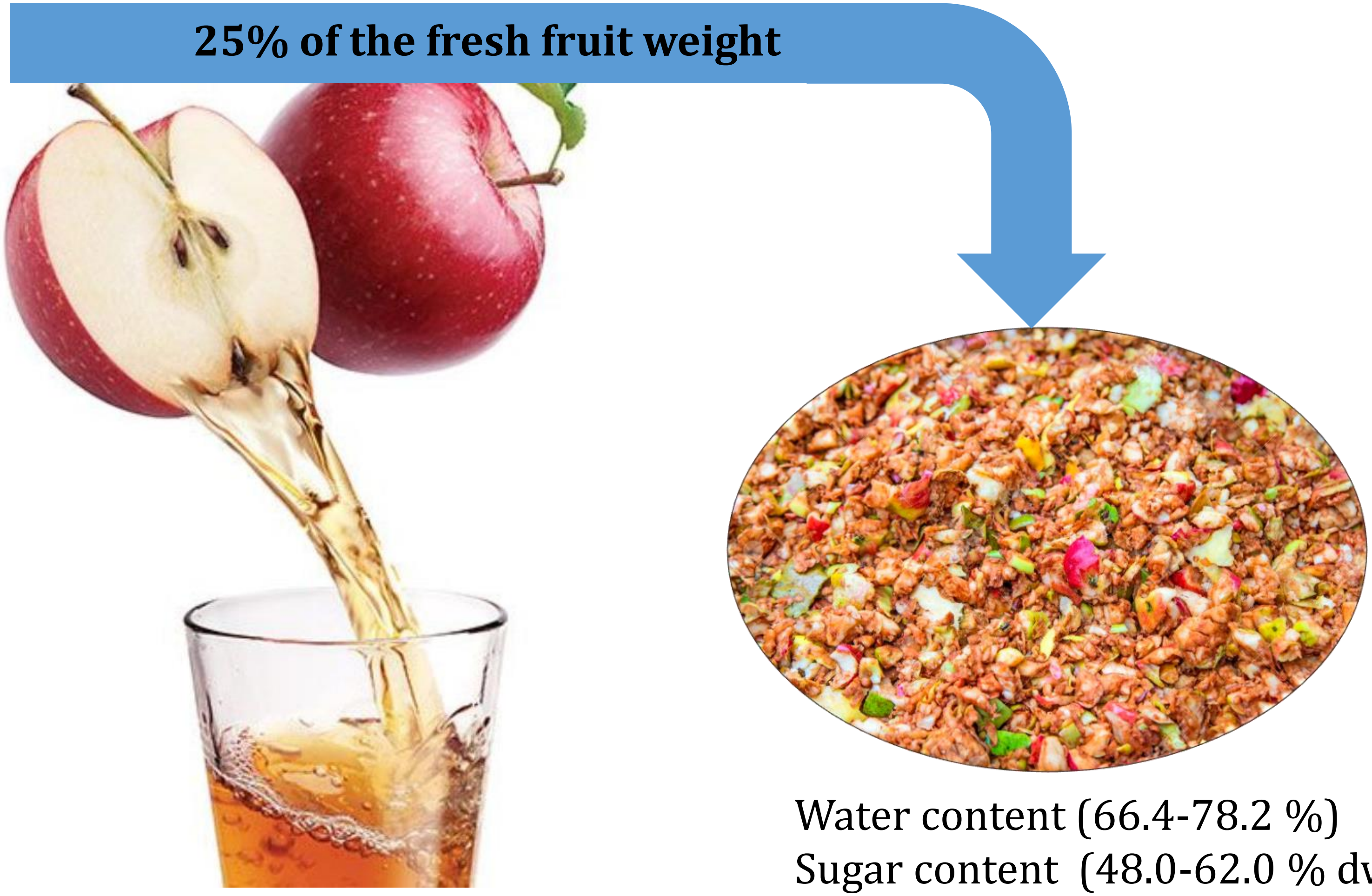
[b] Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE), Department of Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

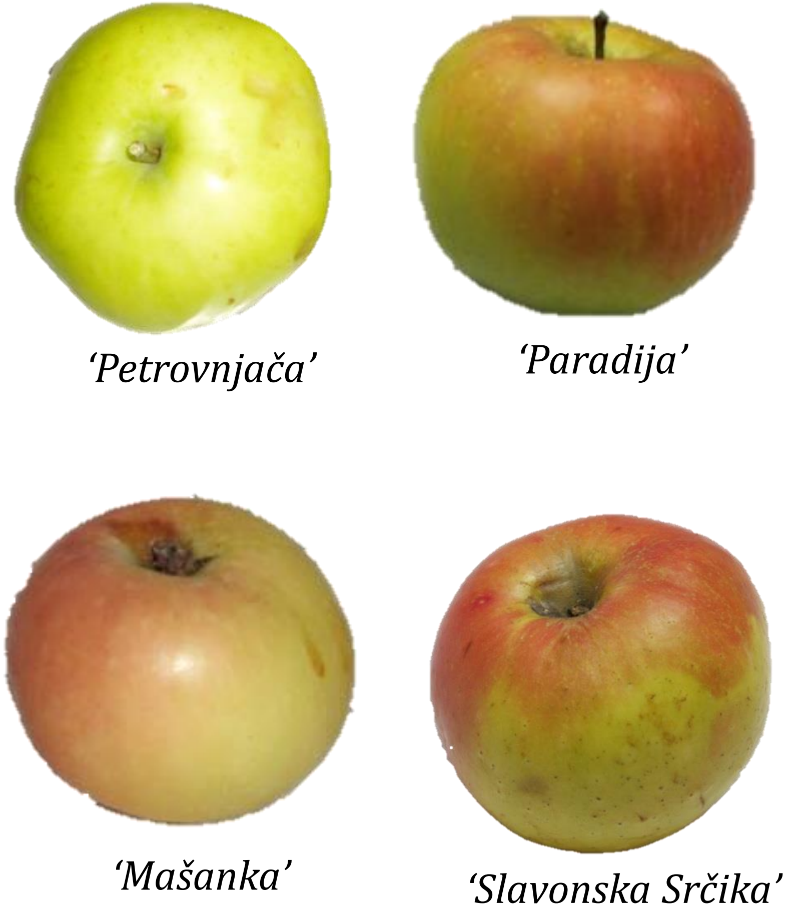


Kua, Y. L., Gan, S., Morris, A., & Ng, H. K. (2016). Ethyl lactate as a potential green solvent to extract hydrophilic (polar) and lipophilic (non-polar) phytonutrients simultaneously from fruit and vegetable by-products. Sustainable Chemistry and Pharmacy, 4, 21–31. doi:10.1016/j.scp.2016.07.003

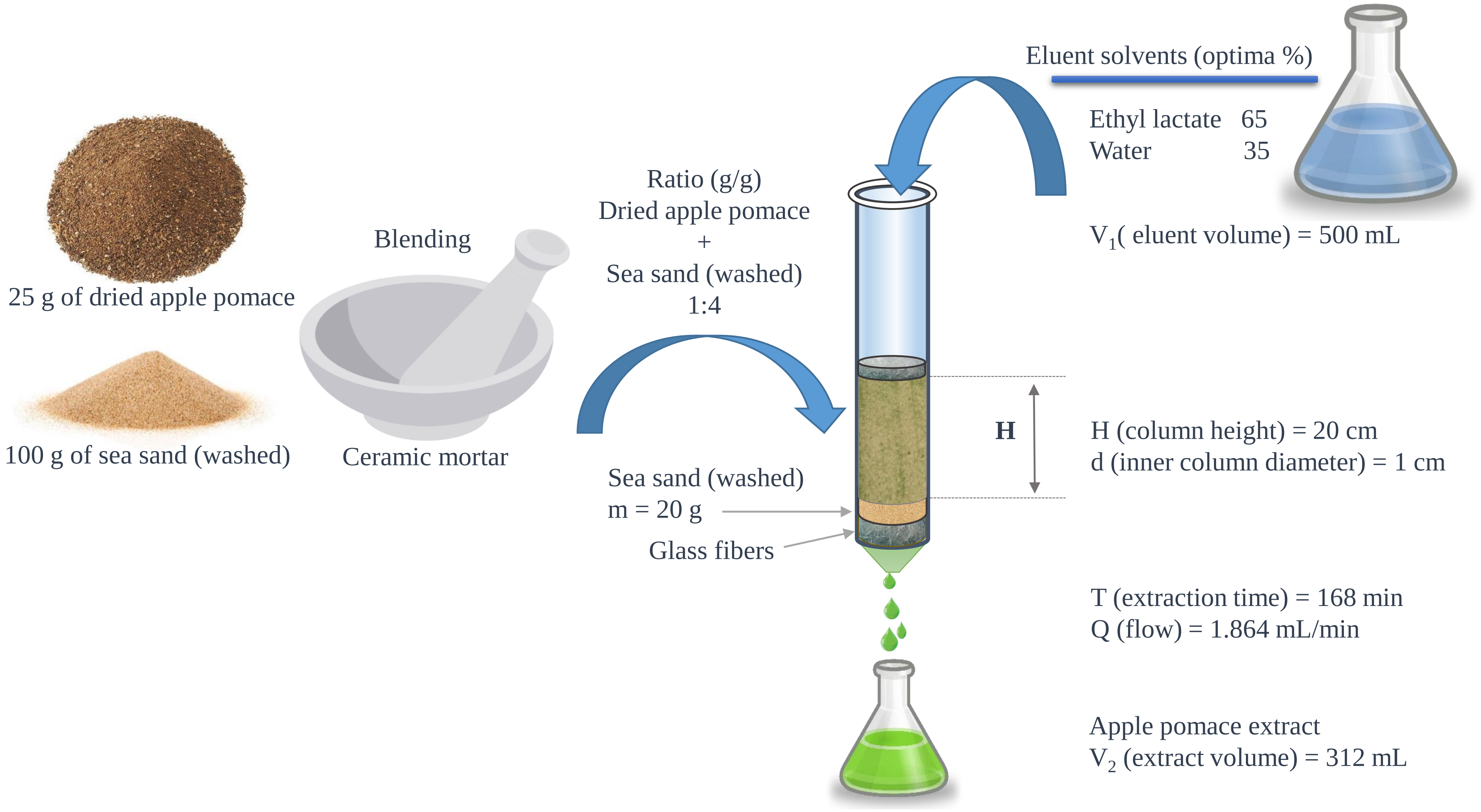
Huge volume of food wastes are generated from the processing industry and these low-value food residues are rich in various phytonutrients worth recovering. This approach of valorisation reduces the generation of food wastes and is cost-effective considering the cheap feedstock, reduced waste management expenses and high market value of extracted compounds.



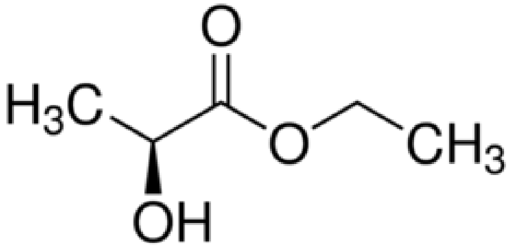
Water content (66.4-78.2 %)
Sugar content (48.0-62.0 % dw)



Matrix solid phase dispersion



Ethyl lactate



Sustainable Chemistry and Pharmacy

Volume 4, December 2016, Pages 21-31

Ethyl lactate as a potential green solvent to extract hydrophilic (polar) and lipophilic (non-polar) phytonutrients simultaneously from fruit and vegetable by-products

Yin Leng Kua [a], Suyin Gan [a], Andrew Morris [b], Hoon Kiat Ng [c]

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- Ethyl lactate is a green and safe agrochemical solvent.
- Ethyl lactate exerts low toxicity, volatility and flammability.
- Ethyl lactate is able to dissolve in polar and non-polar mediums.
- Research studies have shown its potential in extracting various phytonutrients.
- Ethyl lactate has the potential to extract phytonutrients from food wastes.

GENERALLY RECOGNIZED AS SAFE

Volume 500 mL
Solvent: 65% Ethyl lactate
Ratio (g/g): dried apple pomace/sea sand (25g/100g)
Time: 168 min
Extract volume: 312 mL

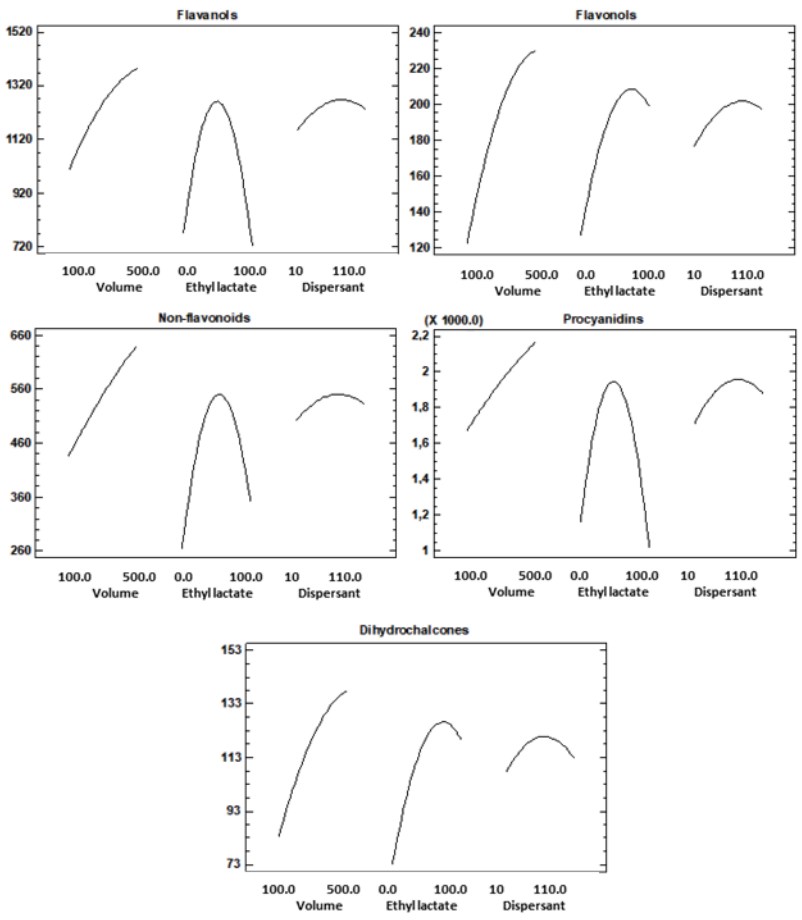

Fig 1. Optimization of extraction of polyphenols from apple pomace

Compounds (µg/mL)	Catechin	Epicatechin	Procyanidine B1
'PETROVNJACA'	28.3 ± 0.26	170.61 ± 2.17	116.3 ± 2.9
'WAGNER'	202.84 ± 5.22	66.71 ± 0.59	53.23 ± 0.1
'KLEKER'	131.37 ± 3.56	143.86 ± 1.3	187.12 ± 6.0
'MASANKA	268.71 ± 2.27	163.49 ± 1.57	91.45 ± 2.1
'ADAMOVKA'	204.07 ± 2.51	313.54 ± 1.3	84.76 ± 0.9
'AMOVKA'	67.55 ± 0.62	213.88 ± 2.59	50.68 ± 1.1
'SRCIKA'	57.04 ± 0.45	242.53 ± 1.27	244.51 ± 4.0
'PARADIJA'	56.24 ± 2.08	162.34 ± 1.01	58.44 ± 0.2

Compounds (µg/mL)	Quercetine	Myrecetin	Quercetin 3-β-D-glucoside
'PETROVNJACA'	1167.89 ± 9.45	35.25 ± 0.82	19.93 ± 0.12
'WAGNER'	103.43 ± 0.98	27.78 ± 0.16	26.9 ± 0.07
'KLEKER'	744.35 ± 18.58	26.94 ± 0.46	22.29 ± 0.94
'MASANKA	880.59 ± 15.63	47.32 ± 1.22	35.58 ± 0.09
'ADAMOVKA'	194.63 ± 7.33	21.47 ± 0.26	19.57 ± 0.52
'AMOVKA'	730.8 ± 9.05	20.53 ± 1.29	18.84 ± 0.36
'SRCIKA'	469.93 ± 7.13	37.72 ± 1.93	21.26 ± 0.36
'PARADIJA'	958.03 ± 6.3	33.9 ± 1.36	22.14 ± 0.79

Compounds (µg/mL)	Chlorogenic acid	4-Hydroxycinnamic acid	2-6-dimethoxybenzoic acid
'PETROVNJACA'	1429.22 ± 16.03	48.64 ± 1.01	37.38 ± 0.75
'WAGNER'	639 ± 1.71	7.45 ± 0.14	25.69 ± 0.31
'KLEKER'	1131.27 ± 16.58	32.7 ± 0.48	25.36 ± 0.77
'MASANKA	1657.98 ± 14.01	14.97 ± 0.61	11.7 ± 0.42
'ADAMOVKA'	900.28 ± 7.24	64.58 ± 0.4	36.83 ± 0.72
'AMOVKA'	1194.39 ± 21.63	22.29 ± 0.24	16.65 ± 0.51
'SRCIKA'	1715.54 ± 13.38	73.56 ± 0.76	32.15 ± 0.28
'PARADIJA'	2383.49 ± 47.14	28.8 ± 0.31	33.47 ± 0.23

Compounds (µg/mL)	Phloridzin	Phloretin	Total dihidrochalcones
'PETROVNJACA'	101.58 ± 1.25	18.22 ± 0.55	119.
'WAGNER'	155.11 ± 1.61	30.14 ± 0.09	185.2
'KLEKER'	121.58 ± 2.02	20.06 ± 1.29	141.6
'MASANKA'	311.97 ± 1.09	40.36 ± 1.64	352.3
'ADAMOVKA'	49.26 ± 1.07	11.93 ± 0.29	61.
'AMOVKA'	39.6 ± 0.91	17.27 ± 1.87	56.8
'SRCIKA'	161.37 ± 1.67	20.75 ± 0.25	182.1
'PARADIJA'	85.23 ± 0.57	21.98 ± 0.61	107.2

Trends in Food Science & Technology 90 (2019) 76–87

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Review

Bioactive and functional compounds in apple pomace from juice and cider manufacturing: Potential use in dermal formulations

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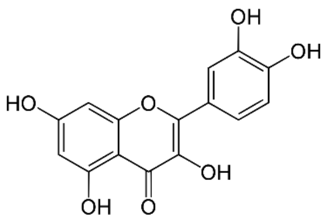
Table 1
Variation in phenolic content in the pomace of different apple cultivars (Ramirez-Ambrosi et al., 2013; Sánchez-Rabaneda et al., 2004).

Compound	Concentration (g/kg DM)	Reference
Phenolic acids		
Protocatechuic acid	0.03–0.26	(Diñeiro García, Valles, & Picinelli Lobo, 2009; Suárez et al., 2010)
Caffeic acid	0.02	(Suárez et al., 2010; Ćetković et al., 2008)
p-Coumaroylquinic acid	0.09	Andreas Schieber et al. (2003)
p-Coumaric acid	0.02	Andreas Schieber et al. (2003)
Ferulic acid	0.02	Andreas Schieber et al. (2003)
Chlorogenic acid	0.03–0.40	(Diñeiro García et al., 2009; Andreas Schieber et al., 2003; Suárez et al., 2010; Ćetković et al., 2008)
Caffeic acid	0.28	Lu and Foo (1997)
Flavonoids		
Epicatechin	0.02–0.64	(Diñeiro García et al., 2009; Lu & Foo, 1997; Andreas Schieber et al., 2003; Suárez et al., 2010; Ćetković et al., 2008)
Catechin	0.12	Andreas Schieber et al. (2003)
Procyanidin B2	0.18–0.45	(Diñeiro García et al., 2009; Andreas Schieber et al., 2003; Suárez et al., 2010)
Quercetin-3-galactoside	0.57–1.61	(Lu & Foo, 1997; Andreas Schieber et al., 2003)
Quecetin-3-glucoside	0.25–0.87	(Lu & Foo, 1997; Andreas Schieber et al., 2003)
Quercetin-3-xyloside	0.09–0.53	(Lu & Foo, 1997; Andreas Schieber et al., 2003)
Quercetin-3-rutinoside	0.06–0.48	(Andreas Schieber et al., 2003; Ćetković et al., 2008)
Quercetin-3-arabinoside	0.98	Lu and Foo (1997)
Quercetin-3-rhamnoside	0.05–0.47	(Lu & Foo, 1997; Andreas Schieber et al., 2003)
Hyperin	0.11–0.23	(Diñeiro García et al., 2009; Suárez et al., 2010)
Isoquercitrin + rutin	0.06–0.16	(Diñeiro García et al., 2009; Suárez et al., 2010)
Reynoutrin	0.02–0.08	(Diñeiro García et al., 2009; Suárez et al., 2010)
Avicularin	0.09–0.26	(Diñeiro García et al., 2009; Suárez et al., 2010)
Quercitrin	0.09–0.19	(Diñeiro García et al., 2009; Suárez et al., 2010)
Di-hydrochalcones		
3-Hydroxyphloridzin	0.27	Lu and Foo (1997)
Phloretin-2′-xyloglucoside	0.02–0.40	(Diñeiro García et al., 2009; Lu & Foo, 1997; Andreas Schieber et al., 2003; Suárez et al., 2010)
Phloridzin	0.01–2.00	(Diñeiro García et al., 2009; Lu & Foo, 1997; Andreas Schieber et al., 2003; Suárez et al., 2010; Ćetković et al., 2008)

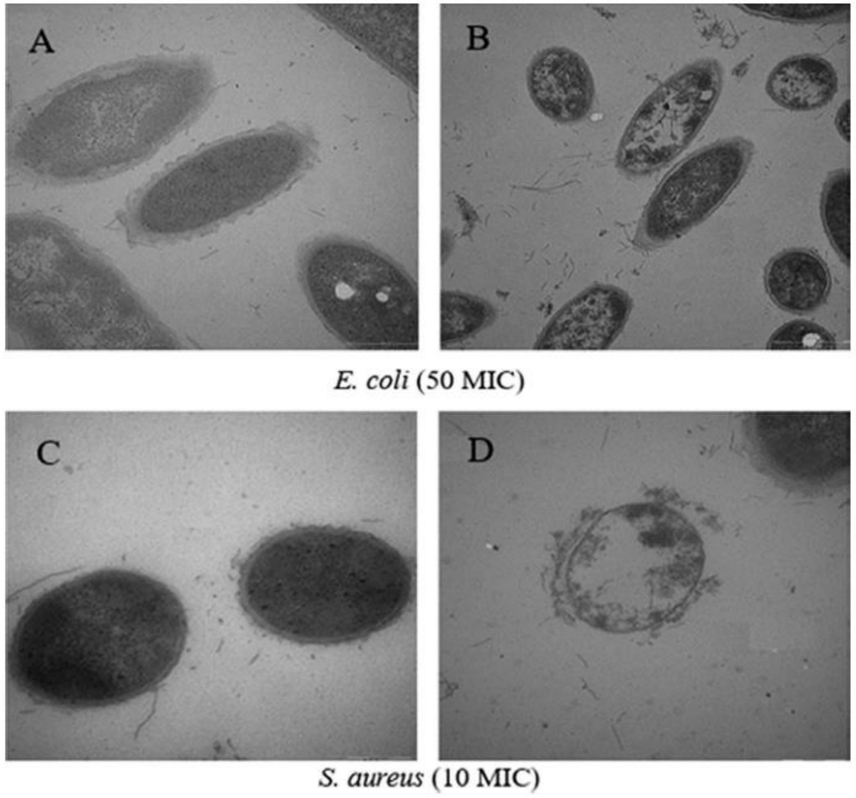
Antimicrobial Activity



Quercetin

Escherichia coli

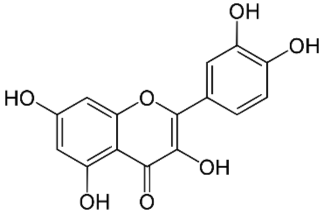
Staphylococcus aureus



Effects of quercetin on cell wall ultrastructure of *E. coli* and *S. aureus*. (A) *E. coli* without quercetin; (B) E. coli with quercetin; (C) S. aureus without quercetin; (D) *S. aureus* with quercetin (Wang et al. 2018).

- **Quercetin** damaged the structure of the bacterial cell wall and cell membrane, leading to increased permeability of these structures.
- The endochylema contents of the cell were released and the activity of ATP was affected.
- Quercetin decreases the synthesis of bacterial proteins, affected the expression of proteins in the cell, and finally resulted in cell lysis and death.

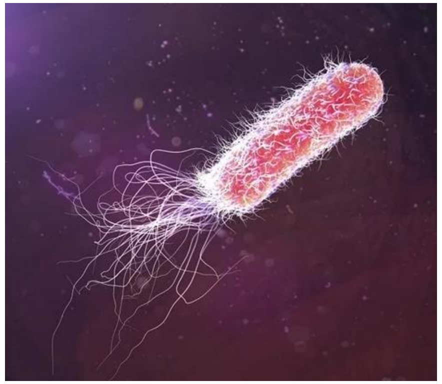
Wang, S.; Yao, J.; Zhou, B.; Yang, J.; Chaudry, M.T.; Wang, M.; Xiao, F.; Li, Y.; Yin,W. Bacteriostatic efect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. J. Food Prot. **2018**, 81, 68–78.

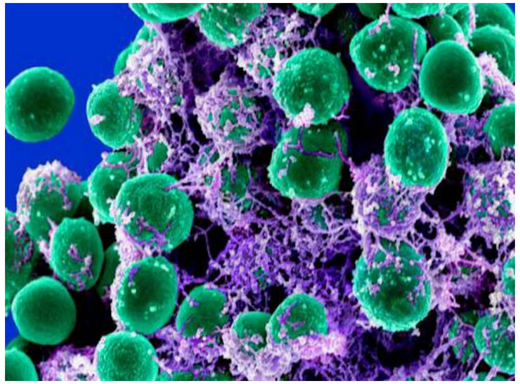


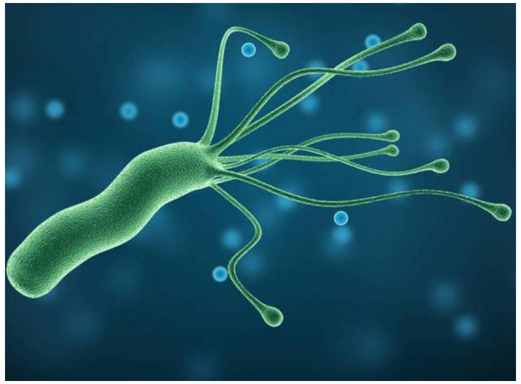
Quercetin

Quercetin exhibits potent bacteriostatic activity against different strains of bacteria, however with more effectiveness against Gram-positive than Gram-negative bacteria.


Campylobacter jejuni


Pseudomonas aeruginosa


Staphylococcus epidermidis


Helicobacter pylori

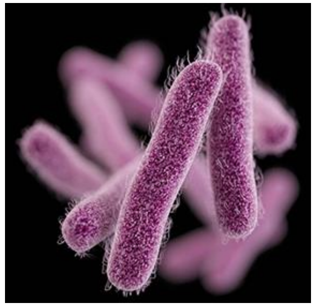

Salmonella enterica

Wang, S.; Yao, J.; Zhou, B.; Yang, J.; Chaudry, M.T.;Wang, M.; Xiao, F.; Li, Y.; Yin,W. Bacteriostatic efect of quercetin as an antibiotic alternative in vivo and its antibacterial mechanism in vitro. J. Food Prot. **2018**, 81, 68–78. [CrossRef]

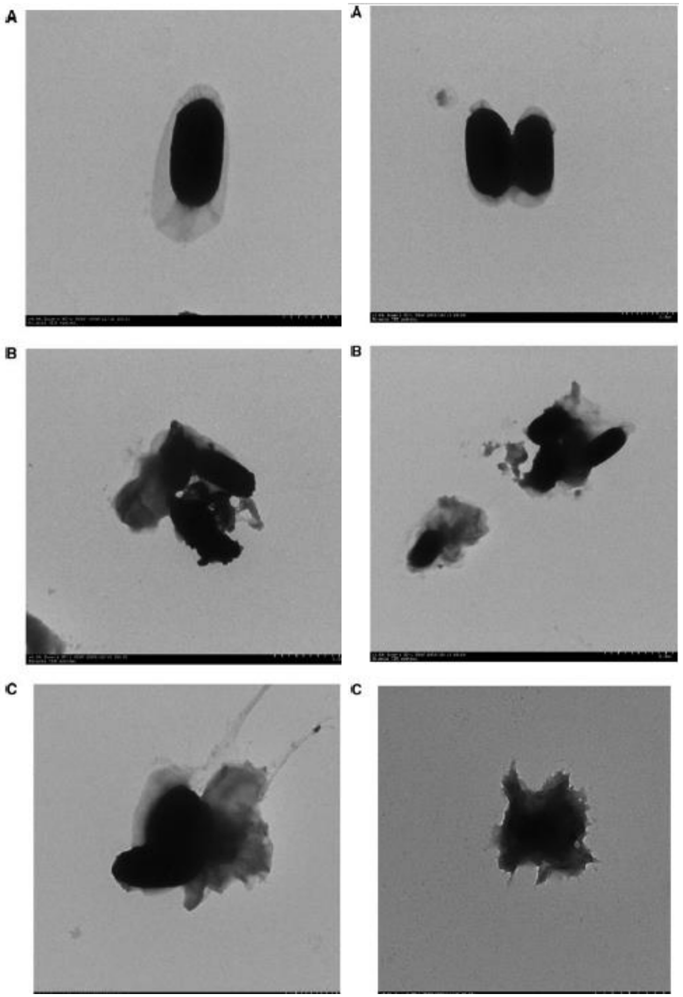
Jaisinghani, R. Antibacterial properties of quercetin. Microb. Res. **2017**, 8. [CrossRef]

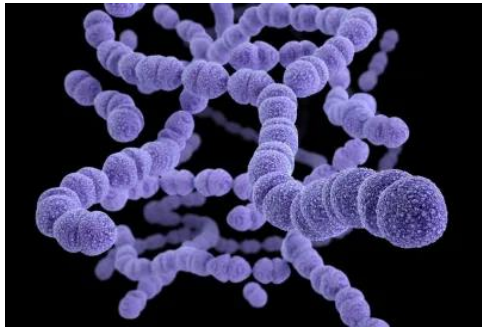
Osonga, F.J.; Akgul, A.; Miller, R.M.; Eshun, G.B.; Yazgan, I.; Akgul, A.; Sadik, O.A. Antimicrobial activity of a new class of phosphorylated and modified flavonoids. ACS. Omega **2019**, 4, 12865–12871. [CrossRef]

Chlorogenic acid - effectively inhibited the growth of bacterial pathogens;



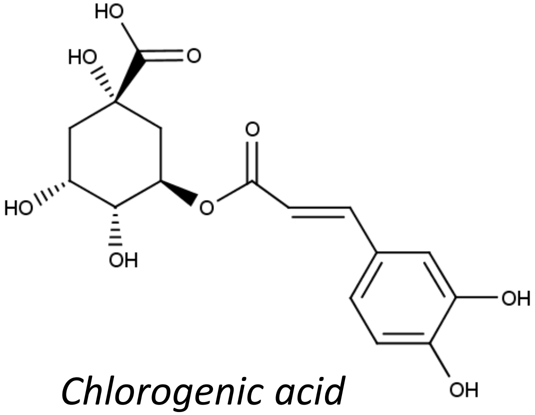
Shigella dysenteriae





Streptococcus pneumoniae

Transmission electron micrographs of *Shigella dysenteriae (left) Streptococcus pneumoniae (right)* treated by chlorogenic acid for 90 min (B) and 3 h (C), (A) control (Lou et al. 2011).



Chlorogenic acid

Journal of Food Science
A Publication of the Institute of Food Technologists

Antibacterial Activity and Mechanism of Action of Chlorogenic Acid

Zaixiang Lou, Hongxin Wang, Song Zhu, Chaoyang Ma, Zhouping Wang

First published: 02 June 2011 | https://doi.org/10.1111/j.1750-3841.2011.02213.x | Citations: 248

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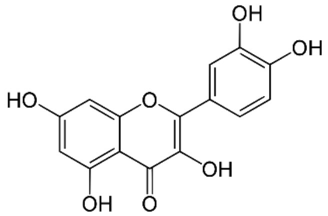
Mechanism of action - significantly increased the outer and plasma membrane permeability, resulting in the loss of the barrier function, even inducing slight leakage of nucleotide, which led to cell death.

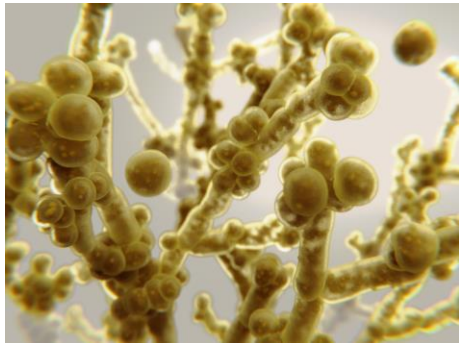
Minimum inhibitory concentration - 20 to 80 μg/mL

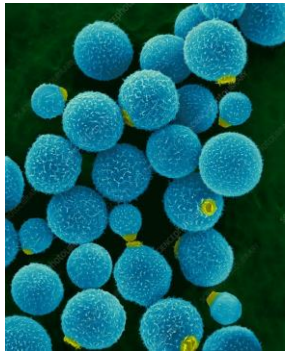
Antifungal Activity

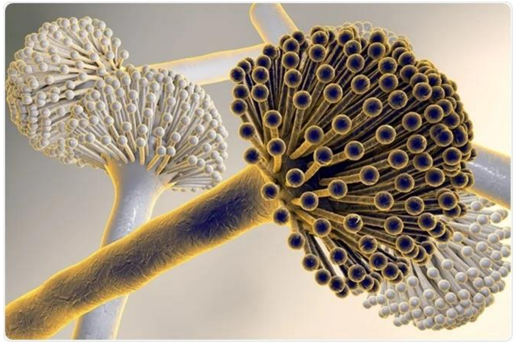


Quercetin

<u>Quercetin</u> revealed the strongest antifungal activities against (Abd-Allah et al. 2015):


Candida albicans

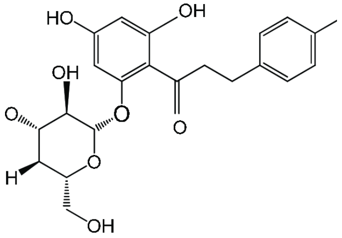

Cryptococcus neoformans


Aspergillus niger

<u>Phloridzin & Phloretin</u> (Shim et al., 2010)

Magnaporthe grisea


Phytophthora infestans

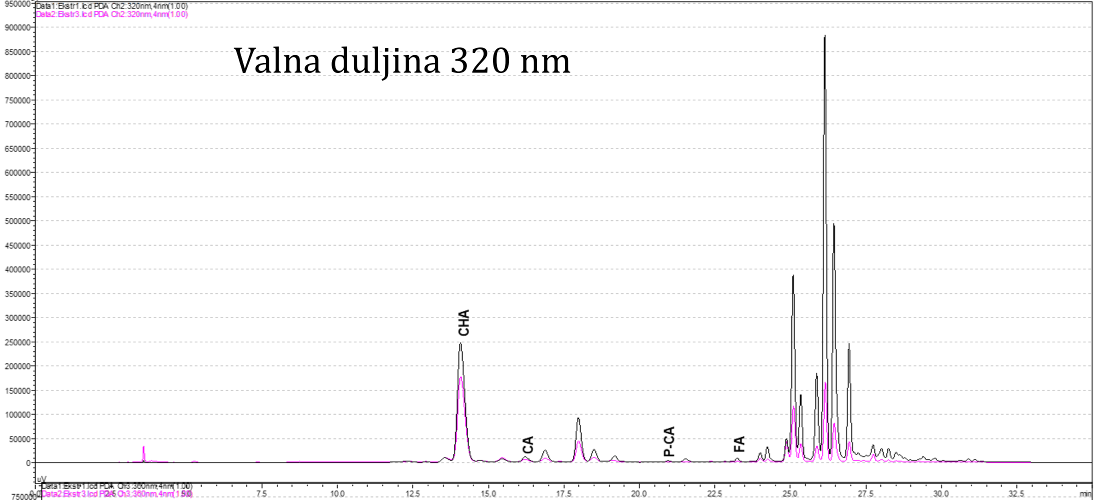


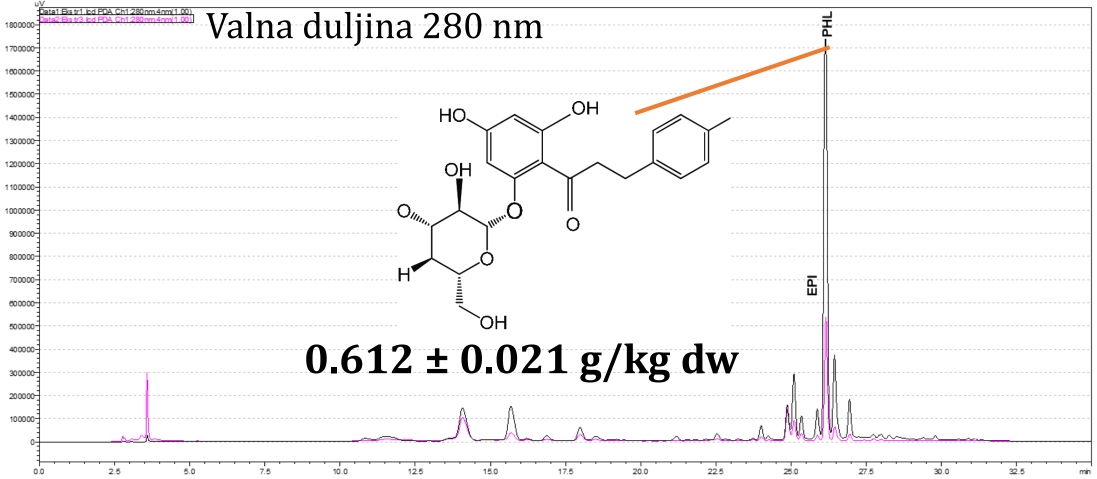
Phloridzin

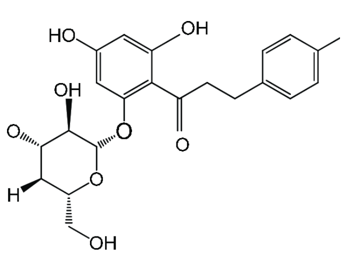
Minimum inhibitory concentration - 67 to 200 μg/mL

Abd-Allah, W.E.; Awad, H.M.; Abdel Mohsen, M.M. HPLC analysis of quercetin and antimicrobial activity of comparative methanol extracts of Shinus molle L. Int. J. Curr. Microbiol. Appl. Sci. **2015**, 4, 550–558.

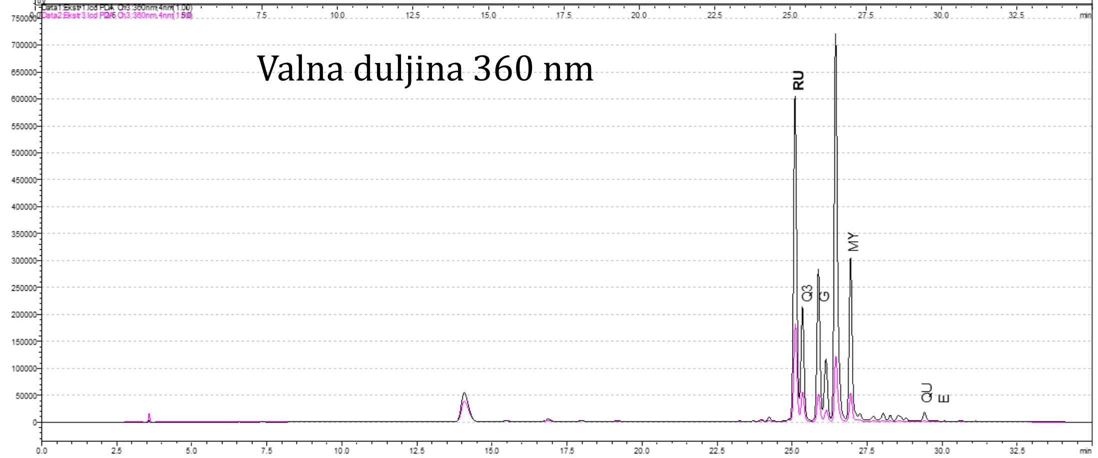
Sang-Hee Shim, Su-Jung Jo, Jin-Cheol Kim and Gyung Ja Choi. Control Efficacy of Phloretin Isolated from Apple Fruits Against Several Plant Diseases Plant Pathol. J. 2010., 26(3):280-285

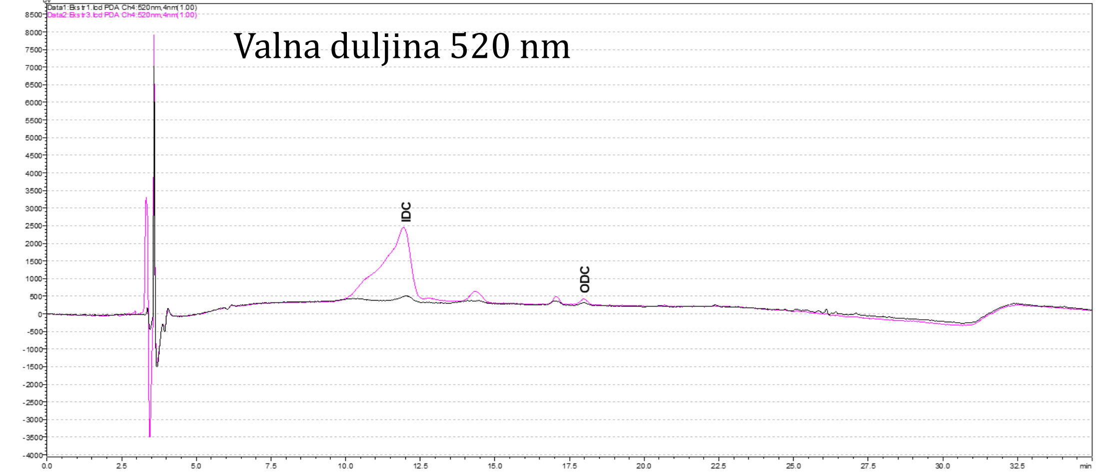




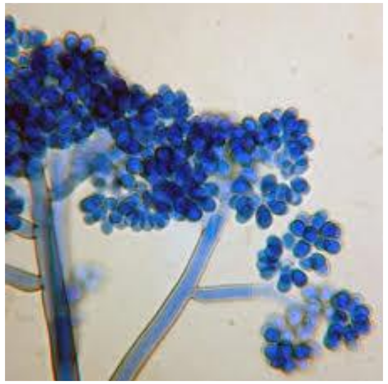


Phloridzin

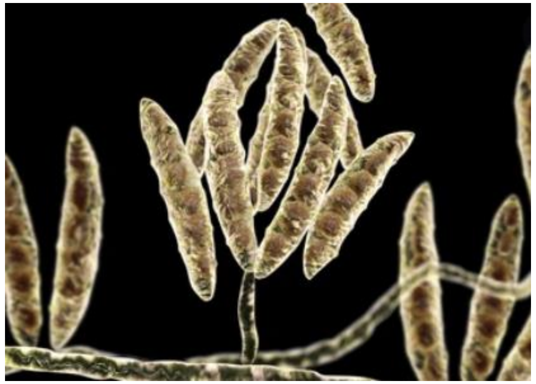




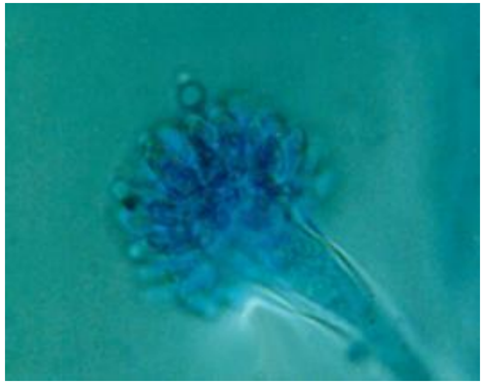
<u>Phloridzin</u> - plays a major role in apple in the resistance to fungal infection. It is metabolized to phloretin and then, to the next oxidation products such as o-quinone, which are fungitoxic (Oleszek et al. 2019).



Botrytis cinerea



Fusarium oxysporum



Neosartorya fischeri

Minimum inhibitory concentration <500 µg/mL

By-Product	Type of application	Target Microorganisms	References
Apple skin	Chitosan edible film	*E. coli, S. enterica and L. monocytogenes*	Riaz et al. 2018
Apple skin (powder, extract)	Composite films ASP/CMC	*L. monocytogenes, S. aureus, S. enterica and S. flexner*	Choi et al. 2017
Apple pomace	Apple phenolic fractions aceton/ethanol	*L. monocytogenes, S. aureus, E. coli, S. Typhimurium*	Zhang et al. 2016
Apple pomace	Phenolic extract ethyl acetate	*S. aureus, E. coli*	Raphaelli et al., 2019
Apple peel	Phenolic extract Ethanol/water	*Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, E. coli, Bacillus cereus, Staphylococcus aureus*	Fratianni et al. 2011
Apple pomace	Fraction of phenolic extract	*Botrytis cinerea; Fusarium oxysporum; Neosartorya fischeri*	Oleszek et al. 2019
Apple pomace	Isolated phloretin	*Phytophthora capsici; Alternaria panax; Magnaporthe grisea; Sclerotinia sclerotiorum; Rhizoctonia solani*	Shim et al. 2010

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Conclusions

- MSPD method proves to be an inexpensive and simple method for polyphenol extraction, it could allow the separation of individual polyphenol fractions

- Ethyl-Lactate gives extract rich in polyphenols

- Croatian apple cultivars, ‘Petrovnjača’, ‘Mašanka’, ‘Paradija’ contains polyphenols (quercetin, phloridzin, and chlorogenic acid, respectively) with potential antimicrobial and antifungal activity

Hvala na pažnji i lijepi pozdrav iz Santiaga de Compostele!