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Chemical composition, antioxidant and cytogenotoxic effects of *Ligularia sibirica* (L.) Cass. roots and rhizomes extracts

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Abstract. Through time, in the traditional medicine *Ligularia* genus has been used as a remedy to cure several diseases and affections. The paper represents an essential step in offering more information about the antioxidant activity, chemical composition and cytogenetic activity of *L. sibirica* (L.) Cass. rhizomes and roots extracts. The antioxidant activity of the extracts has been achieved by analyzing the total phenolic content, total flavonoids, the organic chemical compound and trolox equivalent antioxidant capacity and their major polyphenolic constituents were quantified by liquid chromatography electrospray ionization-tandem mass spectrometry. The extracts were obtained by the Supercritical Fluid Extraction (SFE-CO2) technique, SFE-CO2 extraction with co-solvent and absolute ethanol extraction. The best results for the antioxidant activity have been fulfilled through the last two techniques. High Performance Liquid Cromatography (HPLC) has been applied in order to identify and quantify selected phenolic acids and flavonoids in the ethanolic extracts of *L. sibirica* (L.) Cass. The cytogenotoxic effects of the extracts completed the present study, with a furtherance of antiproliferative potential highlighted by the statmokinetic effect and an additional genoprotective effect.

Keywords. antioxidant activity, phenols, SFE extraction, HPLC, cytogenotoxic effects, Ligularia sibirica.

Abbreviation: TP - total phenols, TF - total flavonoids, DPPH - the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl, TEAC - Trolox Equivalent Antioxidant Capacity, FTIR - Fourier Transform Infrared spectroscopy, HPLC - High-performance liquid chromatography, SFE - Supercritical fluid extraction, MIR - middle infrared region, ATR - Attenuated total reflection, GAE - gallic acid equivalents, ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), MI - mitotic index.

INTRODUCTION

The genus *Ligularia* from the *Asteracae* family has been extensively researched from phytochemical point of view especially in the last years, having a real significance in the traditional medicines as a remedy for bronchitis, asthma, tuberculosis, haemoptysis, soothing pain, rheumatoid arthritis, coughs, inflammations, jaundice, scarlet fever and hepatic diseases (Pinglin *et al*. 2008; Xie *et al*. 2010). *Ligularia* genus has many biological activities such as antibacterial activity, antifeedant and insecticidal activities, antihepatotoxicity, antioxidant, antithrombotic and anticoagulant activity (Yang *et al*. 2011). The pharmaceutical studies as well as the chemical studies on *Ligularia* species demonstrate the specific presence of constituents such aseremophilane-type sesquiterpenes (Wu *et al*. 2016).

Over the glacial and interglacial period, a large number of species migrated from the Asian continent to Europe, including the glacial relict *Ligularia sibirica* (L.) Cass. which is the target of the present study. *L. sibirica* (L.) Cass., a perennial hemicryptophyte species, has a short and thick rhizome with long lateral fasciculated roots, leaves with long petioles, and inflorescence stem straight up to 200 cm. The blooming period starts from July till the end of August (Pop, 1960; Šmídová *et al*. 2011).

An overall small number of studies have been published with regards to the chemical composition and cytogenotoxic effects of *L. sibirica* (L.) Cass. Scientific literature highlights the use of this species as a cure for treating phlegm and for reducing cough (Liao *et al*. 1002; Tori *et al*. 2008; Yuan *et al*. 2013).

It is worth to mention here that there is not a clear situation regarding the *L. sibirica* (L.) Cass. populations in Romania related to its distribution in the Natura 2000 sites (Brînzan *et al*. 2013), and the protection of the species through the Bern Convention, Annex I - Strictly Protected Flora Species (Berne Treaty No. 104), complemented by the IUCN threat status, Data Deficient (Bilz *et al*. 2011). Due to the fact that the species of community interest is protected by the Habitats Directive through Annexes IIb and IVb, GEO 57/2007 (Law 49/2011): Annexes 3, 4 A and mentioned in the Carpathian List of Endangered Species in the category Near Threatened (Mihăilescu *et al*. 2015), the material used in our study was collected after a complex survey regarding the quantitative and qualitative analysis of *L. sibirica* (L.) Cass. population in each site, as follows: Apa Roşie Peat Bog, Hărman Marsh and Zănoaga Gorges. The necessary material for study was harvested in minimum amounts, in order to preserve the existing vegetal communities.

In the present study, we analyzed the chemical composition, antioxidant activity and cytogenotoxic properties of ethanol extracts obtained from roots and rhizomes of *L. sibirica* (L.) Cass. Our study represents a first step in estimating the possible phytotherapeutic applications of *L. sibirica* (L.) Cass., and to understand to what extent this species can be incorporated into farming systems as a medicinal herb.

MATERIALS AND METHODS

Plant material

Roots and rhizomes of *L. sibirica* (L.) Cass were harvested in August 2017, from 3 distinct sites, both in terms of habitat, pedo-climatic conditions, but also with the same level of anthropogenic activity, in order to decide which of them hold a higher potential for further studies. The first sample was collected from Zănoagei Gorges (Lat N45°28', Long E25°25'), which are part of Bucegi Natural Park an interesting limestone mountain system, the studied area being characterized by a cool-wet climate type, the average annual temperature being of 4.9°C where the rainfalls varies with the altitude, covered by rendzina soils (Beldie, 1967). The second sample of *L. sibirica* (L.) Cass. was collected from Hărman Marsh (Lat N45°43', Long E25°39'), an eutrophic marsh with hydric and alluvial soils and $CaCO_3$ deposits, located in the Braşov Depression, with an annual temperature of 7°C and low rainfall. The last sample was collected from Apa Roşie Peat Bog (Lat N46°10', Long E26°15'), located in Nemira Mountains and characterized by a cool-wet climate type rich in precipitations, with an average annual temperature of 2–4°C, occupying hydric soils without $CaCO_3$ deposits (Brînzan *et al*. 2013).

After weighing the plant material, the fresh rhizomes and roots were washed in tap water to remove the soil, rinsed well in distilled water, pat dried with paper towel, and then minced at room temperature.

Reagents and Chemicals

The reagents used: gallic acid monohydrate ACS reagent ≥ 98% and Folin Ciocalteu's phenol reagent of 2M concentration, caffeic, cinnamic, ferulic, rosmarinic and syringic, catechin, myricetin, naringenin, methanol, and ethanol were from Sigma-Aldrich (USA). Chlorogenic acid and quercetin were from Alfa Aesar (Germany) and Fluka, respectively. Ethanol (HPLC degree) was obtained from Merck Co. (Darmstadt, Germany). The used water was double-distilled. The carbon dioxide (99.5% purity)

used in SFE extraction and the rest of the reagents used in the experiments were purchased from commercial sources. The weight of the samples was measured on an analytical balance of Shimadzu Corporation with a precision of 0.1 mg.

Extraction Procedures

Ethanolic extract was prepared by mixing 5 g of each type of plant material, roots and rhizomes, in 50 mL absolute ethanol stored for 8 h at room temperature (22°C). Using Whatman filter paper no. 1, the extracts were filtered and the resulted filtrates were used to indicate their cytogenotoxic potential using *Allium cepa* test.

The SFE extracts were achieved through the specific equipment, a pilot unity called SFT-110 Supercritical Fluid Extractor (Supercritical Fluid Technologies, Inc.). The temperature of the restrictor valve was adjusted to 50° or 60°C for all extraction processes. The extract was collected in a 50 mL glass vial. The output of CO_2 in the system was of 6.0 g/min. The yields of extraction were around 2%. For the co-solvent extraction (sample 4), using 1% and 2% ethanol in relation to the CO_2 the experiment was performed in conditions of 50°C, 250 bar and flow of CO_2 at 6 g/min (Erdogan *et al.* 2011). In this case the extraction yield was 2.5% from the raw material.

Five samples of extracts were prepared: Hărman Mash – **1**, Zănoagei Gorges – **2** and Apa Roşie Peat Bog – **3** through SFE extraction with CO_2, then it was added a SFE extraction with CO_2 and EtOH as cosolvent from Apa Roşie Peat Bog – **4** and the last one was the absolute ethanol extraction from Apa Roşie Peat Bog – **5**. The extracts of every single assay were individualized by studying the antioxidant activity through: TP, TF, DPPH and TEAC. The functional groups were analysed using FTIR in the region 4000–400 cm^{-1}. The ethanolic extract (sample **5**) was used to evaluate cytogenotoxic activity.

Evaluation of chemical composition of the rhizome and roots extracts FT-MIR

The extracts of *L. sibirica* (L.) Cass. were investigated for their chemical composition: extract with CO_2 (**1**, **2**, **3**), CO_2 and ethanol (**4**), ethanolic extract (**5**). For FTIR spectroscopy, was used a Jasco 6300 spectrometer. The FTIR spectroscopy of each rhizomes and roots extract was recorded in the MIR region. An ATR accessory equipped with a diamond crystal (Pike Technologies) was used for sampling. Samples were carried out at 100 scans with resolution of 4 cm^{-1} in the regions of 4000-400 cm^{-1}. Spectra Manager II software was used for processed the spectra data.

Total phenolic content

In order to assess the TP content of the material extract, it has been used the Folin-Ciocalteu method (Singleton and Rossi, 1965). The TP content was brought out by mixing 0.5 mL of sample or standard (gallic acid) with 5 mL of Folin - Ciocalteu reagent and 4 mL of 1M sodium carbonate. It was measured the absorbance at 746 nm (Shimadzu UV-1800 Spectrophotometer) and the calibration curve with standard solutions of gallic acid concentrations ranging from 0.05 to 1 mgL^{-1} was traced. Equation of standard curve was y=0.0067x+0.0105 (R2=0.9960). The results were expressed as mg of GAE in 100 g of the rhizomes and roots.

Extract constituents

The identification and quantification of selected phenolic acids and flavonoids in the plant extracts was performed by high performance liquid chromatography (HPLC) using a Varian 310-MS LC/MS/MS triple quadrupole mass spectrometer (USA) fitted with an electrospray ionization interface (ESI). A sample of each dry plant extract was dissolved in HPLC-grade methanol. The mobile phase was double distilled water/methanol, f_r = 20:80. A Varian C18 column (150 mm 9 3.0 mm; 5 mm) was used at a flow-rate of 0.6 ml/min, in an isocratic elution. The injection volume was 20 ml and triplicate injections were used for each sample. The drying gas was air at a pressure of 131 kPa and 250 °C, and the nebulizing gas was nitrogen at 276 kPa. The capillary voltage was set at the potential -4500 V for negative ionization. The resulting deprotonated molecular ion was selected by the first quadrupole; in the second quadrupole, the deprotonated molecular ion was fragmented by collision with an inert gas (argon) at a pressure of 0.2 Pa; the fragments were analyzed in the third quadrupole. Prior to these experiments, the tuning of the mass spectrometer was performed using a polypropylene glycol standard for both positive and negative modes. The determinations have been carried out in triplicate. The results are expressed as µg of selected phenolic acid or flavonoid per 1 g of dry extract.

Determination of antioxidant activity

1. **DPPH Antioxidant activity**. In order to determine the radical scavenging activity of the ethanol

extract of *L. sibirica* (L.) Cass. the Brand-Williams DPPH assay has been used (Brand-Williams *et al.* 1995). DPPH is stable organic nitrogen radical, with a purple colour solution, which reacts with the antioxidant compounds. The method it stands on the measurement of the reducing ability of antioxidants toward this radical. The ability can be evaluated by measuring the decrease of DPPH absorbance at 517 nm. The percentage of the DPPH remaining was calculated using the equation: I% = [(Ablanck-Asample)/Ablanck] x100, where: Ablank is the absorbance for the blank (ethanol - DPPH. ethanolic solution) and Asample is the absorbance for the sample mixed with 0.04 mg/mL DPPH solution.

The results were expressed as IC50 (the extract concentration which inhibits the activity of DPPH by 50%). In short, to 4 mL of extract at 1 mL of DPPH (0.04 mg/mL) ethanol solution was added. After incubating for 30 min in dark, the absorbance of the resulting solutions was measured at 517 nm using UV-VIS Jasco 730 Spectrophotometer (Kedare and Singh 2011).

2. **TEAC assay**. A stock solution of ABTS•+ was obtained after reacting the ABTS chemical compound with potassium persulfate. Then the mixture has been left at dark at room temperature for 12–16 hours before use. The ABTS•+ used solution was prepared by dilution of this solution with ethanol till the absorbance was around 0.70 (Pellegrini *et al* 2003). The absorbance was measured at 734 nm. The calibration curve was made with Trolox (with concentrations between 0.125 and 2 mM). The results were done in mmol of Trolox per 100g rhizomes and root. Equation of standard curve was y=45.432x+20.334 (R2=0.9896).

Evaluation of cytogenetic effects of L. sibirica ethanol extract

The two samples extracted with ethanol (sample **4** and **5**) showed the best antioxidant properties, taking into account a higher volume of extraction, the sample **5** was studied in order to evaluate the cytogenotoxic effects of *L. sibirica* (L.) Cass. extracts.

The cytogenotoxic effects were evaluated through the changes of the mitotic indices and through the frequency of the phases of mitosis (prophase, metaphase, anaphase, telophase), as well as through the chromosomal aberrations frequency and the nuclear anomalies induced in root tip cells of *A. cepa L.*

The onion bulbs (a local variety), about 4 cm diameter, have been checked to fit in the phytosanitary standards. In order to expose root primordia, the outer scales have been gently removed and the bulbs were placed in 30 mL containers, with the discoid stem being in contact with distilled water. The *Allium* test was performed through static exposure, initially at the action of distilled water for 24 or 48 hours as well as at various concentrations of *L. sibirica* (L.) Cass. ethanol extracts for 24 or 48 hours. Further the samples were defined by the extracts concentration, respectively 5%, 10%, and 15%, as well as by the incubation time of onion roots, 24 hours and 48 hours (L 5% 24h, L 5% 48h, L 10% 24h, L 10% 48h, L 15% 24h, L 15% 48h).

For the cytogenetic analysis roots with a length of about 10 mm were used. The roots were fixed in a mixture of absolute ethanol: glacial acetic acid 3:1 overnight and then transferred to 70° ethanol for long-term preservation. For each experimental variant, a number of 5 roots were subjected to attenuated hydrolysis with HCl 1N for 15 minutes at 60°C. Fixed and macerated roots were stained with aceto-orcein solution 1% at 60°C for 15 minutes. The root tips were cut on a glass slide, in a drop of 45% glacial acetic acid, and used to perform microscopic slides using the squash technique. The coverslips were glued with several layers of nail polish, and the resulted microscopic slides were analyzed on an Olympus CX-31 microscope, at a 400x magnification. The microscopic analysis aimed at establishing the numbers of cells at different stages of mitosis, the frequency of chromosomal and nuclear aberrations related to about 3000 cells for each experimental sample. The MI was determined as the percentage ratio between the number of cells in mitosis and the total number of analysed cells (Tedesco *et al.* 2012). Depending on the total number of cells that carry out mitosis, it was determined the percentage of the cells mitosis stages. The frequency of chromosomal aberrations and nuclear abnormalities was calculated as the percentage between the number of damaged cells and total number of cells in the appropriate stage of the cell cycle and mitosis.

The results were statistical analysed using the IBM SPSS Statistics 20 program. Significant differences among samples were determined using the analysis of variance (one-way ANOVA), as well as the Duncan multiple comparison test. The $P \leq 0.05$ values were considered statistically significant. The graphs and tables were elaborated based on average values ± the standard error (SE) of more independent experiments.

RESULTS AND DISCUSSION

Evaluation of chemical composition of the rhizome and roots extracts by FT-MIR

The ATR-MIR spectra (4000-400 cm^{-1}) of each extract was registered and the specific wavenumbers

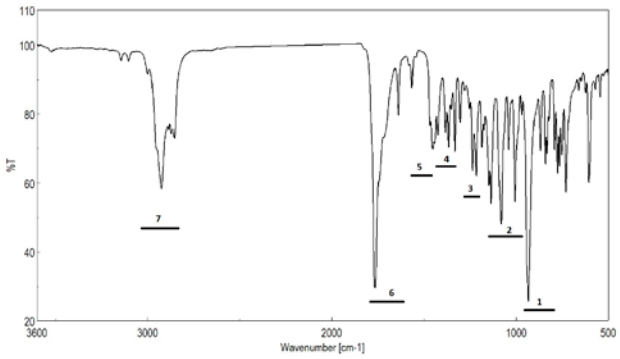

Figure 1. ATR-MIR spectra registered for sample **3** (SFE extraction with CO_2).

and intensities were considered in order to present the FTIR-ATR spectra of alcoholic and CO_2 extracts (Figure S1 in the Supplementary Material), as well as the FT-IR absorption bands for rhizomes and roots extracts (Table S1 in the Supplementary Material). The vibrational assignments for extracts were compared with literature data (Szymanska-Chargot and Zdunek 2013). The identification of the functional groups was based on the FTIR peaks attributed to stretching and bending vibrations. Eight areas were identified in the MIR domain and the fingerprint region was localized between 900 and 1760 cm^{-1} (areas 1-6) (Zavoi *et al*. 2011). Figure 1 shows the spectral regions 1-7 for sample analysis.

Area 1 (< 1000 cm^{-1}) corresponds to C-H bending vibrations from isoprenoids, area 2 (997-1140 cm^{-1}) to stretching vibrations C-O of carbohydrates, with signals at 1005, 1009, 1040, 1080 and 1136 cm^{-1}, while area 3 (1150-1270 cm^{-1}) corresponds to stretching vibrations of carbonyl C-O or O-H bendings. The intense peaks at 1040 cm^{-1} and to nearly 1080 cm^{-1} are attributed to characteristic functional groups of polyphenols. C-O (amide) and C-C stretchings vibrations appear in the region 4 (1300-1450 cm^{-1}) Region 5 (1500-1600 cm^{-1}) corresponds to aromatic group and N-H bending vibrations. Domain 6 is a complex one (1600-1760 cm^{-1}) and corresponds to bending vibrations N-H (amino acids), C=O stretchings (aldehydes and ketones, esters) as well to free fatty acids (1766 cm^{-1}) and glycerides (1738 cm^{-1}). Area 7 (2800-2900 cm^{-1}), corresponds to C-H stretching vibrations of CH_3 and CH_2 from lipids, lipid derivatives, C-H (aldehydes). Region 8 (3350-3600 cm^{-1}) is assigned to stretching vibrations of OH (from water, alcohols, phenols, carbohydrates).

The spectra show relatively more bands in the region of 400-700 cm^{-1}. The inorganic constituents can be observed in the region between 470-480 and 530-540 cm^{-1}. The variation may be due to the differences in the extraction and purification methods.

Antioxidant activity and phenol content of plant extracts

There are a few studies about compounds with antioxidant activity from the genus *Ligularia*. According to them, *L. fischeri* (Ledeb.) Turcz. showed high total phenolic contents (215.8 ± 14.2 mg gallic acid equivalent/g) with low contents of total flavonoid (86.9 ± 3.8 mg rutin equivalent/g) (Lee *et al*. 2013). Liu (2010) has reported the isolation and structural elucidation of the furanoeremophilane-type sesquiterpenes and benzofuran derivatives (part of them with phenol groups) from *L. veitchiana* (Hemsl.) Greenm., and some results about its biologic activity (Liu *et al*. 2010). *L. macrophylla* (Ledeb.) DC. has been reported to contain at least two flavonoids: 6-acetyl-8-methoxy-2,3-dimethylchromen-4-one and 4-14 (2S)-3'-hydroxy-5',7-dimethoxyflavanone in root and rhizome, while in *L. duciformis*'s root have been identified some derivatives of sinapyl alcohol and coniferyl alcohol, with known antioxidant activities (Yang *et al*. 2011). From our knowledge, phenols, flavonoids or other compounds with antioxidant activity from *L. sibirica* (L.) Cass. were not assessed. The Table 1 presents the TP and TF content of *L. sibirica* (L.) Cass. rhizome and root extracts and its TEAC. The highest concentrations of TP and TF were obtained for extracts of *L. sibirica* (L.) Cass. prepared by the co-solvent method (EtOH), as expected.

Our results have also shown that supercritical CO_2 extractions were the least efficient, predictable since phenols are polar compounds and thus are less extractible by CO_2. Something more effective than this was the extraction method at cold temperatures with ethanol (method used for extraction of phenols from vegetal matrix (Santos-Buelga *et al*. 2012).

The polyphenol constituents present in extract 5, as identified and quantified by LC–ESI–MS/MS, are listed in Table 2. Based on these results, it was concluded that the antioxidant properties of the extract originate in

Table 1. TP, TF and TEAC assay for *L. sibirica* rhizome and root extracts.

Sample	TP (mg gallic acid equivalent/100g root)	TF (mg quercetol equivalent/100g root)	TEAC assay (mmoli Tolox equivalent/100g root)
1	2.13	n. d. [a]	0.03
2	3.08	n. d.	0.04
3	10.01	n. d.	0.09
4	494.26	5.25	1.42
5	49.25	n. d.	0.47

[a] not detected.

Table 2. The phenolic profile of the plant extract-**5**, as determined by LC–ESI–MS/MS.

Class compound	Compound	Concentration (µg compounds/g dry extract)
Phenolic acids	Caffeic acid	69.778±1.0229
	Chlorogenic acid	189.114±1.7604
	Cinnamic acid	0.438±0.0177
	Ferulic acid	1.856±0.0764
	Gallic acid	7.420±0.1071
	Rosmarinic acid	25.359±0.1292
	Syringic acid	2.532±0.0955
Flavonoids	Catechin	6.901±0.0124
	Myricetin	0.343±0.0158
	Naringenin	0.704±0.0283
	Quercetin	13.032±0.6992

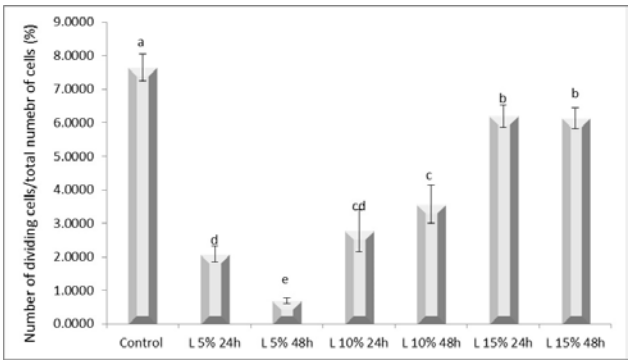

Figure 2. The influence of *L. sibirica* extracts on the mitotic index in root meristem cells of *Allium cepa* L. (The data are the averages ± SE of three repetitions; a, b, c, d, e, - the interpretation of statistical significance and of significant differences through the Duncan test, $p < 0.05$).

two main classes of compounds, namely flavonoids (catechin, myricetin, naringenin, quercetin) and, to a greater extent, phenolic acids (caffeic, chlorogenic, cinnamic, ferulic, gallic, rosmarinic, syringic acid).

Evaluation of cytogenetic effects of L. sibirica ethanol extracts

Starting with Fiskejio (1988) the *Allium* test was considerably used for the evaluation of cytotoxic and genotoxic effects, as well as for the genoprotective potential of various natural or synthetic chemical compounds. The *Allium* test follows some endpoints, such as the mitotic index, chromosomal aberrations and the nuclear anomalies (Bonciu *et al*. 2018).

For each experimental sample, the results were compared with the control. Figure 2 shows the MI variation in onion roots exposed for 24 or 48 hours at the action of *L. sibirica* (L.) Cass. extracts of 5%, 10% and 25% concentration. Statistical analyzis of the microscopic results revealed that ethanol extracts of *L. sibirica* (L.) Cass. have determined a significant decrease in the frequency of cells in various phases of mitosis. The highest MI was determined for the control for which the calculated percentage was 7.64%. *L. sibirica* (L.) Cass. extracts had a statistically important mitoinhibitory effect, when compared to the control, showing an indirect correlation with their concentration. The lowest MI (0.7%) was calculated after the roots incubation in the extract with the concentration of 5% for 48h. This decreased frequency of cells in the mitosis was followed at a statistically significant difference by that determined in the experimental sample, defined by the 5% concentration, respectively 24h. In the root tip cells incubated in *L. sibirica* (L.) Cass. extracts, the highest MI with a 6.19% value was calculated for the L 15% 24h experimental sample. The overall interpretation of the microscopic results revealed that the variation of the mitotic index was independent of the exposure time. The decrease of the MI in *A. cepa* L. meristematic root cells demonstrates the presence of bioactive substances with antiproliferative potential.

The effects of *L. sibirica* (L.) Cass. extracts on the distribution of the mitotic division phases in the onion meristem cells are summarized in Figure 3. The frequency of prophase and metaphase has significantly varied, in a concentration dependent manner. Therefore L 5% 24h has induced the highest percentage of prophases, significantly different compared to the other tested concentrations. When compared to the control, the ethanol extracts of *L. sibirica* (L.) Cass. have induced an increase and a significant increase of metaphases frequency. The

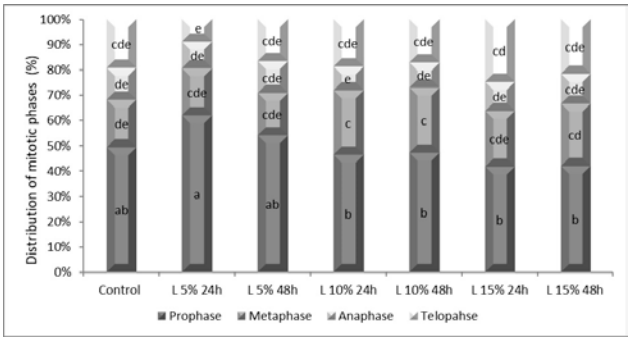

Figure 3. The influence of *L. sibirica* extracts on the distribution of mitotic division phases in the radicular meristem cells of *A. cepa* L. (the data are ± SE averages of three repetitions; a, b, c, d, e, f, g, h, i, j, k – the interpretation of statistical significance and of significant differences through the Duncan test, $p < 0.05$).

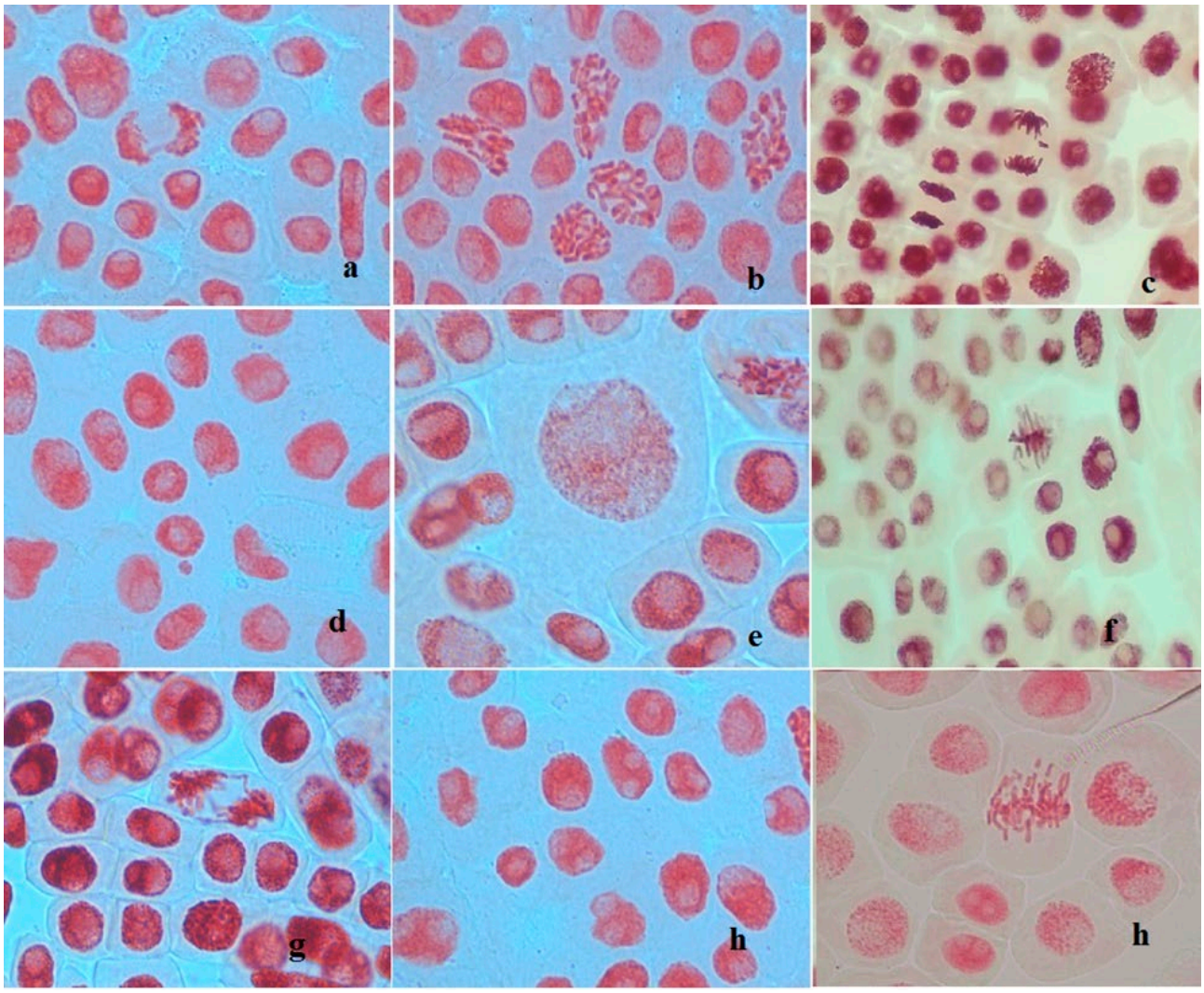

Figure 4. Chromosomal aberrations and nuclear abnormalities identified in meristematic root cells of *A. cepa L.* (a) telophase bridge – L 5% 24h; (b) C-mitosis – L 5% 48h; (c) laggards – L 5% 24h; (d) micronucleus and nuclear buds - L 5% 48h; (e) giant cell – L 10% 48h; (f) vagrant – L 10% 24h; (g) anaphase bridges – L 15% 24h; (h) altered nuclear shape – L 10% 48h; (h) fragments – L 5% 24h.

highest metaphases percentage was determined in L 10% 24h and L 10% 48h variants. The calculated percentage values for anaphase and telophase were not significantly different compared to the control.

On the basis of these observations it can be appreciated that the MI decline is due to cells arresting in the metaphase at a 5% extracts concentration. The overall interpretation of the results also reveals a global slowdown of mitotic progression at a higher concentration of the extracts. The decrease of the MI may be reflecting a cytogenotoxic effect of *L. sibirica* (L.) Cass. ethanol extracts and could be interpreted as cellular death (Yuet Ping *et al.* 2012). According to Nieva-Moreno *et al.* (2005) cited by Stojković *et al.* (2013) the main mechanisms of cancer chemoprevention are anti-mutagenesis and anti-proliferation or mitotic anti-progression.

An extensive review by Yang *et al.* in 2011, as well as other recent studies (Dong *et al.* 2015) shows that among the bioactive chemical constituents of *Ligularia* species, the most common phytochemical types are sesquiterpenes, which have demonstrated a strong cytotoxic or inhibitory activity on some tumor cell lines.

Among the volatile compounds identified by Gas chromatography with mass spectrometry detection in the *L. sibirica* (L.) Cass. extracts obtained by microwave assisted hydrodistillation, there were sabinene, limonene and terpinolene (monoterpenoids), as well as alkaloids that were most likely tussilagine and isotussilagine. In

our study, the statmokinetic effect may be attributed to these terpenoids. Many of the isolated terpenoids from natural sources have chemopreventive effects (Akihisa *et al*. 2003; Yang *et al*. 2011).

Nuclear and chromosomal aberrations observed in the root tip meristem cells of *A. cepa* L. are shown in Figure 4. Their frequency in the various experimental samples is shown in Supplementary Material (Table S2).

The average percentage value of the nuclear and chromosomal aberrations calculated for the control it was only 0.35 ± 0.07, and the percentages 1.53 ± 0.12, 3.46 ± 0.55, 3.25 ± 0.94, 1.83 ± 0.22, 1.25 ± 0.42 and 1.26 ± 0.35 for L 5% 24h, L 5% 48h, L 10% 24h, L 10% 48h, L 15 % 24h, L 15% 48h. The significant higher frequency of the chromosomal and nuclear aberrations in L 5% 48h, L 10% 24h, L 10% 48h variants, may be attributed to the potentially lower antioxidant activity as a result of the dilution of the tested extracts. This antimutagenic activity may be attributed to the secondary metabolites or to synergistic action of the antioxidant compounds. Besides that, the FT-MIR analysis has revealed the existence of OH group characteristic for phenols, to which may be due the genoprotective activity. Oxidative DNA damage can contribute to single double strand breaks formation or to oxidation of the purines or pyrimidines bases, inducing a genomic instability and also the development of cancer (Chobotokova 2009). The biologically active phenols are known for the antioxidant properties exerted by the absorption of the free radicals (Hidalgo and Almajano 2017; Shahidi *et al* 2015) as well as for the wide spectrum of biological and physiological actions (Durazzo 2017).

Mitoinhibitory effect of *L. sibirica* (L.) Cass. extracts was supported by the C-mitosis high percentage, which indicates the spindle disturbance in metaphase (Firbas and Amon 2014; Bonciu 2018). However, the nuclear aberrations identified in the meristematic root cells were much more varied and had a higher frequency compared to chromosomal aberrations. The nuclear abnormalities defined by the nuclear morphology alterations in the interphase, such as micronuclei, nuclear buds, altered nuclei shape may be an indicator of some processes such as cell death or tumorigenesis (Pellegrini 2003; Nefic *et al*. 2013).

In our study, except for the L 10% 24h experimental sample, changes in the shape of the nucleus was observed with a very low frequency. Nuclear envelope proteins ensure the nucleus rigidity to the distortion associated forces caused by the cytoplasmic microtubules. These changes may be the consequence of microtubule generated forces in the cytoplasm, if any of the membrane nuclear proteins is inactive (Nefic 2013; King *et al*. 2008). Other studies have shown that the inactivation of the associated proteins with the endoplasmic reticulum affects the nucleus form (Higashio *et al*. 2000; Matynia *et al*. 2002; Webster *et al*. 2009).

Moreover, the chromosomal aberrations and nuclear abnormalities identified by microscopic analysis are indicative of both clastogenic and aneugenic effects and of genotoxic damage. In this context, for a deeper estimation of the potential of this species additional *in vitro* and *in vivo* studies are required, aiming the extraction conditions, extract concentration and exposure time.

CONCLUSION

The paper represents an essential step in offering more information about the antioxidant activity, chemical composition and cytogenotoxic activity of ethanol extracts obtained from roots and rhizomes of *L. sibirica* (L.) Cass. The best results for phenols and flavonoids extraction from roots and rhizomes of *L. sibirica* (L.) Cass. were obtained in supercritical CO_2 extraction with cosolvent (ethanol) followed by ethanol extraction. The ethanol extracts of *L. sibirica* (L.) Cass. have demonstrated a very strong mitoinhibitory effect on *in vitro* root meristem cells of *A. cepa L*. Chromosomal aberrations and nuclear abnormalities identified by the microscopic analysis are key indicators of both clastogenic and aneugenic effects, and of genotoxic damage.

SUPPORTING INFORMATION

The version of this article contains supplementary material: vibration assignments for the rhizome and roots of *L. sibirica* (L.) Cass. extracts; the FTIR fingerprint of the studied rhizome and roots extracts, the chromosomal aberrations and nuclear anomalies observed in root meristem cells of *A. cepa* L. incubated in *L. sibirica* (L.) Cass. ethanolic extracts.

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