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Approach regarding the biosafety evaluation of black and red currant pomace extracts using *Allium cepa* test

Luminiţa GROSU,[1] Elisa FERENȚ,[2] Daniela NICUŢĂ,[*2] and Irina-Claudia ALEXA[†1]

[1] *"Vasile Alecsandri" University of Bacău, Faculty of Engineering, Department of Chemical and Food Engineering 157, Calea Mărăşeşti, Bacău, 600115, Romania*
[2] *"Vasile Alecsandri" University of Bacău, Faculty of Sciences, Department of Biology, 157, Calea Mărăşeşti, Bacău, 600115, Romania*

Abstract. In the present work, biosafety evaluation of black and red currant pomace extracts was conducted using *in vivo* plant test system, such as *Allium cepa*, a sustainable method which can provide valuable information on the cytotoxic and genotoxic effect of extracts from natural sources in relation with their phytochemical composition. In this view, different aqueous and hydroethanolic extractions from black and red currant pomaces were carried out. For revealing the differences in the phytochemical profile of the studied extracts, rapid, efficient and easy-to-operate analytical techniques such as colorimetry, UV-Vis spectrometry and electrometry were used. Cytogenetic analysis of pomace extracts was achieved using *Allium cepa* test by scoring the mitotic index, the limit value of cytotoxicity, the phase index of mitosis and presence of chromosomal aberrations for all samples and comparing to the control (tap water). The results have shown that the cytogenetic response depends both on the type of pomaces and on the experimental extraction conditions. Compared to the control sample (22.58 %), a decrease in mitotic index for each analyzed sample was observed. As well, an increase in the cells with chromosomal aberrations was detected in onion root tips exposed to the tested extracts compared to the control (0.69 %). The lowest value of mitotic index (12.44 %) and the highest value of chromosomal aberrations (1.91 %) were recorded from the root tip cells of onion bulbs exposed to the hydroalcoholic extract obtained from red currants pomace using water/ethanol (60:40 v/v). Even if the mitotic index decreased, the limit value of cytotoxicity was higher than 50 %, level considered as a sublethal condition for the organisms. At the same time, the increase of the frequency of chromosomal aberrations is not so significant, and in correlation with the cytotoxicity limit, it does not indicate a genotoxic effect on onion cells. Following these results, it can be concluded that black and red currant pomace extracts can be safely used for possible therapeutic benefits.

***Keywords*:** by-products; berry pomace extracts; mitotic index; chromosomal aberrations; *Allium cepa* assay.

1. Introduction

In recent years, the interest on by-products, which remain after processing of fruit and vegetable in food industry, has increased worldwide [1, 2].

During the production of juice, seeds, peels and pulp rests end up in the press-residue which still contains a huge amount of bioactive compounds. As a pressing residue of the juice industry, pomace represents a valuable source of potentially healthy phytochemicals such as tannins, phenolic acids, flavonoids, anthocyanins, etc. which are well-known that possess antioxidant and radical scavenging capacities [3-5].

The pomaces valorization can occur in different ways and in various fields. The most available use of pomace is the use in animal feed [6, 7] and as a fertilizer [8, 9].

Another method to reuse the pomaces consists in the extraction of the bioactive compounds using different technologies in view to obtain the compounds of interest for developing valuable products: food additives as an alternative to synthetic additives, functional foods, dietary supplements, pharmaceutical and cosmetic products, etc. [4, 5, 10, 11].

Our research approaches have been lately focused on finding sustainable methods of valorizing some by-products and waste of agro-food origin, either by obtaining new functional food products, through the grape marc powder's incorporation [12], or by investigating the potential application of the pomace extracts on *in vitro* plant growth and development [13].

With the aim of continuing to find innovative applications for other by-products from the beverage industry, our research group also became interested in black and red currants pomace, due to their composition rich in bioactive molecules and to their therapeutic potential [14, 15].

The interest of researchers in berry by-products, such as black and red currant pomaces, has recently increased due to their content in bioactive compounds, especially flavonoids and anthocyanins, as well as rich source of dietary fiber for obtaining food ingredients with functional properties [20, 21].

[*] Corresponding author. *E-mail address:* daniela.nicuta@ub.ro (Daniela Nicuţă)
[†] Corresponding author. *E-mail address:* irinaalexa@ub.ro (Irina-Claudia Alexa)

Therefore, one approach to exploit the black and red currant pomaces is to obtain their extracts in view of using them to positively modulate the key indicators of the health status of a consumers' body. For example, in their investigation, Untea *et al.* presented the therapeutic potential of the black currant pomace in human digestion using a simulated *in vitro* model [22]. Also, some studies reported the ability of black currant pomace to attenuate the intestinal tract changes using rabbits or rats as *in vivo* model [23, 24].

It is well-known that *in vitro* and *in vivo* tests represent an important step in the biosafety assessment of the plant-based extracts in view to establish a correlation between their phytochemical profile and the possible involvement and contribution of their use in preservation on human health. In order to evaluate the biosafety and effect of different compounds, drugs or supplements, researchers may use plant-based tests before moving on to more laborious and expensive methods which may include cell lines, animals or humans [25, 26].

As a part of a recent research, our research group turned its attention to a plant-based assay used for biosafety screening, namely *Allium cepa* test which is proven to be a sustainable method [27]. This highly sensitive and reproducible bioassay represents an efficient bioindicator in genotoxicity testing [28-30] and can provide valuable information on the cytotoxic and genotoxic effects of different plant extracts in relation with their phytochemical composition [27, 31, 32].

In this context, our research has focused on bringing new arguments regarding the effectiveness of this test in the assessment of the cytotoxicity and genotoxicity of extracts from plant by-products with therapeutic potential generated by the presence of bioactive compounds. To the best of our knowledge, following the literature review, no study has been reported so far on the comparative effects of black and red currant extracts on cell division using the *Allium cepa* test.

Furthermore, rapid, efficient and easy-to-operate analytical methods such as electrometry, colorimetry and UV-Vis spectrometry were used for revealing the differences in the phytochemical profile of the investigated extracts.

Our research may represent a part of a contribution in view of pomaces valorization and for a better knowledge of the biosafety potential of black and red currant pomace aqueous and hydroalcoholic extracts on onion meristematic cells, by scoring the mitotic index (MI) and chromosomal aberrations (CA) for all samples and comparing to the control sample.

Therefore, the present study was carried out aiming to investigate the influence of black and red currant pomace extracts on root cells of *Allium cepa*, evaluating their cytotoxic and genotoxic effects in order to assess their safety for possible therapeutic purposes.

2. Experimental

2.1. Materials and reagents

Analytical grade chemicals used for the present study were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany), *i.e.* Folin–Ciocalteu phenol reagent, sodium carbonate, gallic acid, glacial acetic acid, absolute ethanol, hydrochloric acid and carbol-fuchsin. The absolute ethanol used for the hydroalcoholic extraction of pomaces was purchased from Chemical Company S.A. (Iași, Romania).

2.2. Preparation of black and red currant pomace extracts

Black currant (*Ribes nigrum* L.) and red currant (*Ribes rubrum* L.) berries were purchased from local market. The extraction of the juice from the berry fruits was performed using household juice extractor (Heinner TurboMax 1000, China). After the complete removal of the juice, the obtained pomace mainly contains peels and seeds. For the extraction process, the obtained pomaces were used as such.

The moisture content of black and red currant pomaces (70.38 ± 0.52 %) was established using a Moisture Analyser (KERN MLB 50-3, Germany) by drying at 105 °C until a constant mass. Moisture was calculated by the difference in the mass of the sample before and after drying; the result was expressed in percentage of moisture.

Aqueous and hydroalcoholic extractions using water or hydroethanolic mixture were carried out, the ratio between pomace mass (g) and solvent volume (mL) being 1:100 (*i.e.* 2 g : 200 mL). Tap water was used for aqueous extracts and commercial ethanol for the hydroalcoholic extracts. The aqueous extractions were carried out by infusion and decoction, respectively. The hydroalcoholic extractions were performed using two different hydroalcoholic mixtures by classical extraction under agitation (Table 1). Filtration through Whatman filter paper No. 2 was realized after extraction.

The detailed extractions conditions for black and red currant pomace extracts are summarized in Table 1.

Table 1. Experimental extraction conditions for prepared pomace extracts.

Samples code	Pomace	Extraction conditions		
		Operator Mode	Solvent	Time
IBC	Black currant	Infusion	200 mL boiling tap water [27]	5 min at rt** [27]
IRC	Red currant			
DBC	Black currant	Decoction		5 min boiled and 5 min at rt** [27]
DRC	Red currant			
E_1BC	Black currant	Classical extraction under agitation	200 mL HA* (water/ethanol 80:20 v/v) [27]	stirred at rt** 150 rpm for 30 min [27]
E_1RC	Red currant			
E_2BC	Black currant		200 mL HA* (water/ethanol 60:40 v/v) [27]	
E_2RC	Red currant			

*HA - hydroalcoholic mixture; **rt - room temperature.

Until the initiation of the *Allium cepa* assay, the obtained extracts were kept at 4 °C in the refrigerator, for 2-3 days. These were used as such, without further concentration or dilution.

2.3. Pomace extracts analysis

For each prepared pomace extracts, several physicochemical parameters such as *p*H, total dissolved solids (TDS) and electrical conductivity (EC) were determined using Thermo Scientific™ Orion™ Versa Star Pro™ Multiparameter Benchtop Meter (Thermo Fisher Scientific, USA).

As well, a phytochemical screening was realized by UV-Vis scanning the studied pomace extracts using wavelength ranging from 190 - 1100 nm with UV-Vis Spectrophotometer (Shimadzu UV-1280, Japan).

Color intensity ($CI = A_{420} + A_{520} + A_{620}$) of pomace extract samples was calculated [33, 34]. The spectrophotometric absorbances at 420 nm (A_{420}), 520 nm (A_{520}) and 620 nm (A_{620}) were recorded using UV-Vis Spectrophotometer (Shimadzu UV-1280, Japan).

Also, the total polyphenols content (TPC) expressed as mg gallic acid equivalents (GAE) / 100 mL extract was evaluated using the Folin-Ciocalteu method. For this purpose, for each sample, 100 µL of extract was used then 7.9 mL distilled water and 500 µL Folin–Ciocalteu reagent were added and mixed well. After 5 minutes, 1.5 mL sodium carbonate (20 %) was added. The mixture was allowed to stand for 30 minutes, at room temperature (20 °C) [35]. Absorption determination at 750 nm was made spectrophotometrically (UV-Vis Spectrophotometer - Shimadzu UV-1280, Japan). A calibration curve was plotted ($y = 0.0009x - 0.0047$; $R^2 = 0.9957$) using standard gallic acid (50-500 mg/L) [27].

All measurements were realized in triplicate.

The analysis carried out as well as the equipment used is illustrated in Figure 1.

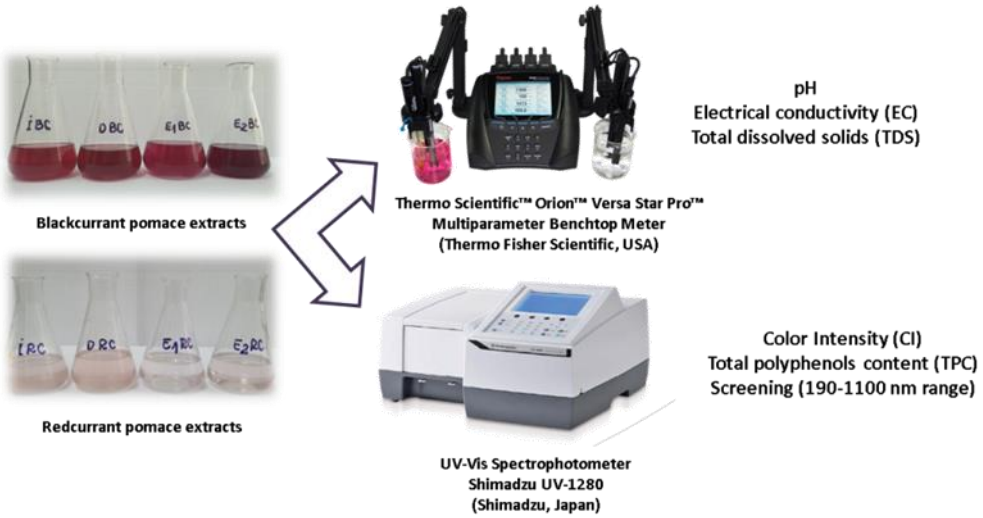

Figure 1. Black and red currant pomace extracts analysis.

2.4. Allium cepa test and cytogenetic analysis

Allium cepa bioassay experiment is schematically presented in Figure 2, and was conducted following the procedure described in details in our previously study [27].

Briefly, the main steps for cytogenetic preparation consisted in:

- keeping the onion bulbs in contact with the pomace extracts in controlled conditions (in a Sanyo growth chamber - model MLR-351, Japan: temperature = 22 °C; time = 24 h in the dark) [27];
- harvesting and fixation of the roots in Farmer's solution (18 h in refrigerator);
- hydrolyzing the roots (by immersing them for 10 minutes in a 50 % hydrochloric acid solution, at room temperature);
- coloring the chromosomes (using carbol-fuchsin staining solution 10 %);
- preparing the roots for microscopic evaluation by the "squash" technique.

Tap water was used for placing onion bulbs in the case of the control sample.

In order to determine different parameters of root meristematic cells as indicators of cytotoxicity and genotoxicity, cytogenetic investigation of microscopic slides was performed using an optical microscope Novex (model AP-8 LED, binocular, The Netherlands) at 40x and 100x magnification.

Through this analysis the type and frequency of cells in the division phases were examined as well as the type and frequency of chromosome aberrations. For each variant, 30 microscopic fields were examined and all measurements were realized in triplicate.

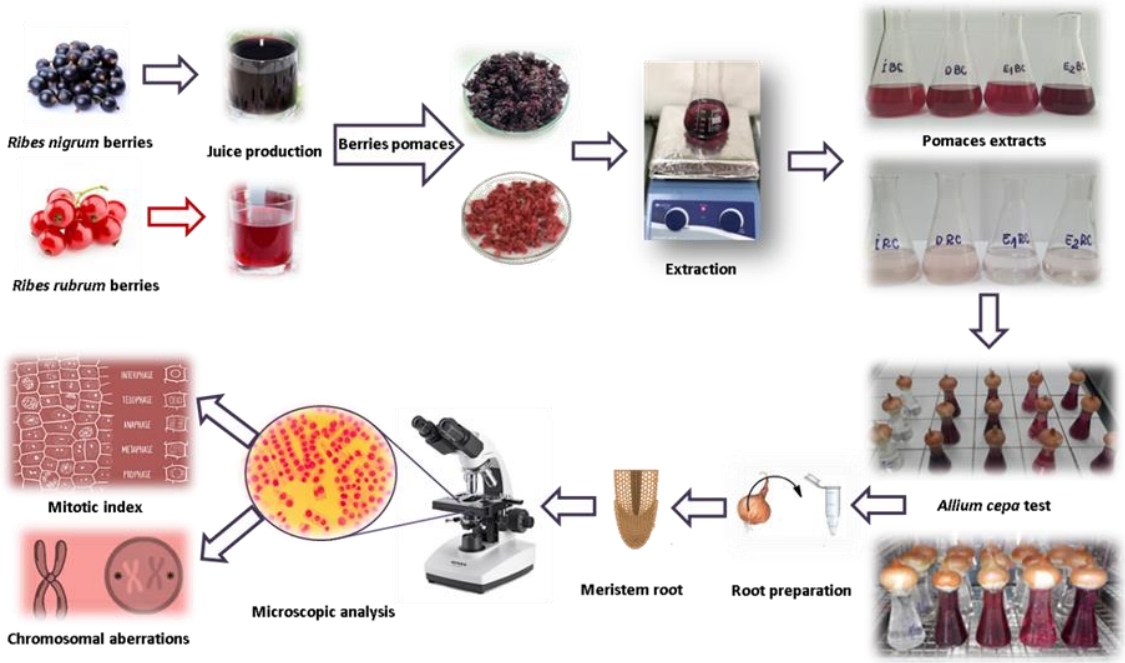

Figure 2. Schematic diagram of *Allium cepa* experiment.

Four parameters were evaluated for performing cytogenetic analysis: mitotic index, phase index, limit value for cytotoxicity and chromosomal aberration. The calculation formulas for each parameter are resumed in Table 2.

Table 2. Calculation formula for cytogenetic parameters.

Cytogenetic parameter	Calculation formula
Mitotic index	$\mathbf{MI}\ \% = \frac{Number\ of\ cells\ undergoing\ mitosis}{Total\ number\ of\ analyzed\ cells}\ x\ 100$
Phase index	$\mathbf{PI}\ \% = \frac{Number\ of\ cells\ in\ each\ mitotic\ phase}{Total\ number\ of\ analyzed\ cells}\ x\ 100$
Limit value for cytotoxicity	$\mathbf{LVC}\ \% = \frac{Mitotic\ index\ of cells\ exposed\ to\ pomace\ extracts}{Mitotic\ index\ of cells\ exposed\ to\ water\ (control)}\ x\ 100$
Chromosomal aberration	$\mathbf{CA}\ \% = \frac{Total\ chromosonal\ aberration}{Total\ number\ of\ analyzed\ cells}\ x\ 100$

2.5. Statistical analysis

For the descriptive statistics (mean, standard deviation - SD) and Pearson correlation analysis, Microsoft Office Excel 2013 (15.0.5589.1000) software of the Windows XP operating system was used.

The correlogram representing the Pearson correlation coefficient matrix was made using Origin 2024 10.1.0.170 (Academic - OriginLab Corporation).

3. Results and discussions

3.1. Pomace extracts analysis

The values summarized in Table 3 represent the analyzed physicochemical parameters such as *p*H, total dissolved solids (TDS) and electrical conductivity (EC) of black and red currant pomace extracts, as well as the color intensity (CI) and total phenolic content (TPC).

It can be noticed that the *p*H values for black currant pomace extracts are between 5.27-5.68, while those from red currants pomace are much more acidic with values between 3.47-3.85.

Regarding TDS and EC, it is observed that the hydroalcoholic extracts present the lowest values, while in the aqueous extracts these values are considerably increased (IRC and DRC, followed by DBC and IBC), being 5 - 9 times higher than the hydroalcoholic extracts. The maximum value for TDS (238.16 ppm) is recorded for the sample obtained from red currant pomace by infusion (IRC), the same sample having the highest EC value (477.06 µS/cm). The E_2RC sample obtained by hydroalcoholic extraction with water/ethanol (60:40 v/v) shows the lowest values for both TDS (27.61 ppm) and EC (56.4 µS/cm).

The highest TPC values are acquired in the case of black currant pomace extracts compared to red currant pomace extracts. The obtained values are in agreement with other researches [10].

The DBC aqueous extract obtained by decoction present a maximum value for TPC (204.85 mg GAE /

100 mL extract) followed by IBC extract obtained by infusion (108.92 mg GAE / 100 mL extract). The E_2BC extract obtained by hydroalcoholic extraction using water/ethanol (60:40 v/v) has a value close to that of the IBC extract (105.22 mg GAE / 100 mL extract). The E_1RC sample presented the lowest values for TPC (18.18 mg GAE / 100 mL extract) and CI (0.20), respectively.

The highest value for the color intensity corresponds to the DBC sample obtained by decoction from black currant pomace (1.98) and is four times higher than the similar sample DRC obtained from red currant pomace (0.47).

Table 3. Measured physicochemical and chemical parameters of black and red currant pomace extracts.

Samples	Characteristics				
	***p*H at 25 °C**	**TDS [ppm]**	**EC [µS/cm]**	**TPC [mg GAE / 100 mL extract]**	**CI***
IBC	5.68±0.003	153.86±0.15	312.9±0.79	108.92±1.69	0.97±0.001
DBC	5.27±0.005	186.76±0.13	380.63±0.25	204.85±2.31	1.98±0.001
E_1BC	5.45±0.003	74.30±0.13	151.73±0.15	67.44±1.11	0.79±0.003
E_2BC	5.54±0.004	39.57±0.16	80.60±0.254	105.22±1.11	0.93±0.002
IRC	3.62±0.01	238.16±1.80	477.06±1.33	37.81±1.69	0.36±0.001
DRC	3.47±0.01	225.46±0.51	458.23±0.25	45.22±1.92	0.47±0.002
E_1RC	3.60±0.03	45.08±0.04	91.97±0.02	18.18±1.69	0.20±0.003
E_2RC	3.85±0.002	27.61±0.01	56.40±0.06	35.96±1.28	0.31±0.001

Values are expressed as mean ± SD. Each value represents the average from three measurements.
*Calculated as sum of the absorbance at 420 (yellow), 520 (red) and 620 (blue) nm

For the measured physicochemical and chemical parameters of black and red currant pomace extracts, a Pearson correlation analysis was performed (Figure 3).

The strongest positive correlation was observed between the following parameters: TDS-EC (0.999) and TPC-CI (0.992). A positive correlation is also recorded in the case of the *p*H parameter with TPC (0.709) and CI (0.702), respectively.

Moreover, a low positive correlation (0.214-0.235) can be identified between TDS-TPC, EC-TDS, TDS-CI and EC-CI, respectively.

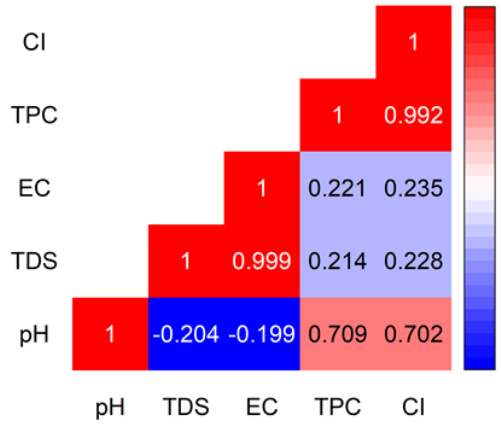

Figure 3. Correlogram representing the Pearson correlation coefficient matrix between the physicochemical and chemical parameters determined for black and red currant pomaces extracts.

Spectroscopic methods prove to be efficient analytical tool for bioactive compounds fingerprinting. Thus, the UV-Vis profiles of aqueous and hydroethanolic pomace extracts were recorded and are presented in Figure 4.

Through comparative UV-Vis analysis of the obtained spectra corresponding to the black currant pomace extracts, the major absorption bands were observed in the range from 260 to 280 nm and from 520 to 535 nm. It can be noticed that the four extracts obtained from black currant pomace show a comparable profile with a quite notable difference at the absorption level, especially in the range 520-535 nm, where the absorbance value is between 0.238 (for E1BC sample) and 0.872 (for DBC sample).

The four spectra of the red currant pomace extracts present an absorption band from 260 to 280 nm, band observed as well in the case of the black currant pomace extracts.

The qualitative UV-Vis spectroscopy of black and red currant pomace samples displayed intense absorption band at around 260-280 nm due to the presence of hydroxycinnamic acids, flavonols, flavanols and the UV absorption part of the anthocyanins. The obtained results were confirmed by comparing the absorption and wavelength values with available research data [36].

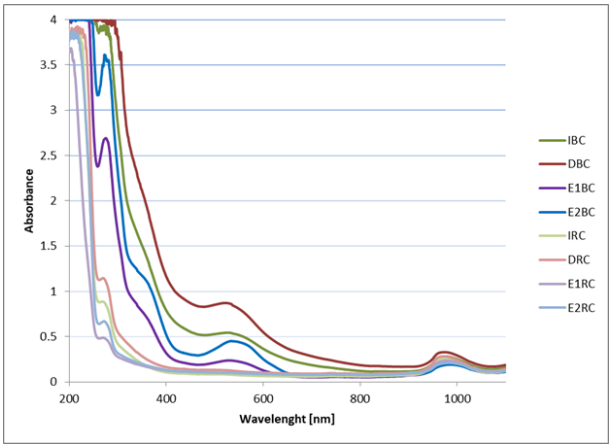

Figure 4. UV-Vis spectra of black and red currant pomace extracts.

It is well known that flavonol aglycons such as quercetin and myricetin and their glycosides are the compounds found in black and red currant pomace [23].

The band observed at 520-535 nm is due to the anthocyanin content especially in the case of black currant pomace extracts. Anthocyanins such as

delphinidin, malvidin and cyanide glycosides are strongly absorbing in this range, their bands overlapping [15, 37, 38]. This indicates that these compounds are present in the extracts obtained from black currant pomace.

3.2. Cytogenetic analysis of the black and red currant pomace extracts

The mitotic index (MI) constitutes an appropriate indicator of cell proliferation. A decrease in this cytogenetic parameter is related to an inhibitory effect, while an increase is induced by a stimulatory influence on the cell cycle. A severe reduction in MI is a sign of cytotoxicity of certain compounds found in the extracts from natural sources and it can be explained as a result of slowing down the rate of cell proliferation or as a consequence of the obstruction of the prophase beginning [39].

The data regarding the mitotic index (MI), phase index (PI) and the limit value for cytotoxicity (LVC) scored for onion roots treated with black and red currant pomace extracts are presented in Table 4.

Table 4. Cytogenetic parameters for onion roots treated with black and red currant pomace extracts.

Samples	MI [%]	LVC [%]	PI [%]			
			Prophase	Metaphase	Anaphase	Telophase
Control	22.58±2.43	-	79.73	5.68	5.03	9.56
IBC	19.71±1.92	87.28	81.1	4.03	4.63	10.24
DBC	18.61±0.98	82.41	79.75	5.30	4.04	10.91
E_1BC	16.15±1.56	71.52	77.63	5.21	5.10	12.06
E_2BC	13.68±0.99	60.58	69.90	7.47	7.58	15.05
IRC	17.80±1.41	78.83	80.30	3.80	5.29	10.61
DRC	14.84±1.03	65.72	82.00	4.61	3.99	9.40
E_1RC	13.89±1.22	61.51	74.87	5.74	4.90	14.49
E_2RC	12.44±0.95	55.09	70.02	8.80	8.53	12.65

Values are expressed as mean ± SD. Each value represents the average of three measurements.

The meristematic cells of *Allium cepa* for the control sample had an average mitotic index of 22.58±2.43 %. In the case of samples exposed to the black currant pomace extracts, the mitotic index range from 19.71 % to 13.68 %, while for those treated with red currant pomace extracts, the MI present values between 17.80 % and 12.44 %.

Thus, overall, for each tested sample, regardless of whether the extract is obtained from black or red currant pomace or it is aqueous or hydroalcoholic, a decrease in the mitotic index value (MI %) was observed compared to the control sample (Figure 5). Furthermore, the mitotic indexes for the hydroalcoholic samples were significantly lower than for the aqueous samples. This aspect is confirmed by other authors [40], cell division being affected by the presence of alcohol in the extraction solvent.

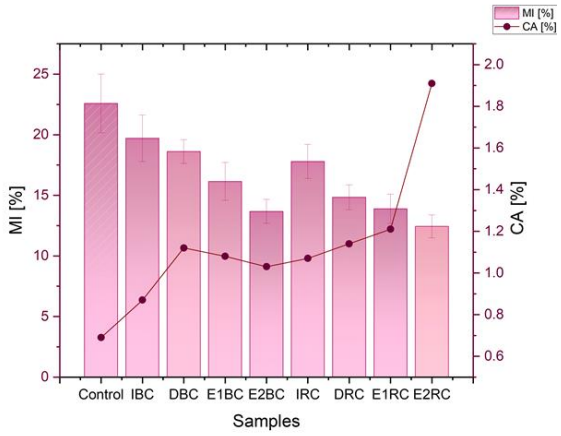

Figure 5. Mitotoc index (MI) and chromosomal aberrations (CA) for analyzed meristematic cells of *Allium cepa* exposed to black and red currant pomace extracts.

The closest mitotic index value (19.71 %) to that of the control sample (22.58 %) was registered in the case of cells treated with aqueous extract IBC obtain by infusion from black currant pomace. The lowest MI (12.44 %) was recorded for the root tip cells of onion bulbs exposed to the hydroalcoholic extract of red currants E_2RC.

It can be also noticed that all samples exposed to red currant pomace extracts have lower values of MI than those treated with black currant pomace extracts.

These results can be correlated with the physicochemical and chemical parameters measured for the pomace extracts presented in the Table 3.

It would have been expected that the higher content of polyphenols in the extracts obtained from black currant pomace would have a greater influence on the mitotic index decrease compared to the samples from red currant pomace which have a low content of polyphenols. It seems that, in the case of samples exposed to extracts from red currant pomace, the *p*H is the parameter that affects the mitosis rate of onion cell more than the total polyphenol content. Decreasing level of *p*H in the growth medium conducts to the reduction of mitotic index which is in accordance to literature [41].

A decrease of MI value by 50 % compared to the control constitutes the limit value for cytotoxicity (LVC); while a decrease under 22 % compare with the control is considered as a lethal condition for the organisms [29-31]. As it can be noticed from results presented in Table 4, not any extracts used in this study have an LVC lesser than 50 %, the lowest value of this parameter being calculated for the hydroalcoholic extract of red currants E_2RC (55.09 %).

According to the obtained results, a ranking of the samples that correlates the extraction method with the

highest MI values can be highlighted: IBC and IRC extracts followed by DBC and DRC and then by the hydroalcoholic extracts (E_1BC and E_1RC, E_2BC and E_2RC). This ranking show that the most advantageous extracts with a non-disruptive effect on cell division is the ones obtained by infusion.

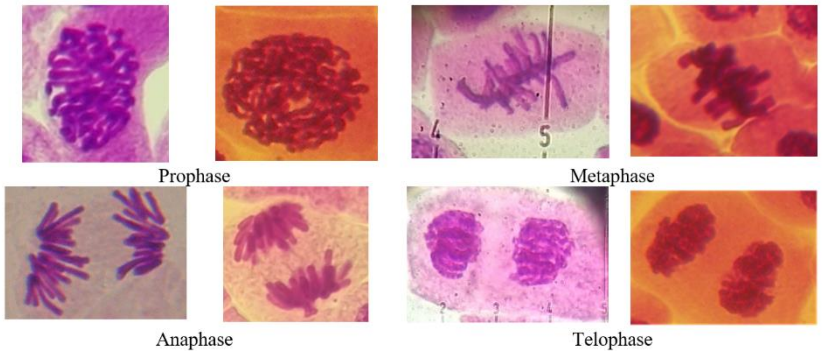

Figure 6. The four basic stages of mitotic division in cells of onion roots exposed to black and red currant pomace extracts.

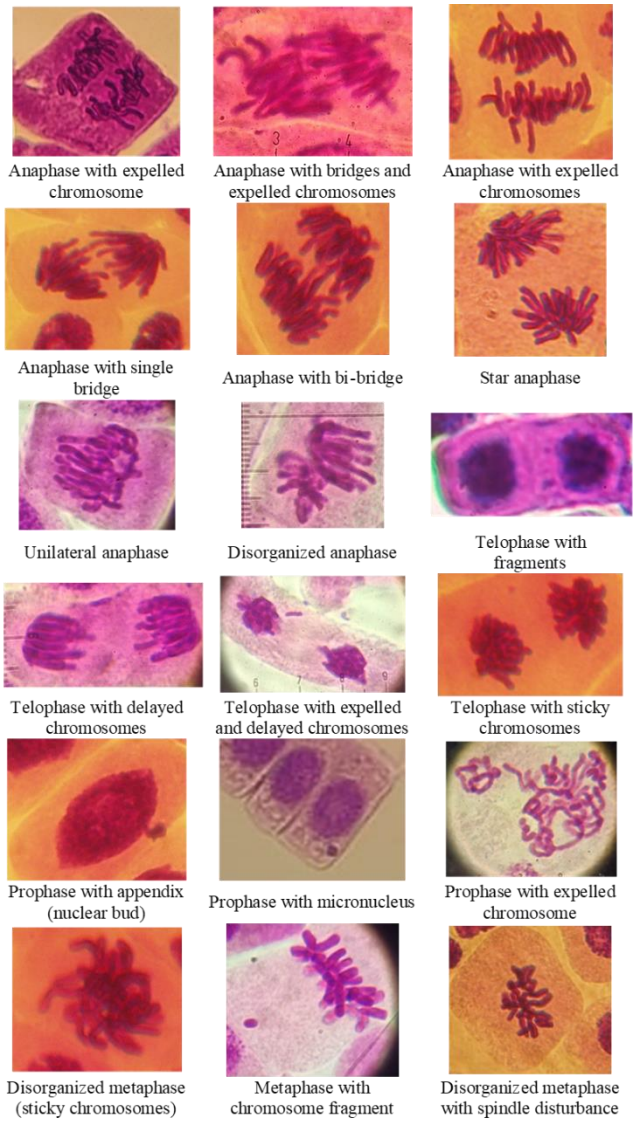

Figure 7. Cells with chromosomal aberrations of onion exposed to black and red currant pomace extracts.

Analyzing the results from Table 4 it can be observed that all phases of cell division were clearly identified, some changes of the phase index values liable to the type of extract used were observed.

It can be noticed that for all samples (including the control), the prophase index is reasonably the highest (69.90-82.00 %), followed by the telophase index (9.30-15.05 %). The metaphase index of *Allium cepa* root meristematic cells exposed to the investigated pomace extracts ranged from 3.80 to 8.80 % and the anaphase index from 9.99 to 8.53 %.

Concerning the prophase index, comparing to the control, a decrease was observed in the case of all hydroalcoholic samples (69.90-77.63 %), whether are obtained from black or red currant pomaces, while for

all aqueous samples an increase of this index was scored (79.75-82.00 %).

On the contrary, in the case of majority of samples the telophase indexes increased comparing to the control sample (9.56 %) with values comprises between 10.24-15.05 %. Only the samples DRC obtained by decoction from red currant pomace suffered an insignificant reduction in the index phase of telophase (9.40 %).

Figure 6 shows the four basic normal phases of mitotic division.

In some cytogenetic studies, to facilitate investigations, the analysis of different types of chromosomal aberrations is performed only for cells in anaphase and telophase of cell division [42].

In the case of our study, the analysis of cells was carried out in all phases of mitosis, including interphase for all analyzed and the control samples. This allows a more accurate assessment of the cytotoxic potential on *Allium cepa* cells. Our approach is accepted by other authors, because it implies a more comprehensive evaluation, therefore a better investigation of the action of the tested agents [40, 43, 44].

For all samples including the control, some chromosomal abnormalities were observed in all the mitotic stages.

Different types of cytological abnormalities at the appropriate mitotic stage identified in *Allium cepa* root cells treated with black and red currant pomace extracts are showed in Figure 7.

The percentage of the total chromosomal aberrations (CA %) and several types of chromosomal abnormalities are presented in Table 5.

Table 5. Chromosomal aberrations for onion roots treated with black and red currant pomace extracts.

Samples	CA [%]	Aberrations type						
		Micronucleus at prophase	A-T-B	A-T-DC	A-T-EC	A-T-CF	Disorganized anaphases	Abnormal metaphases
Control	0.69±0.04	0.12	0.15	0.03	0.09	0.15	0.09	0.06
IBC	0.87±0.09	0.03	0.24	0.17	0.24	0.05	0.10	0.04
DBC	1.12±0.08	0.07	0.17	0.17	0.31	0.17	0.19	0.04
E_1BC	1.03±0.11	0.05	0.23	0.20	0.27	0.12	0.07	0.09
E_2BC	1.08±0.07	0.10	0.19	0.07	0.39	0.14	0.12	0.07
IRC	1.07±0.07	0.10	0.10	0.24	0.24	0.07	0.19	0.13
DRC	1.14±0.11	0.08	0.18	0.08	0.34	0.09	0.29	0.08
E_1RC	1.21±0.06	0.17	0.15	0.20	0.11	0.11	0.30	0.17
E_2RC	1.91±0.07	0.17	0.33	0.29	0.33	0.20	0.38	0.21

Values are expressed as mean ± SD. Each value represents the average of three measurements.
A-T-B – ana-telophase with bridges; A-T-DC – ana-telophase with delayed chromosomes; A-T-EC – ana-telophase with expelled chromosomes; A-T-CF – ana-telophase with chromosomal fragments.

From the comparative analysis of the cytogenetic results, it can be noticed that the incidence of cells with chromosomal abnormalities depends both on the type of pomace (black and red currant pomace) and on the solvent used for extraction (aqueous / hydroalcoholic) in all samples analyzed.

The results presented in Table 5 reveals that all tested extracts increase the percentages of abnormal dividing cell of the onion root meristems, the chromosomal aberration for the samples treated with black currant pomace extracts range from 0.87 % to 1.08 %, while for those treated with red currant pomace extracts, the CA present values between 1.07 % and 1.91 %.

Thus, regardless of the type of pomace (black pomace or currant), the treatment of onion roots with the hydroethanolic extracts led to an increase in the percentage of abnormal cells, the highest CA value being recorded for the E_2RC sample (1.91 %) almost three times higher than the control sample (0.69 %).

The cytogenetic analyzes determined by the hydroethanolic extracts (E_1 and E_2) obtained from the two types of pomace, showed differences between them as well.

It can be noticed that the hydroalcoholic extracts with water/ethanol (60:40 v/v) showed a more pronounced disruptive effect on cell division compared to the hydroalcoholic extracts using water/ethanol (80:20 v/v) as solvent, because the percentage of abnormal cells highlighted was higher. These results are similar to those obtained in other studies, showing the influence of a larger volume of alcohol in the extractant solution [27, 40].

Moreover, unexpectedly the hydroalcoholic extracts obtained from red currant pomace induced a higher percentage of cells with aberrations (1.21 % for E_1RC and 1.91 % for E_2RC) compared to those obtained from black currant pomace (1.03 % for E_1BC and 1.08 % for E_2BC).

These results can be correlated both with those obtained for the mitotic index (Table 4) and with the physicochemical and chemical parameters measured for the pomace extracts (Table 3).

It can be supposed that the presence of alcohol as well as a low *p*H [41] in the case of red currant pomace extracts (E_1RC and especially E_2RC) determined the increase in the frequency of cells with aberrations.

Analyzing the results obtained both for the mitotic index and the frequency of abnormal cells in the sample exposed to hydroalcoholic extract from red currant pomace (E_2RC), even if the frequency of cells with aberrations was higher in this case, because the mitotic index does not reach the limit value for cytotoxicity, it allowed to consider that this sample have both non-cytotoxic and non-genotoxic effects on onion cells. In similar cytogenetic studies, a significantly low MI

correlated with a significantly high CA compared to the control, indicated a cytotoxic and genotoxic effect [45].

The analysis of the cytogenetic results of the aqueous extracts obtained by black current and red current pomace indicated that in the samples treated with extract obtained by decoction (DBC and DRC) percentages of abnormal cells were higher compared to those exposed to the extract obtained by the infusion (IBC and IRC).

From the results presented above, making the correlation between the extraction method and the CA frequency, it can be seen that the IBC extract stands out with the lowest CA value compared to the other samples, followed in the ranking by the E_1BC, IRC and E_2BC extracts with very close values. Therefore, the extracts obtained by infusion prove to be the most advantageous both in terms of non-cytotoxic and non-genotoxic effects.

Different types of cell abnormalities were identified and quantified by microscopic observations.

The most numerous cells with chromosomal aberrations were identified in anaphase and telophase (A-T) of mitotic division: cells with bridges, with expelled chromosomes, with delayed chromosomes, with chromosome fragments and disorganized anaphase cells, respectively. The frequency of these cells varied among the samples tested.

The highest percentage of abnormal cells represented by A-T with expelled chromosomes was calculated for sample E_2BC (0.39 %) four times greater than the control sample. Ana-telophase with bridges was also observed, the highest percentage being recorded in the E_2RC sample (0.33 %), compared to the control sample (0.15 %).

As well, in the onion roots treated with E_2RC the highest values for the following types of abnormal cells were recorded: disorganized anaphases (0.38 %), A-T cells with delayed chromosomes (0.29 %), A-T with chromosomal fragments (0.20 %).

Also, a reduced number of cells with chromosomal aberrations were highlighted in metaphase and prophase of mitotic division. Thus, some cells in metaphase presented unoriented or expelled chromosomes, and in prophase, cells with micronucleus were observed. The presence of these abnormal metaphase and prophase cells varies depending on the samples tested. The most frequent abnormal metaphase cells were calculated in the case of the sample E_2RC (0.21 %) and the prophases with micronucleus were observed especially in samples E_1RC and E_2RC (0.17 %).

5. Conclusions

The present study reports the possibility of valorization and safe utilization of some by-products from the beverage industry, namely the pomace extracts obtained from the fruits of two currant species - *Ribes nigrum* L. and *Ribes rubrum* L., by *in vivo* evaluation using the *Allium cepa* assay which is a sensitive and informative cytogenetic tool for the rapid screening of chemical and natural environmental substances interacting with human body.

Investigation of physicochemical and chemical parameters, as well as the phytochemical screening of the studied extracts allowed highlighting the differences in the phytochemical profile using rapid, efficient and easy-to-operate analytical techniques. The highest bioactive compounds content revealed by the UV-Vis spectrophotometric fingerprint and also the TPC values was observed for the black currant pomace extracts. The samples obtained from the red currant pomace presented the lowest *p*H values.

Regarding *Allium cepa* assay, our study showed that all phases of cell division were clearly identified in the onion root meristems exposed to the currant pomace extracts, indicating that the process of cell multiplication was not affected.

Different stages of mitotic division and several types of chromosomal aberrations were observed. The results have shown that the cytogenetic response depends both on the type of pomaces (from black or red currant) and on the extraction method / solvent used.

Even if the mitotic index for all extracts was inferior to the control sample, the limit value for cytotoxicity (LCV) is not reached (50 %) so that a cytotoxic effect induced by the tested extracts cannot be taken into account. At the same time, the increase of the frequency of chromosomal aberrations was not so significant, and in correlation with the cytotoxicity limit, it does not indicate a genotoxic effect on onion cells exposed to the pomace extracts. Following the ranking of the tested samples, the extracts obtained by infusion prove to be the most advantageous both in terms of non-cytotoxic and non-genotoxic effects.

The results of the present research validate the efficiency of the *Allium cepa* assay and recommend it as a useful tool for evaluating the cytotoxicity and genotoxicity of extracts from plant by-products with therapeutic potential. Overall, the study was carried out with environmentally friendly methods, with minimal costs, the results being easily reproducible.

It can be concluded that the black and red currant pomace extracts can be safely used for possible therapeutic purposes.

Conflict of interest

The authors declare no conflicts of interest.

References

[1]. C.V. Berenguer, C. Andrade, J.A.M. Pereira, R. Perestrelo, J.S. Câmara, Current challenges in the sustainable valorisation of agri-food wastes: A Review, Processes 11 (2023) 20. Doi: 10.3390/pr11010020

[2]. Z. Liu, T.S.P. de Souza, B. Holland, F. Dunshea, C. Barrow, H.A.R. Suleria, Valorization of food waste to produce value-added products based on its bioactive compounds, Processes 11 (2023) 840. Doi: 10.3390/pr11030840

[3]. N.A. Sagar, S. Pareek, S. Sharma, E.M. Yahia, M.G. Lobo, Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization, Comprehensive Reviews in Food Science and Food Safety 17 (2018) 512-531. Doi: 10.1111/1541-4337.12330

[4]. A. Patra, S. Abdullah, R.C. Pradhan, Review on the extraction of bioactive compounds and characterization of fruit industry by-products, Bioresources and Bioprocessing 9 (2022) 14. Doi: 10.1186/s40643-022-00498-3

[5]. Y. Chang, X. Shi, F. He, T. Wu, L. Jiang, N. Normakhamatov, A. Sharipov, T. Wang, M. Wen, H.A. Aisa, Valorization of food processing waste to produce valuable polyphenolics, Journal of Agricultural and Food Chemistry 70 (2022) 8855-8870. Doi: 10.1021/acs.jafc.2c02655

[6]. T.J. Erinle, D.I. Adewole, Fruit pomaces - their nutrient and bioactive components, effects on growth and health of poultry species, and possible optimization techniques, Animal Nutrition 9 (2022) 357-377. Doi: 10.1016/j.aninu.2021.11

[7]. C.M. Mnisi, G. Mhlongo, F. Manyeula, Fruit pomaces as functional ingredients in poultry nutrition: A review, Frontiers in Animal Science 3 (2022) 883988. Doi: 10.3389/fanim.2022.883988

[8]. A. Nosalewicz, M. Maksim, M. Brzezińska, J. Siecińska, A. Siczek, M. Nosalewicz, M. Turski, M. Frąc, B. Przysucha, J. Lipiec, The use of apple pomace as a soil amendment enhances the activity of soil microorganisms and nitrogen transformations and affects crop growth, Journal of Soil Science and Plant Nutrition 21 (2021) 1831-1841. Doi: 10.1007/s42729-021-00483-3

[9]. S. Korz, S. Sadzik, C. More, C. Buchmann, E. Richling, K. Munoz, Effect of grape pomace varieties and soil characteristics on the leaching potential of total carbon, nitrogen and polyphenols, Soil System 7 (2023) 49. Doi: 10.3390/soilsystems7020049

[10]. J.M. Ueda, M.C. Pedrosa, S.A. Heleno, M. Carocho, I.C.F.R. Ferreira, L. Barros, Food additives from fruit and vegetable by-products and bio-residues: A comprehensive review focused on sustainability, Sustainability 14 (2022) 5212. Doi: 10.3390/su14095212

[11]. A. Frum, C.M. Dobrea, L.L. Rus, L.I. Virchea, C. Morgovan, A.A. Chis, A.M. Arseniu, A. Butuca, F.G. Gligor, L.G. Vicas, O. Tita, C. Georgescu, Valorization of grape pomace and berries as a new and sustainable dietary supplement: Development, characterization, and antioxidant activity testing, Nutrients 14 (2022) 3065. Doi: 10.3390/nu14153065

[12]. E.M. Suceveanu, L. Grosu, I.C. Alexa, A. Fînaru, Valorisation potential of *Feteasc*ă *neagr*ă grape pomace for obtaining honeybased fortified innovative product, Scientific Study & Research - Chemistry & Chemical Engineering, Biotechnology, Food Industry 21 (2020) 243-252.

[13]. P.E. Bran, D. Nicuţă, L. Grosu, O.I. Patriciu, I.C. Alexa, Investigation regarding the potential application of grape pomace extracts on *in vitro* plant growth and development, Ovidius University Annals of Chemistry 33 (2022) 135-142. Doi: 10.2478/auoc-2022-0020

[14]. B. Lapornik, M. Prosek, A.G. Wondra, Comparison of extracts prepared from plant by-products using different solvents and extraction time, Journal of Food Engineering 71 (2005) 214-222. Doi: 10.1016/j.jfoodeng.2004.10.036

[15]. M.J. Jara-Palacios, A. Santisteban, B. Gordillo, D. Hernanz, F.J. Heredia, Comparative study of red berry pomaces (blueberry, red raspberry, red currant and blackberry) as source of antioxidants and pigments, European Food Research and Technology 245 (2019) 1-9. Doi: 10.1007/s00217-018-3135-z

[16]. V. Vorobyova, M. Skiba, G. Vasyliev, O. Chygyrynets, Component composition and antioxidant activity of the blackcurrant (*Ribes nigrum* L.) and apricot pomace (*Prunus armeniaca* L.) extracts, Journal of Chemical Technology and Metallurgy 56 (2021) 710-719.

[17]. B. Kruszewski, E. Boselli, Blackcurrant pomace as a rich source of anthocyanins: ultrasound-assisted extraction under different parameters, Applied Sciences 14 (2024) 821. Doi: 10.3390/app14020821

[18]. S. Farooque, P.M. Rose, M. Benohoud, R.S. Blackburn, C.M. Rayner, Enhancing the potential exploitation of food waste: extraction, purification, and characterization of renewable specialty chemicals from blackcurrants (*Ribes nigrum* L.), Journal of Agricultural and Food Chemistry 66 (2018) 12265-12273. Doi: 10.1021/acs.jafc.8b04373

[19]. S. Struck, M. Plaza, C. Turner, H. Rohm, Berry pomace - a review of processing and chemical analysis of its polyphenols, International Journal of Food Science and Technology 51 (2016) 1305-1318. Doi: 10.1111/ijfs.13112

[20]. I. Jurevičiūtė, M. Keršienė, L. Bašinskienė, D. Leskauskaitė, I. Jasutienė, Characterization of berry pomace powders as dietary fiber-rich food ingredients with functional properties, Foods 11 (2022) 716. Doi: 10.3390/foods11050716

[21]. A.M. Reißner, S. Al-Hamimi, A. Quiles, C. Schmidt, S. Struck, I. Hernando, C. Turner, H. Rohm, Composition and physicochemical properties of dried berry pomace, Journal of the Science of Food and Agriculture 99 (2019) 1284-1293. Doi: 10.1002/jsfa.9302

[22]. A.E. Untea, A.G. Oancea, P.A. Vlaicu, I. Varzaru, M. Saracila, Blackcurrant (fruits, pomace, and leaves) phenolic characterization before and after *in vitro* digestion, free radical scavenger capacity, and antioxidant effects on iron-mediated lipid peroxidation, Foods 13 (2024) 1514. Doi: 10.3390/foods13101514

[23]. A. Jurgoński, J. Juśkiewicz, Z. Zduńczyk, P. Matusevicius, K. Kołodziejczyk, Polyphenol-rich extract from blackcurrant pomace attenuates the intestinal tract and serum lipid changes induced by a high-fat diet in rabbits, European Journal of Nutrition 53 (2014) 1603-1613. Doi: 10.1007/s00394-014-0665-4

[24]. A.L. Molan, Z. Liu, M. Kruger, The ability of blackcurrant extracts to positively modulate key markers of gastrointestinal function in rats, World Journal of Microbiology Biotechnology 26 (2010) 1735-1743. Doi: 10.1007/s11274-010-0352-4

[25]. G.A. Van Norman, Limitations of animal studies for predicting toxicity in clinical trials: Is it time to rethink our current approach?, JACC: Basic to Translational Science 4 (2019) 845-854. Doi: 10.1016/j.jacbts.2019.10.008

[26]. T. Răşanu, M. Mehedinţi-Hâncu, M. Alexianu, T. Mehedinţi, E. Gheorghe, I. Damian, Carnitine deficiency, Romanian Journal of Morphology & Embryology 53 (2012) 203-206.

[27]. D. Nicuță, L. Grosu, I.C. Alexa, A.L. Finaru, Sustainable characterization of some extracts of *Origanum vulgare* L. and biosafety evaluation using *Allium cepa* assay, Horticulturae 10 (2024) 504. Doi: 10.3390/horticulturae10050504

[28]. E. Bonciu, P. Firbas, C.S. Fontanetti, J. Wusheng, M.C. Karaismailoğlu, D. Liu, F. Menicucci, D.S. Pesnya, A. Popescu, A.V. Romanovsky, S. Schiff, J. Ślusarczyket, C.P. de Souzaal, A. Srivastava, A. Sutan, A. Papini, An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay, Caryologia 71 (2018) 191-209. Doi: 10.1080/00087114.2018.1503496

[29]. W.M. Dimuthu Nilmini Wijeyaratne, P.G. Minola Udayangani Wickramasinghe, Chromosomal abnormalities in *Allium cepa* induced by treated textile effluents: Spatial and temporal variations, Journal of Toxicology (2020) 8814196. Doi: 10.1155/2020/8814196

[30]. W.M.D.N. Wijeyaratne, L.G.Y.J.G. Wadasinghe, *Allium cepa* bioassay to assess the water and sediment cytogenotoxicity in a tropical stream subjected to multiple point and nonpoint source pollutants, Journal of Toxicology (2019) 5420124. Doi: 10.1155/2019/5420124

[31]. Y. Ouzid, M.N. Kaci-Boudiaf, A. Zeghouini, A.O. Madi, N. Smail-Saadoun, K. Houali, Antimitotic and genotoxic effect of methanolic extracts of leaves of *Peganum harmala* L. On the meristematic cells of *Allium cepa* L., Bioagro 35 (2023) 97-104. Doi: 10.51372/bioagro352.2

[32]. G.O. Ihegboroa, A.J. Alhassanb, C.J. Ononamadua, T.A. Owolarafe, M.S. Sule, Evaluation of the biosafety potentials of methanol extracts/fractions of *Tapinanthus bangwensis* and *Moringa oleifera* leaves using *Allium cepa* model, Toxicology Reports 7 (2020) 671-679. Doi: 10.1016/j.toxrep.2020.05.001

[33]. H. Remini, C. Mertz, A. Belbahi, N. Achir, M. Dornier, K. Madani, Degradation kinetic modelling of ascorbic acid and colour intensity in pasteurized blood orange juice during storage, Food Chemistry 173 (2015) 665-673. Doi: 10.1016/j.foodchem.2014.10.069

[34]. D. Teneva, D. Pencheva, A. Petrova, M. Ognyanov, Y. Georgiev, P. Denev, Addition of medicinal plants increases antioxidant activity, color, and anthocyanin stability of black chokeberry (*Aronia melanocarpa*) functional beverages, Plants 11 (2022) 243. Doi: 10.3390/plants11030243

[35]. E. Laczkó-Zöld, A. Komlósi, T. Ülkei, E. Fogarasi, M. Croitoru, I. Fülöp, E. Domokos, R. Ștefănescu, E. Varga: Extractability of polyphenols from black currant, red currant and gooseberry and their antioxidant activity, Acta Biologica Hungarica 69 (2018) 156-169. Doi: 10.1556/018.69.2018.2.5

[36]. K. Määttä, A. Kamal-Eldin, R. Törrönen, Phenolic compounds in berries of black, red, green, and white currants (Ribes sp.), Antioxidants and Redox Signaling 3 (2001) 981-993. Doi: 10.1089/152308601317203521

[37]. S.C.A. Cobzac, D. Casoni, M. Badea, B. Balabanova, N. Markova Ruzdik, Ultraviolet-Visible (UV-VIS) spectroscopy and cluster analysis as a rapid tool for classification of medicinal plants, Studia Universitatis Babeș-Bolyai Chemia, 64 (2019) 191-203. Doi: 10.24193/Subbchem.2019.4.14

[38]. J.L. Aleixandre-Tudo, W. du Toit, The role of UV-Visible spectroscopy for phenolic compounds quantification in winemaking, in: R.L. Solís-Oviedo, Á. de la Cruz Pech-Canul (Eds.), Frontiers and New Trends in the Science of Fermented Food and Beverages, IntechOpen, 2019. Doi: 10.5772/intechopen.79550

[39]. S.B. Tedesco, H.D. Laughinghouse in: J.K. Srivastava (Eds.), Bioindicator of Genotoxicity: The *Allium cepa* test in environmental contamination, IntechOpen, 2012, pp. 137-156. Doi: 10.5772/31371

[40]. T.A. Owolarafe, K. Salawu, G.O. Ihegboro, C.J. Ononamadu, A.J. Alhassan, A.M. Wudil, Investigation of cytotoxicity potential of different extracts of *Ziziphus mauritiana* (Lam) leaf *Allium cepa* model, Toxicology Reports 7 (2020) 816-821. Doi: 10.1016/j.toxrep.2020.06.010

[41]. R. Raju, A.G. Paul, U.P. Aguilor, J.T. Capili, The effect of induced acid rain; *Allium cepa* chromosomal aberration assay, Scholars Academic Journal of Biosciences 9 (2021) 89-97. Doi: 10.36347/sajb.2021.v09i03.005

[42]. D. Morais Leme, M.A. Marin-Morales, *Allium cepa* test in environmental monitoring: A review on its application, Mutation Research / Reviews in Mutation Research 682 (2009) 71-81 1383-5742. Doi: 10.1016/j.mrrev.2009.06.002

[43]. V. Prajitha, J.E. Thoppil, Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* assay, South African Journal of Botany 102 (2016) 18-25. Doi: 10.1016/j.sajb.2015.06.018

[44]. G.M. Hassan, A.A.M. Yassein, Cytogenotoxicity evaluation of water contaminated with some textile azo dyes using RAPD markers and chromosomal aberrations of onion (*Allium cepa*) root cells, Egyptian Journal of Genetics and Cytology 43 (2014) 39-57. Doi: 10.21608/ejgc.2014.9932

[45]. T. Aşkin Çelik, Ö.S. Aslantürk, Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* test, Journal of Biomedicine and Biotechnology (2010) 189252. Doi: 10.1155/2010/189252

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