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Extraction of Anthocyanins from Black Currants and In Vitro Testing for the Determination of Antioxidant Activity

Introduction

Black currants

Rich source of vitamin C (50 - 280 mg/100 g)

Antioxidant, antimicrobial, anti-inflammatory, chemoprotective properties

Reducing the risks of cancer and cardiovascular diseases

Anthocyanins

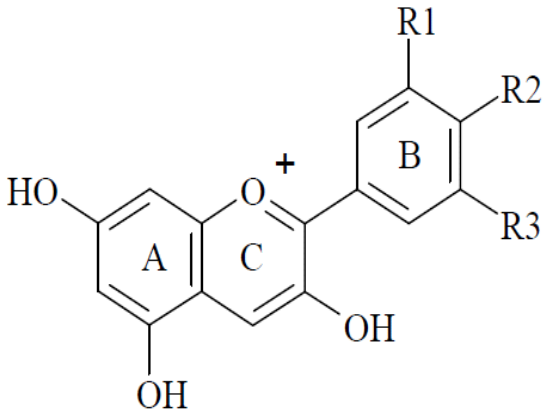
vacuoles of plant cells

Red
Purple
Blue

With increasing hydroxyl (OH) groups

Addition of methoxy groups (OCH3)

antioxidants and free radical scavengers, are able to act as reducing agents in the electron transfer reaction pathway.



General chemical structure and classification of anthocyanins (After Prior and Wu, 2006)

Name	*R1*	*R2*	*R3*
Pelargonidin	H	OH	H
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Peonidin	OCH_3	OH	H
Petunidin	OCH_3	OH	OH
Malvidin	OCH_3	OH	OCH_3

Purpose and objectives

- The purpose of this paper is to investigate the antitumoral effects produced by exposure to blackcurrant extract, rich in anthocyanins, on cervical cancer.

- The objectives of this study are represented by:

obtaining blackcurrant extract

assessment of antioxidant capacity
- DPPH test (2,2-difenyl-1-picrylhydrazyl)
- Oxigen radical absorbance capacity (ORAC)
- The radical neutralization test of NO

evaluation of the viability of the HeLa cell line following exposure to certain concentrations of active compounds and measurement of the NO level

cytometric analysis for evaluate the mode of action on the phases of the cell cycle

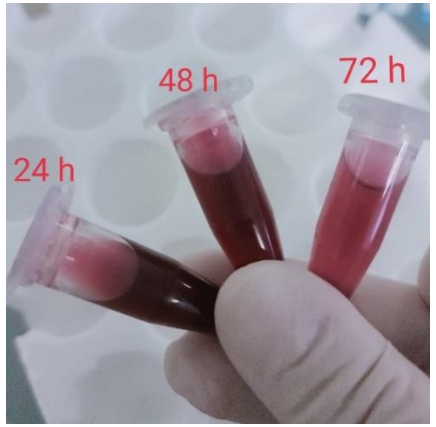
the effect of the extract on the antioxidant defense capacity
- evaluation of catalase activity
- assessment of reduced glutathione levels
- glutathione S-transferase level

Materials and methods

- *Extraction of anthocyanins from blackcurrant powder*
- *Determinations of the antioxidant capacity by the DPPH test*
- *Determination of total polyphenol content*
- *Determination of NO neutralization capacity*

HeLa Cells

- *Viability assay and measurement of nitric oxide levels*
  - ***NO test***
  - ***MTT test***
- *Catalase activity dosage*
- *Glutathione S-transferase dosage*
- *Determination of the concentration of reduced glutathione (GSH)*
- *Cell cycle analysis by flow cytometry*



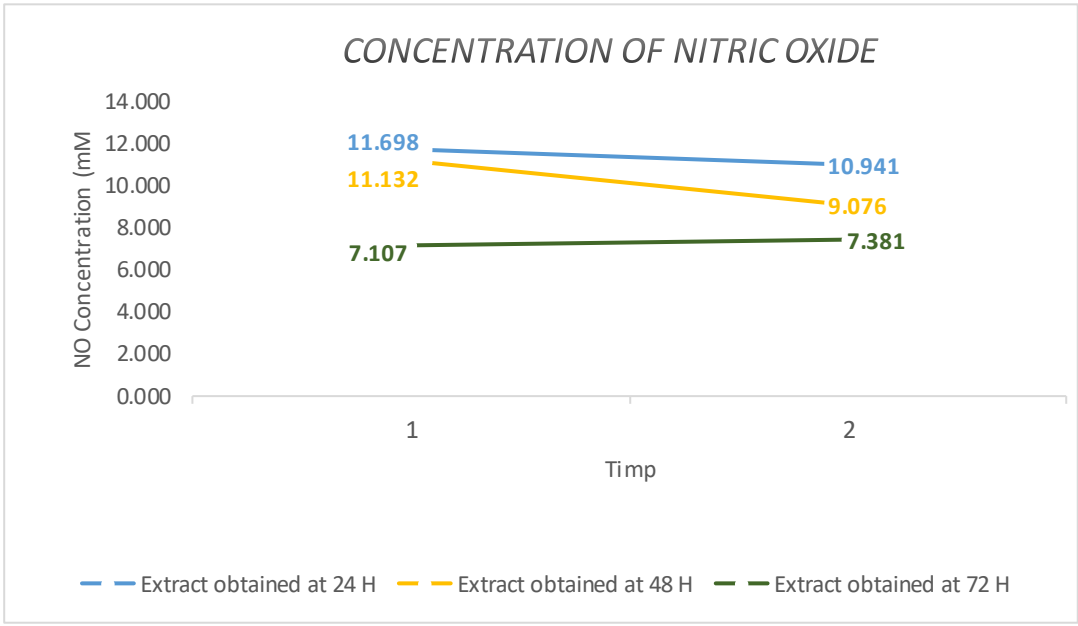
Concentrations: 0,5 µg/mL polyphenol
2,5 µg/mL polyphenol

Time intervals: 24 and 72 hours

Results and discussions

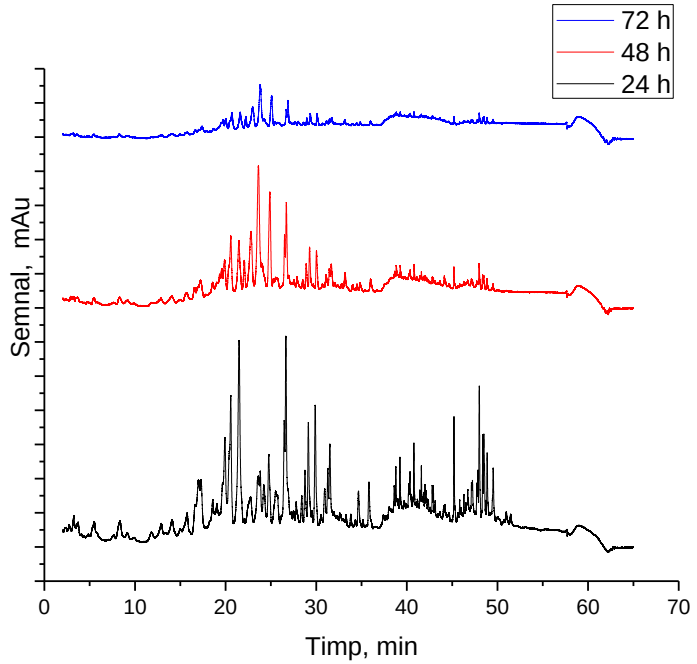
Determination of the antioxidant capacity of blackcurrant extracts

Extract	Anthocyanin content (mg/L)	DPPH test (% inhibition of reactive oxygen species)	ORAC (μM Trolox)	Total polyphenol content (mg GAE/mL)
24 hours	63.465	16%± 0.002	15.6	75.10
48 hours	364.550	8% ±0.21	12.6	51.69
72 hours	0	6% ±0.087	5.6	20.80



Graphic representation of nitric oxide scavenging capacity at 0 minutes (1) and 30 minutes (2)

Chemical analysis of the extracts by the HPLC method

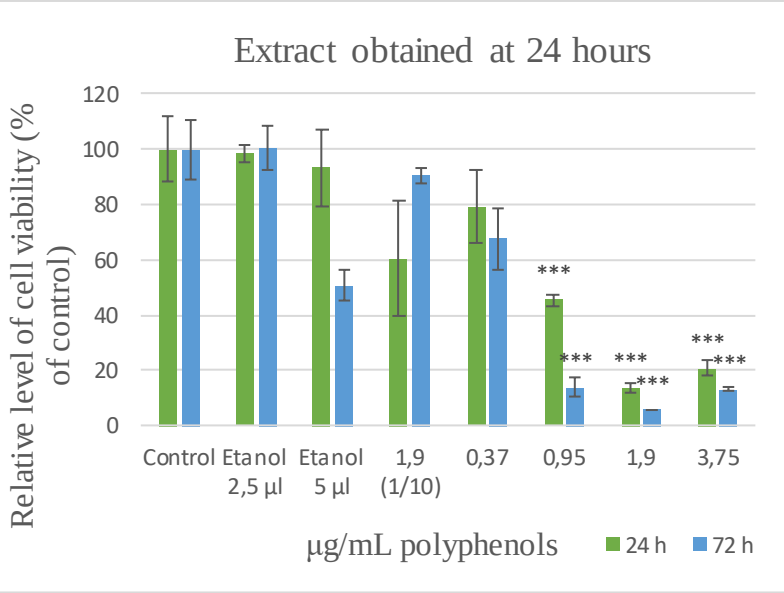


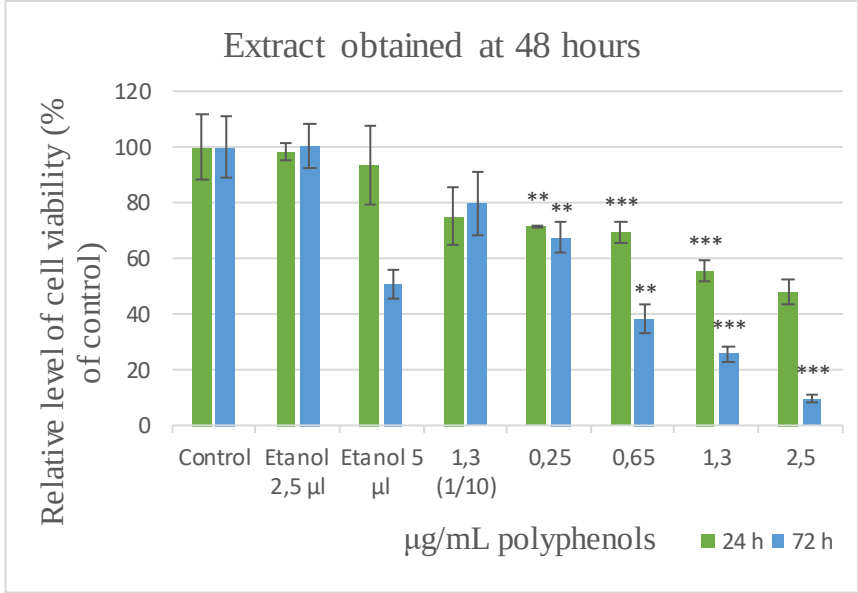
HPLC profiles of the extracts obtained at the 3 time intervals. Peak identifications are suggested based on HPLC standards, mass spectrometry analysis, and literature comparisons

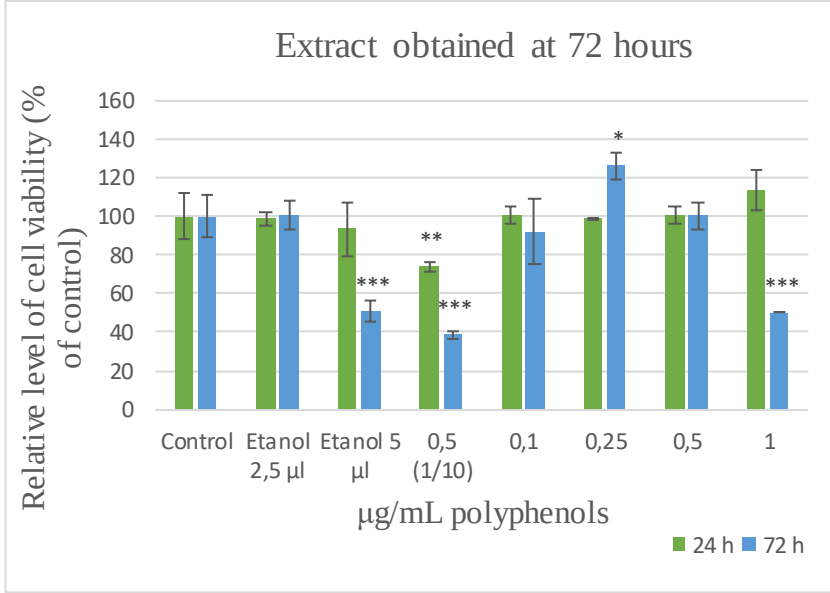
	Extract obtained at 24 hours (mg/L)	**Extract obtained at 48 hours (mg/L)**	**Extract obtained at 72 hours (mg/L)**
Galic acid	3.258	0.628	0
Catechin	41.692	31.513	0
Caffeic acid	0.066	0.412	0
Chlorogenic acid	5.747	13.446	4.632
Epicatechin	180.196	942.155	360.949
Delphinidin	39,985	364.550	0
Coumaric acid	1.621	0	0
Daidzein	14.557	0	0
Hiperosiide	40.879	0	0
Rutin	3.754	1.481	0.640
Naringin	17.301	5.130	0.990
Malvidin	23.478	0	0
Quercitin	1.033	1.127	0
Naringenin	2.254	0	0
Genistein	0	0	0

Viability assay and measurement of nitric oxide (NO) level – HeLa cells

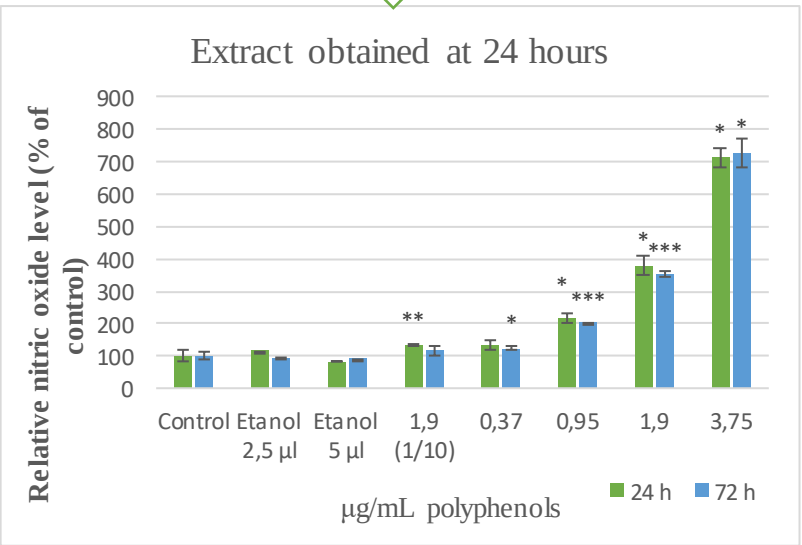
MTT test

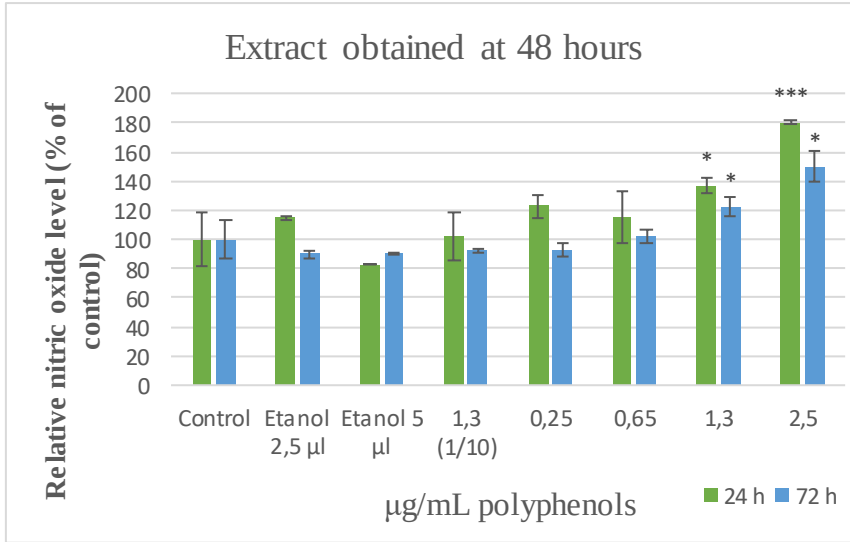


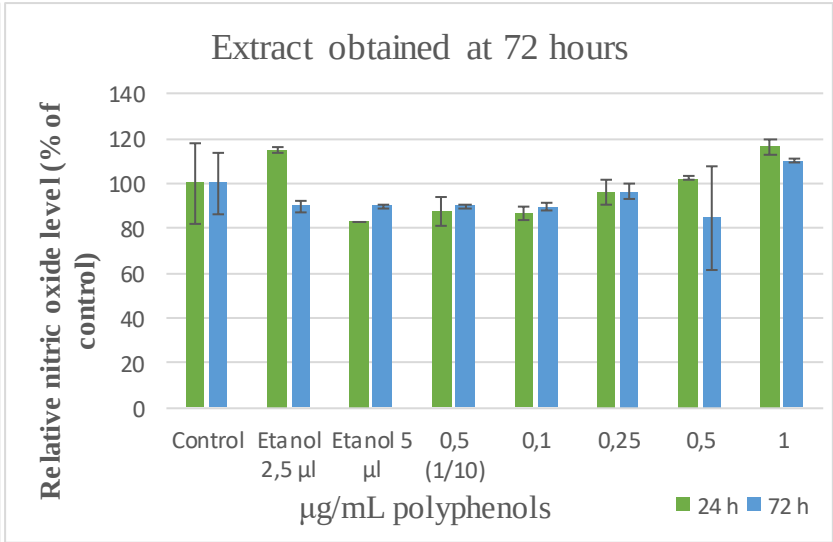




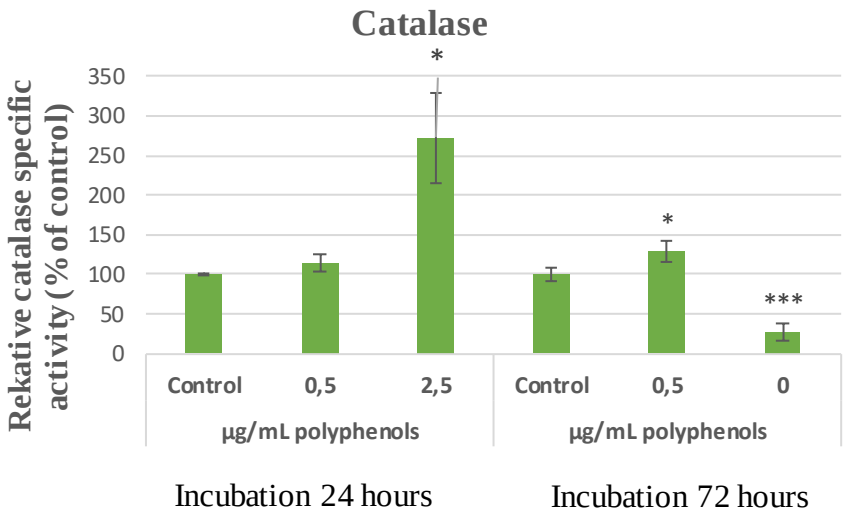
NO test



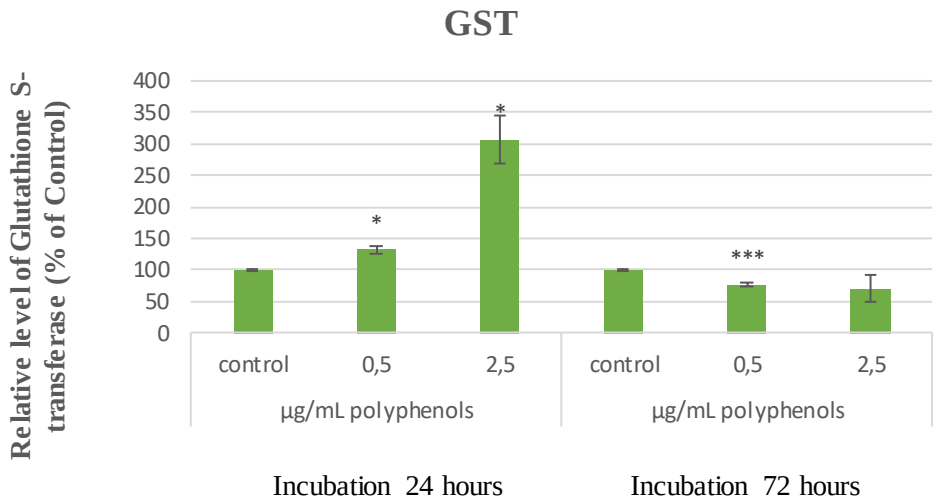




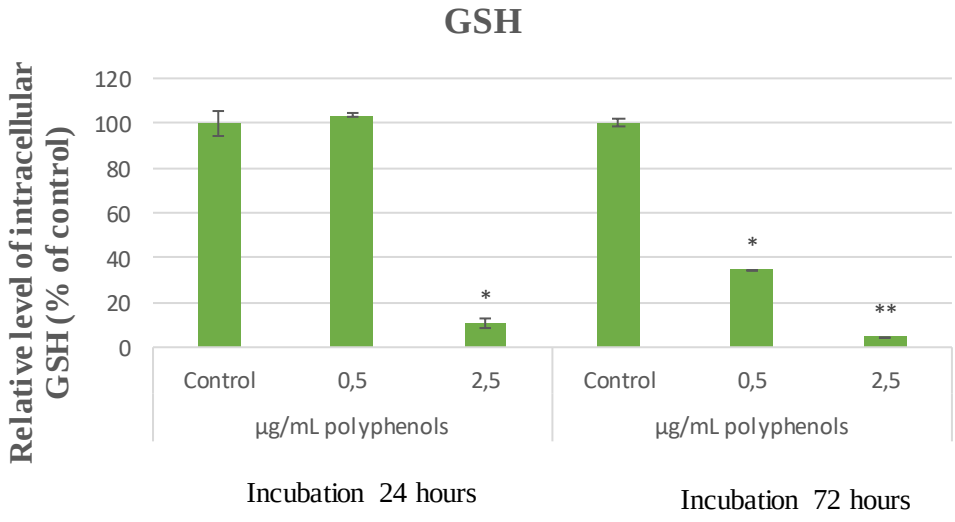
Determination of the antioxidant enzyme activities



Evolution of the catalase activity after exposure for 24 and 72 hours to different concentrations of the extract obtained at 48 hours

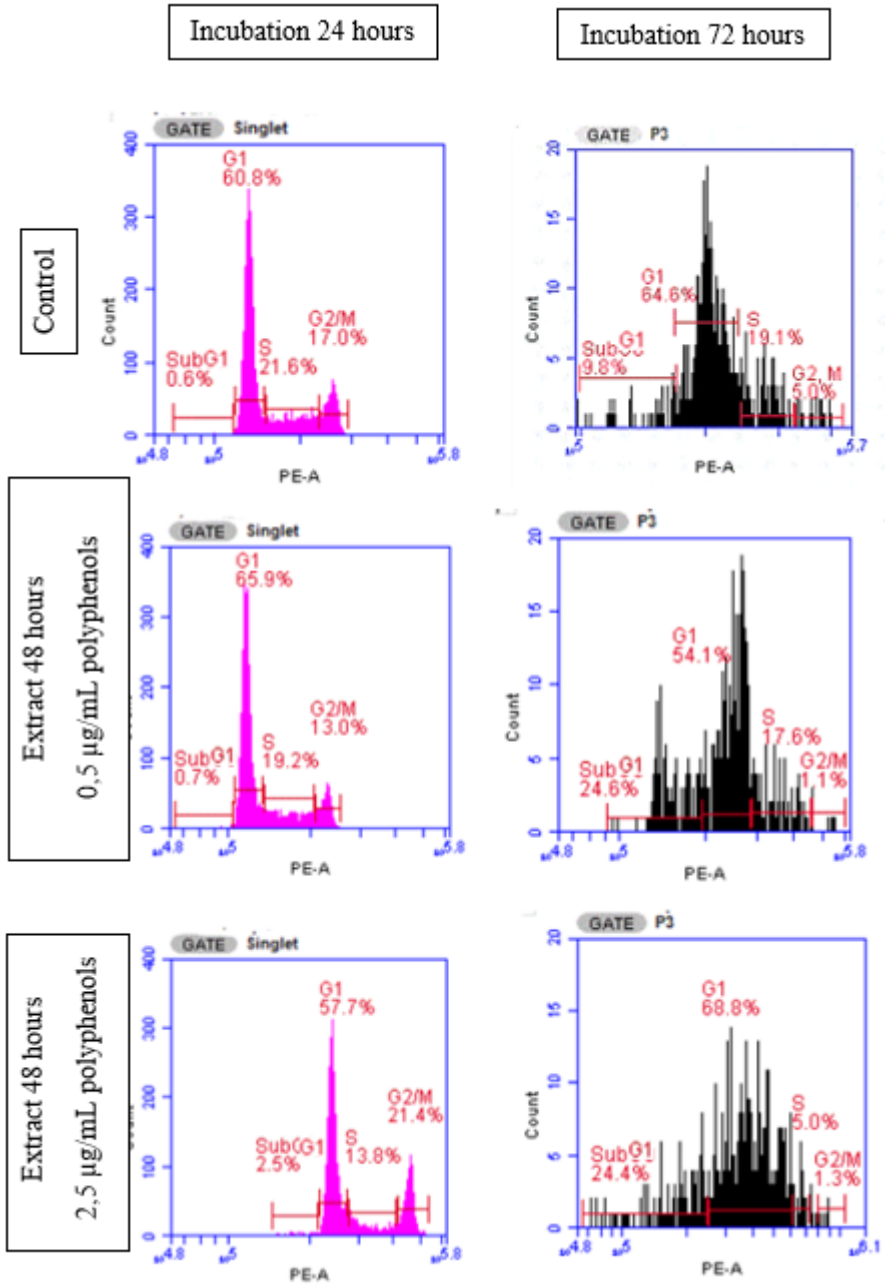


Glutathione-S-transferase (GST) concentration after exposure for 24 and 72 hours to different concentrations of the extract obtained at 48 hours



The concentration of reduced glutation (GSH) after exposure for 24 and 72 hours to different concentration of the extract obtained at 48 hours

Determination of cell cycle phases



Percentage results obtained from flow cytometry analysis at 24 and 72 hours with different concentrations of the extract obtained at 48 hours.

Incubation 24 hours	**Sub-G1 (% cells)**	**G0/G1 (% cells)**	**S (% cells)**	**G2/M (% cells)**
Control	0.65	60.83	21.58	17
0,5 µg/mL polyphenols	0.74	65.93	19.19	12.99
2,5 µg/mL polyphenols	2.51	57.68	13.77	21.44

Incubation 72 hours	**Sub-G1 (% cells)**	**G0/G1 (% cells)**	**S (% cells)**	**G2/M (% cells)**
Control	9.76	64.64	19.09	4.99
0,5 µg/mL polyphenols	24.57	54.13	17.61	1.09
2,5 µg/mL polyphenols	24.35	68.75	4.96	1.29

Conclusions

The extract with the highest concentration of polyphenols, the absorption capacity of O2 radicals (ORAC), but also the ability to inhibit the propagation of free radical reactions was the one obtained at 24 hours.

The extract richest in anthocyanins (delphinidin, malvidin), with an antiproliferative capacity and a higher level of NO neutralization was the one obtained at 48 hours, which is why it was chosen for the in vitro studies with HeLa cells, of cancer cervix.

The decrease in cell viability for the extract obtained at 48 h was dependent on the time and concentration used.

An increase in enzyme activity (catalase and glutathione S-transferase) was noted after the first 24 hours of incubation in the case of the highest concentration tested, which suggests a tendency of the cells to counteract the oxidative stress induced by blackcurrant extract, followed by a significant decrease in the activity of these enzymes, also correlated with the reduced level of GSH.

Incubation of cells with blackcurrant extract resulted in cell cycle arrest in the G1 and sub-G1 phases after 24 and 72 hours, respectively.