Luke
NATURAL RESOURCES
INSTITUTE FINLAND

Enzyme-assisted extraction of blackcurrant press-cake: bioactivities of the extracts

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Luke-URCA webinar on production and characterization of biosourced molecules

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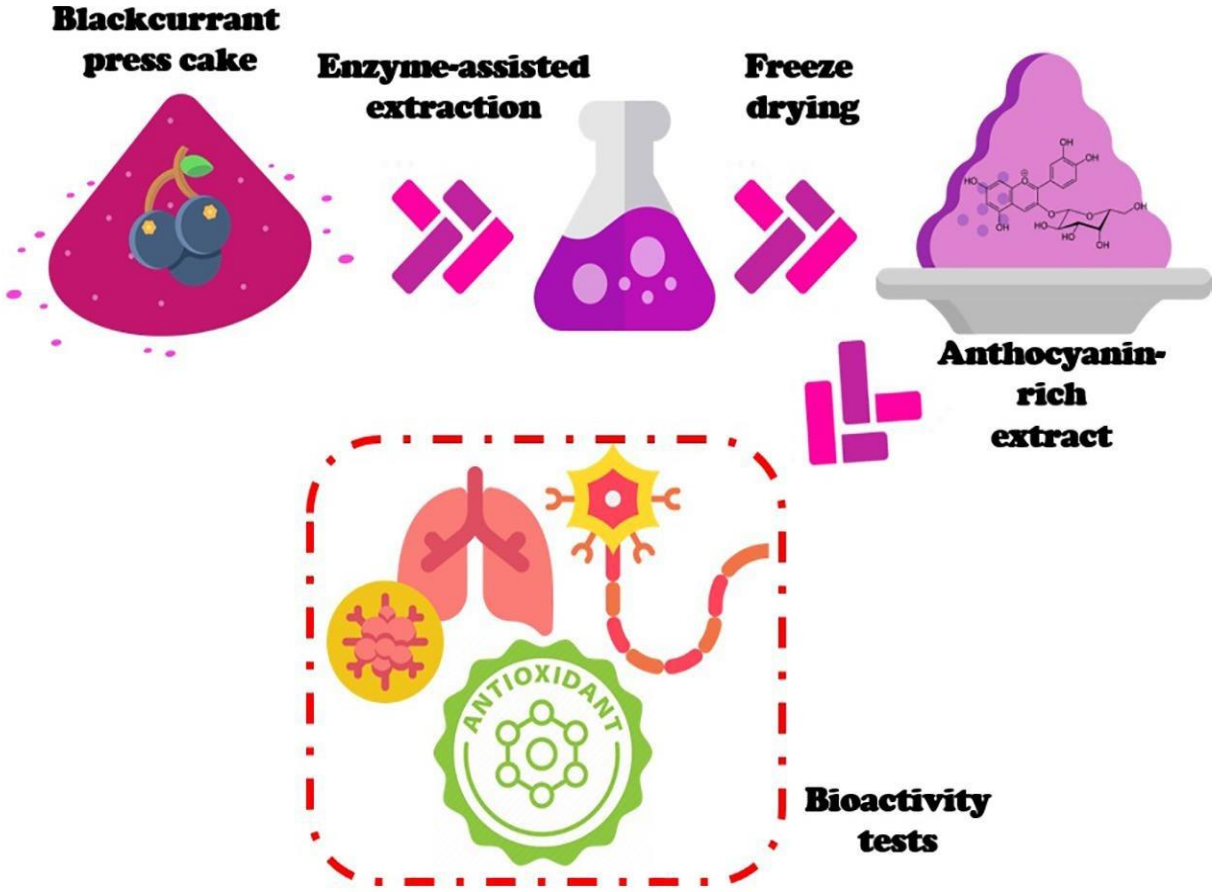
Nutritive factors of blackcurrant (*Ribes nigrum* L.) berry

- Vitamins (niacine, riboflavin, B1, C, A, E, K, carotenoids)
- Minerals (calcium, manganese and iron, iodide, potassium, magnesium, sodium, salts, phosphorus, selenium, zinc)
- Sugars (fructose, glucose, sucrose)
- Dietraty fibre (cellulose, pectin, lignin, hexosan, pentosan)
- Organic and phenolic acids (tartaric acid, malic acid, citric acid, p-coumaric acid, gallic acid, ferulic acid and caffeic acid)
- Fatty acids (linoleic acid, α-linolenic acid, stearidonic acid and γ-linolenic acid
- Anthocyanins (cyanidin and delphinidin glucosides and rutinosides)
- Flavonols (quercetin, kaempferol, myricetin)

Background of the press-cake utilization

- Large volume from the industry
- Study on the green extraction of anthocyanins from the blackcurrant press-cake by microwave-assisted vs. conventional solvent extraction, and maceration vs. pressurized hot water extraction
- Heat sensitivity of the health promoting compounds
- Need for low temperature processing>enzymes
- Exploration of the bioactivities of the press-cake extract: antioxidant activities, antiviral- and anti-inflammatory activities and cytotoxicity towards cancer cells



Blackcurrant press-cake

S:L=1:10
C=200 ppm
T=40 °C
t= 1-5 h
$T_{inactivation}$= 60-80 ° C

S:L=1:4
C=100 ppm
pH < 5.5
T=40-50 ° C
t= 1-5 h
$T_{inactivation}$= 75 °C

S:L=1:4
C=100 ppm
pH < 5.5
T=40-50 ° C
t= 1-5 h
$T_{inactivation}$= 75 °C

Control 60 °C
Pectinase 1
Pectinase 2
Pectin lyase
Control 80 °C
B-glucanase
Cellulase
B-glucanase + Pectinase 1
Cellulase + Pectinase 2

Control 40 °C
Control (past.) 40 °C
Pectin lyase
Control 50 °C
Control (past.) 50 °C
Cellulase
Cellulase (past.)
Cellulase + Pectin lyase
Cellulase + Pectin lyase (past.)

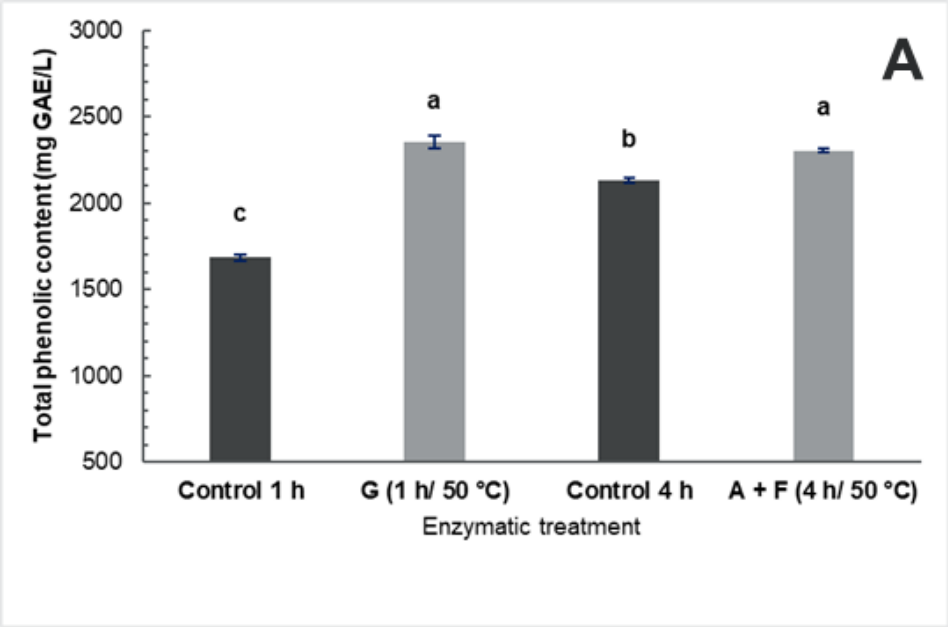
Control 1 h and 4 h
Cellulase
Beta-glucanase+pectin lyase

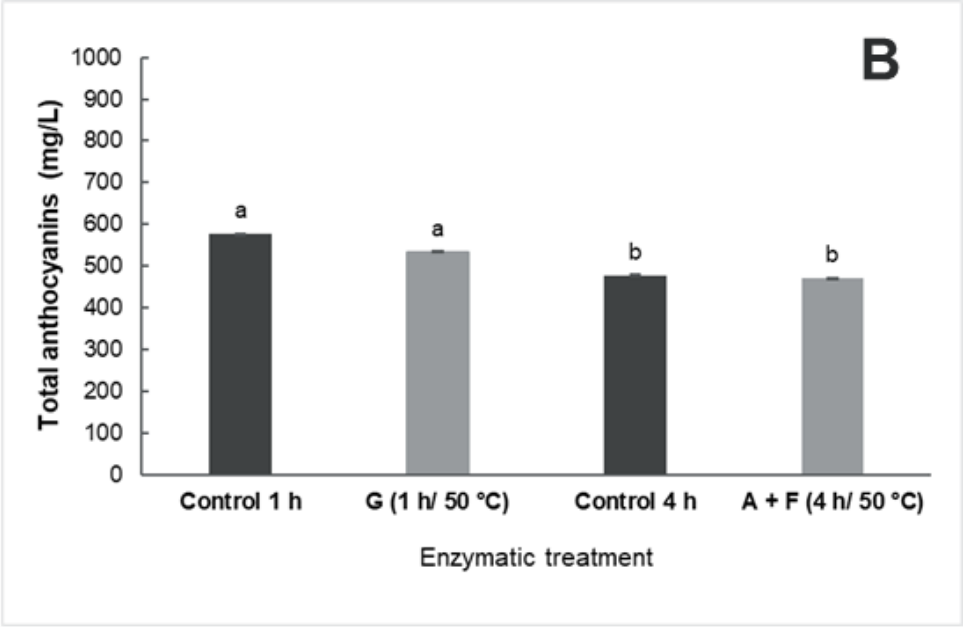
Analyses

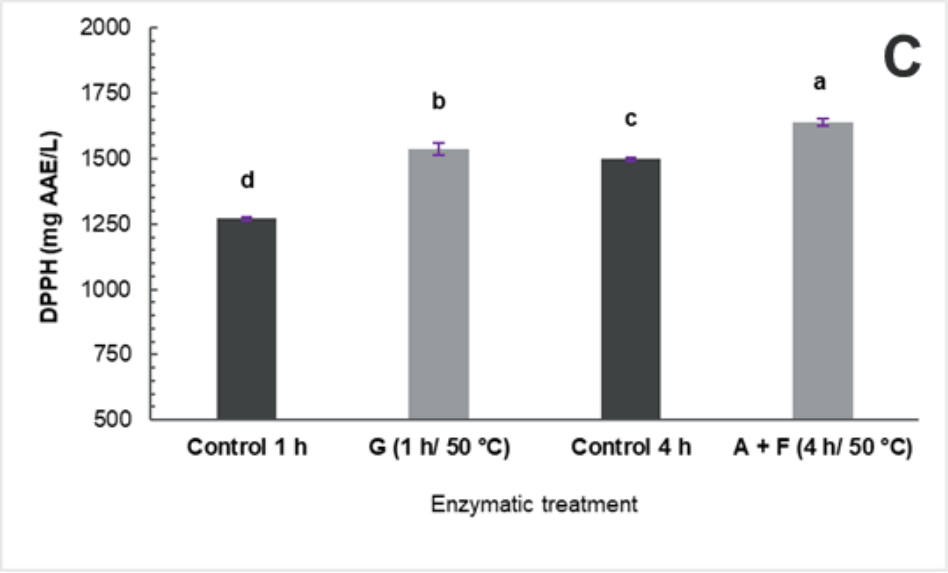
Monomeric anthocyanins
Total phenolics content
DPPH• assay
CUPRAC assay
Total anthocyanins
Anthocyanin composition

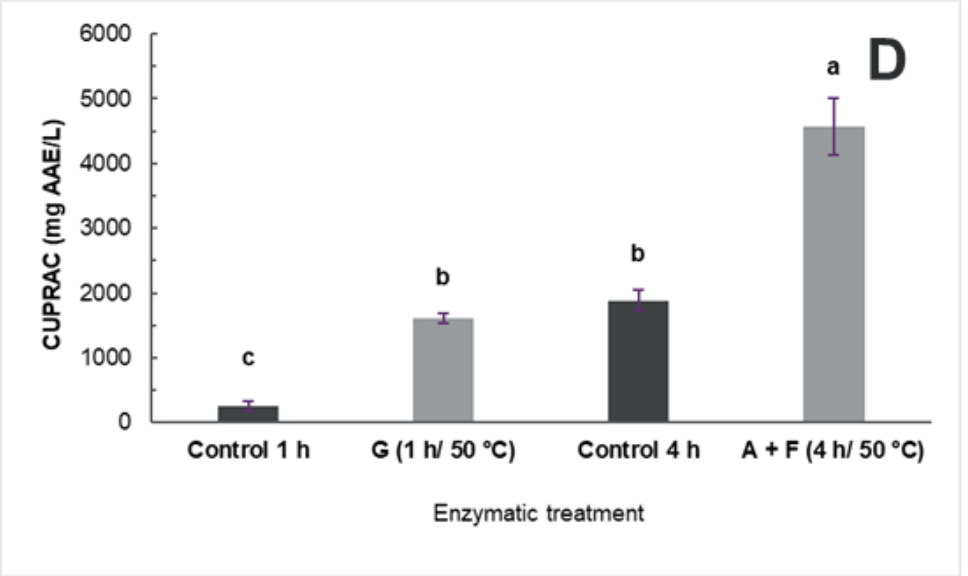
Analyses

Monomeric anthocyanins
Total phenolics content
DPPH• assay
CUPRAC assay
Total anthocyanins
Anthocyanin composition
Antiviral activities
ROS generation
Anti-inflammatory activities

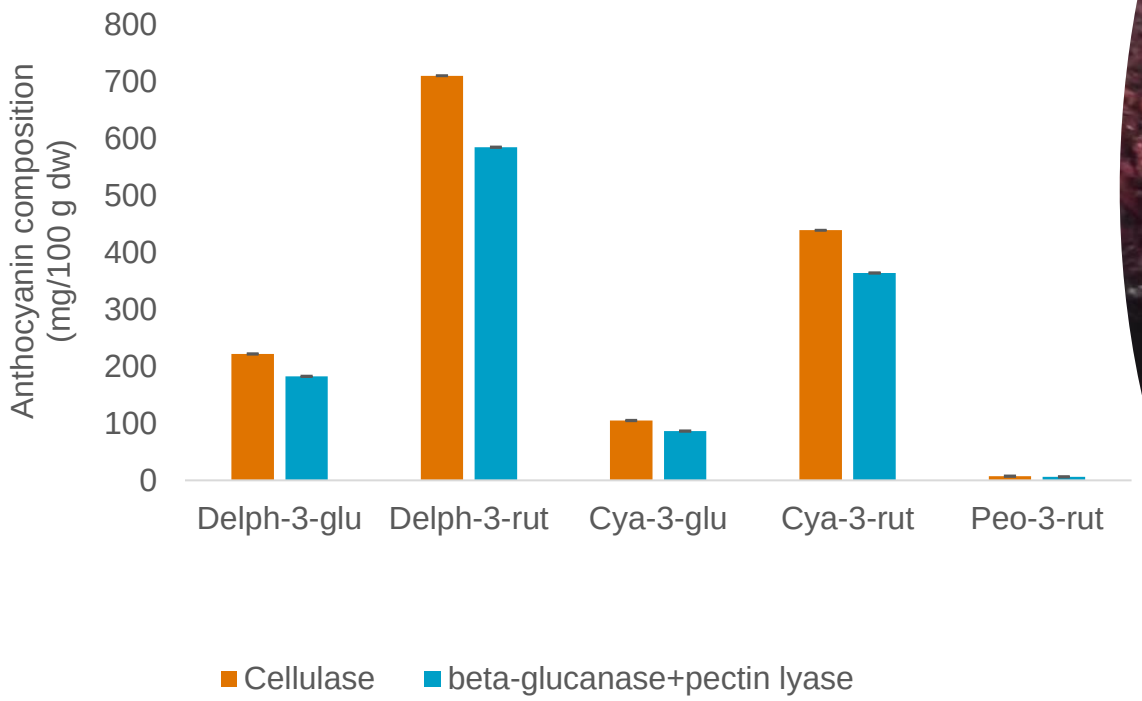








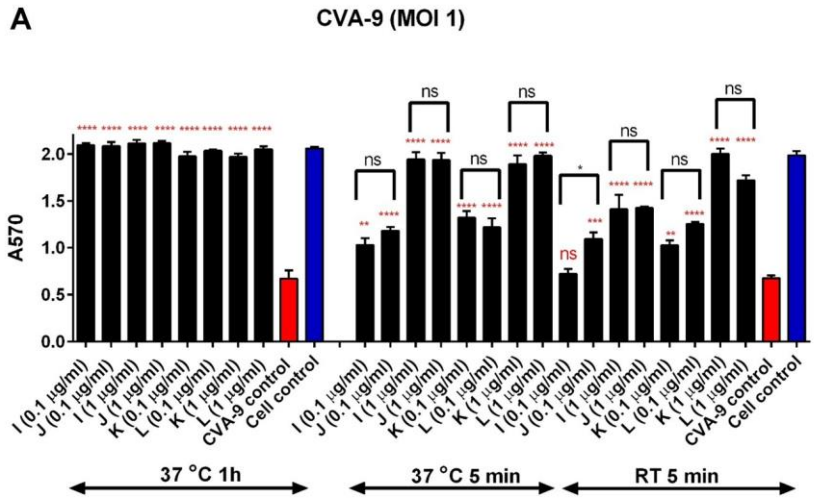
Anthocyanin composition

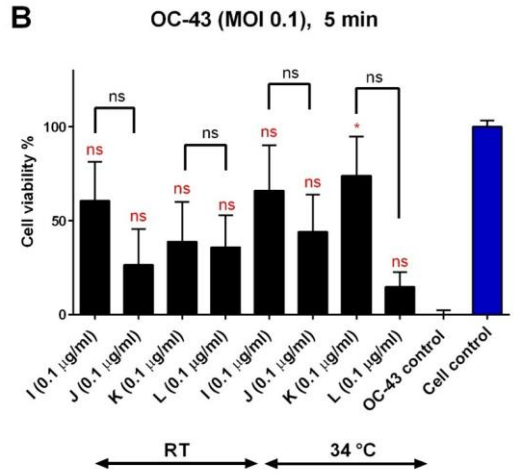


Picture: Reelika Rätsep

Antiviral activity

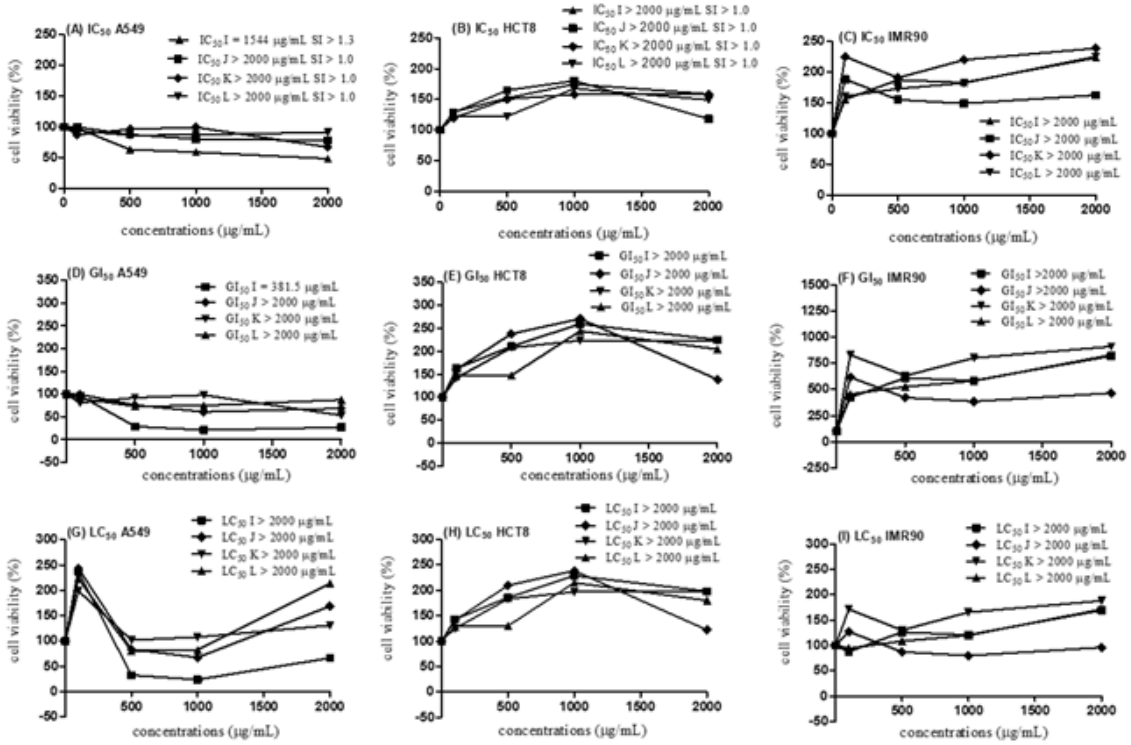
- Coxackievirus A9 (CVA-9) and human coronavirus (OC 43) were treated with the blackcurrant extracts in concentration of 0.1 and 1 µg/mL
- Incubation for 1 h or 5 min in 37 °C or room temperature for CVA-9
- Incubation for 1 h room temperature and 34 °C for OC-43
- All extracts showed antiviral activity without cytotoxicity in A549 cells
- Enzyme assisted extracts were not showing significantly better effects





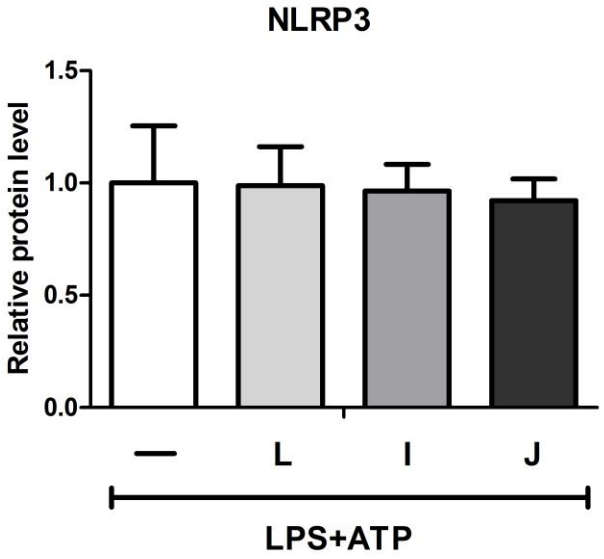
Cell-based viability and ROS generation

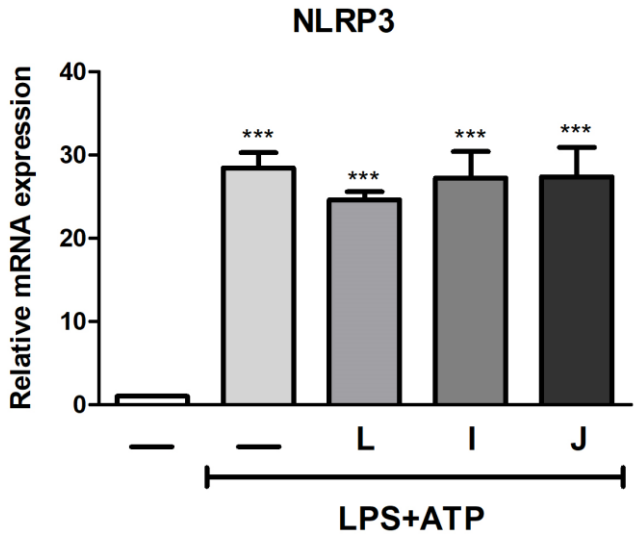
- Backcurrant control sample "I" seemed to be more sensible to A549 cells then HCT8 or IMR90 cells
- 50% reduction in cell viability at 1544 µg/mL and growth at 381.5 µg/mL
- Sample did not have selectivity over the cancer cells
- Decrease in ROS levels when tested on A549 cell line



Anti-inflammatory activity in IMG microglial cells

- Extracts had no protective effect on the cell viability in the IMG microglial cells at 100 µL/mL concentration
- Exracts at 100 µL/mL concentration did not significantly effect the mRNA levels of proinflammatory cytokines
- None of the blackcurrant extracts further had efficacy in expressing NLRP3 inflammasome related proteins
- No observation of the anti—inflammatory effect of the blackcurrant extracts in IMG microglial cells in this study





Highlights

- Blackcurrant press cake (BPC) was extracted with cellulase/beta-glucanase+pectinase
- Enzyme-assisted extraction increased the recovery of phenolics
- BPC extracts showed no anti-inflammatory action in murine microglial cells
- They had antiviral activity in Coxsackievirus A9 and human coronavirus HCoV-OC-43
- They did not show cytotoxicity for normal and cancer human cells

Articles connected to the topic

- Granato, D. et al. **Enzyme-assisted extraction of anthocyanins and other phenolic compounds from blackcurrant (Ribes nigrum L.) press cake: From processing to bioactivities**,Food Chemistry,Volume 391,133240,ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2022.133240.
- Pap,N. et al. (2021).**Toxicological and bioactivity evaluation of blackcurrant press cake, sea buckthorn leaves and bark from Scots pine and Norway spruce extracts under a green integrated approach**,Food and Chemical Toxicology,Volume 153,112284, ISSN 0278-6915,https://doi.org/10.1016/j.fct.2021.112284.
- Pap, N, et al. (2013). **Microwave-assisted extraction of anthocyanins from blackcurrant marc**. Food and Bioprocess Technology, 6 (10), 2666-2674.

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