New Aspects of Biomass Waste Valorization: Spruce Bark Crude Extracts as Plant Growth Regulators

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The effects of spruce bark crude extracts were evaluated relative to basil (*Ocimum basilicum* L.) plantlet development and metabolic processes (cellular division and histo-anatomical modification) involved in breeding. Natural phenolic compounds were separated as crude extracts by conventional and green extraction techniques, in a primary biorefining process, from *Picea abies* L. waste bark. First, the influence of phenolic compounds, in different concentrations, on the basil plants' main biosynthetic processes was analyzed. The mitotic indices and chromosomal aberrations of the plantlets were monitored. There was a significantly positive action of the studied bioproducts on basil root and stem growth, biomass accumulation, and photo-assimilating pigment synthesis. The natural polyphenols triggered an intensification of metabolic processes and cell division, yielding a high mitotic index and good development of vascular bundles. The results supported the possibility of exploiting spruce bark wastes by conversion into valuable bio-compounds, with uses as innovative products in green biotechnology, as simple growth bioregulators, for example, or for biotech crops.

Keywords: Polyphenols; Spruce bark; Green extraction; Picea abies L.; Ocimum basilicum L.

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INTRODUCTION

In the last several decades, biomass of all types (including agricultural crops, forestry products, and household and agro-industrial wastes) has attracted research and commercial interest in finding new solutions for sustainable economic growth. In these circumstances, special attention has been given to superior biomass valorization and biorefining concepts. Separating bioactive compounds by biomass biorefinery remains challenging, taking into account the recent interest in these valuable compounds and the large amounts of secondary products and biomass wastes.

Although spruce (*Picea abies* L.) is a common and widely used material in the wood industry, spruce bark is still regarded as a low-value residual material. The bark is separated as a waste product during wood processing and represents 8 to 10% of the total stem volume. Spruce bark is currently used in combustion. Among other bark extracts from conifers (especially pine and fir), spruce bark extracts are used in traditional medicine to prevent and treat colds, stomach pains, indigestion, and vascular, pulmonary,

neurological, and venereal diseases; they exhibit antitumor, antibacterial, antifungal, anti-ulcerogenic, anti-inflammatory, and antihypertensive properties (Yang *et al*. 2008; García-Pérez *et al*. 2012; Conde *et al*. 2013; Le Normand *et al*. 2014) due to their richness in antioxidants (especially tannins and phenolic acids). In addition to these general uses, a new possible and promising way to valorize spruce bark wastes is to use the extractives (polyphenols) obtained in a primary biorefining process in biotech crops and ecological agriculture (Aşkin and Atik 2016). This idea started to develop during recent years as a necessity for green agriculture expansion (Abdel-Hameed 2009).

In plants, polyphenols play an important role, acting against UV light and attacks from predators and pathogens. Natural polyphenols' utilization in the plant science world is an innovative concept, which shows increasing success. The effects of polyphenolic extracts as antioxidant agents, plant growth regulators, or bioremediation agents have been successfully tested on a large group of plants, such as soybean, sunflower, bean, oat, rape, maize, and tomatoes (Bălaş and Popa 2007; Stingu *et al*. 2009a; Tanase *et al*. 2014; Bujor *et al*. 2015; Talmaciu *et al*. 2015). Also, prior research details the influence of phenolic compounds in seed germination and plant development processes, either as individual compounds or as global extracts recovered from different bioresources (Almaghrabi 2012).

Ocimum basilicum L., the sweet basil plant, belonging to the Lamiaceae family, is an important herb to many cultures and cuisines. It is a common aromatic plant and also a medicinal plant, mainly used in cosmetics and pharmaceutics. Basil plants contain large amounts of essential oils, rosmarinic acid, and antioxidants, which are compounds with a large commercial interest (Taie *et al.* 2010; Mijani *et al*. 2013; Kiferle *et al*. 2014). Moreover, basil is a great source of phytochemicals due to its phenolic acids and aromatic compounds, which present antioxidant, antimicrobial, and antitumor activities (Anwar *et al.* 2010). The economic importance and global dissemination of basil, with its many uses in food industries and folk medicine, make it important to investigate this plant.

Based on these proprieties and the lack of information regarding the cultural uses of these plants, this study focused on the influence of natural extracts obtained by combinations of grinding and conventional and ultrasonic extraction, from *Picea abies* L. waste bark. In a continuation of a previous study (Talmaciu *et al*. 2015), this work evaluated the effects on basil plantlet development and metabolic processes (cellular division and histo-anatomical modification) involved in breeding. The use of wood industry waste as raw material for obtaining natural products with bioregulatory effect in basil plants is the novelty of this study.

EXPERIMENTAL

Materials

Spruce (*Picea abies* L.) bark was provided as waste from a wood processing company (Vatra Dornei, Romania). Prior to extraction, the spruce bark was air dried at room temperature (10.4% humidity) and milled in a GRINDOMIX GM 200 (Retsch GmbH, Haan, Germany) mill up to a mean particle diameter of 0.6 mm. The biomass was directly used without any pre-treatments.

Basil seeds (*Ocimum basilicum* L.) were provided by a seed distribution company (SC Agrosel SRL, Câmpia Turzii, Romania) and used after sterilization (immersion in a

20% HClO solution for 2 min and well washed with tap water). All chemicals and standards were provided by Sigma-Aldrich (St. Louis, MO, USA).

Methods

Extraction methods

Crude extracts were obtained by applying a traditional extraction method (batch water extraction) and a green extraction procedure (ultrasound-assisted extraction). The difference between the two extraction methods is that ultrasound-assisted extraction has a shorter extraction time (15 min) and a lower extraction temperature (70 °C).

Batch water extraction was performed using 10 g of ground and dried spruce bark placed in an Erlenmeyer flask over which 125 mL of distilled water was added. The mixture was introduced to and kept for 45 min in a water bath at 85 to 90 °C, with shaking from time to time. Collected extracts were filtered, and the spruce bark was subjected to a second extraction with fresh distilled water. This operation was repeated 3 times until full spruce bark exhaustion (colorless extract). All extracts were accumulated in a 1000 mL volumetric flask and marked up to volume with distilled water (Tanase *et al*. 2013).

Ultrasound-assisted extraction was performed using 5 g of spruce bark, immersed in 125 mL distilled water in an Erlenmeyer flask, under ultrasound treatment (using an ultrasonic bath, Bandelin Sonorex (Berlin, Germany) at 35 kHz frequency, at 70 °C for 15 min. The operation was repeated four times (until full exhaustion of the spruce bark, *i.e*., colorless extract) and the filtration of the extracts was carried out. The filtrate was transferred to a 500 mL volumetric flask and completed with distilled water (Talmaciu *et al*. 2015).

Characterization of the extracts

Spruce bark aqueous extracts were analyzed in terms of total polyphenols, tannin, flavonoid, and flavonol content using spectrophotometric methods, with all determinations performed in triplicate. The total organic matter content was also determined by evaporation of the extracts at 105 °C followed by a 6 h drying at 600 °C. The total polyphenolic content (TPC) was determined using the Folin-Ciocalteu method, following the protocol described previously (Talmaciu *et al*. 2015). Results were expressed as milligrams of gallic acid equivalents (GAE) per spruce bark gram (mg GAE/g).

The total content of tannins (TTC) was also measured applying the Folin-Ciocalteu method, taking into account the precipitation reaction of tannins with casein (Abdel-Hameed 2009). The total tannin content, given by the difference between the absorbance read for the pure spruce bark extract and the solution containing casein, was expressed as milligrams of GAE per spruce bark gram (mg GAE/g).

The total flavonoid (TFC) and flavonol (TFnolC) contents were determined using the aluminum chloride method, taking rutin as a reference compound (Abdel-Hameed 2009). The amounts of flavonoids and flavonols in the spruce bark extracts were calculated by applying Eq. 1 and expressed as milligrams of rutin equivalents (RE) per spruce bark gram (mg RE/g),

$$X = (A \cdot m_0) / (A_0 \cdot m) \quad (1)$$

where X is the flavonoid/flavonol content (mg RE/g), A is the absorption of the plant extract solutions, A_0 is the absorption of standard rutin solution, m is the weight of the plant extracts (g), and m_0 is the weight of rutin in the solutions (mg).

Experimental design

Experiments on basil seeds were preceded by many lab tests on the influence of polyphenols on the main physiological and biosynthetic processes involved in seed germination and plantlet development. To advance this goal, seed germination tests and physiological, cytogenetic, and histo-anatomical analyses were performed. The sweet basil (*Ocimum basilicum* L.) plants were cultivated in greenhouse conditions. Sweet basil seeds were directly sown into pots. Each sample was replicated in ten pots, with three uniform plants spaced evenly in each pot and allowed to grow. The cultivated soils were wetted daily with 15 mL of solution (tap water for control samples and spruce bark extract for test solutions) for one week, until germination of the plants was observed. From this point, basil plants were wetted every 3 days with the same amount of tested solutions. After 45 days from the beginning of the experiments, the basil plants were separated into roots, stems, and leaves, which were characterized to determine the different effects of the cultivation conditions on their growth and development.

Plant growth and development analysis

To evaluate the influence of polyphenolic extract from spruce bark on plant growth and development, the basil plants were separated into roots, stems, and leaves, followed by biometric measurements of plant elongation and quantitative determinations of biomass. Samples of separated plants were oven dried at 70 °C until constant values of mass were reached, and the dry biomass was quantified. For pigment quantification (Lichtenthaler and Wellburn 1983), 0.05 g of fresh vegetal material was milled with quartz sand and extracted with acetone (80%). The carotenoid and chlorophyll (*a* and *b*) contents were spectrophotometrically determined at wavelengths of 470, 646, and 663 nm and quantified using Eq. 2 to 4:

$$Chlorophyll\ a = 12.21\ A_{663} - 2.81\ A_{646} \quad (2)$$

$$Chlorophyll\ b = 20.31\ A_{646} - 5.03\ A_{663} \quad (3)$$

$$Carotenoids = (100\ A_{470} - 3.27\ [chl\ a] - 104\ [chl\ b]) / 22 \quad (4)$$

where chlorophyll *a*, chlorophyll *b*, and carotenoids are given in micrograms per milliliter (µg/mL) and results were expressed in micrograms per gram fresh vegetal material (µg/g); A_{663}, A_{646}, and A_{470} are the specific absorbances read spectrophotometrically; and [*chl a*] and [*chl b*] are the chlorophyll *a* and chlorophyll *b* contents, respectively.

Cytogenetic analysis

For cytogenetic investigations (Tanase *et al*. 2015), seedling roots (collected when measuring between 10 and 15 mm) were fixed by immersion for 24 h in a 3:1 mixture of absolute ethylic alcohol to glacial acetic acid. As a first step in microscopic slide preparation, the previously fixed roots were hydrolyzed in a 50% (v/v) HCl solution for 8 min, followed by a quick wash in a 1 N HCl solution. For the next step (root coloring), a carbol-fuchsin solution was used for staining, with the samples kept in this solution for 48 h. After coloring, the root tips were chopped into several tiny fragments on the microscopic slide, and 1 to 2 drops of fixative solution was added. The squash method

was used to emphasize chromosome aberrations. Five slides/repetitions were analyzed for each working variant. Chromosomal field photos were captured on a Nikon Eclipse 600 microscope, 100× immersion objective, using a Nikon Eclipse 600 digital camera.

Histo-anatomical analysis

For histo-anatomical observations (Tanase *et al.* 2016), the basil plants were first fixed and preserved in 70% alcohol and were then prepared for further analysis by classic procedure. As a first step, cross sections (ten sections for every sample) through the vegetative organs of the plants and seedlings were made. For the second step, the sections obtained, collected on a watch glass with water, were double stained using iodine green and ruthenium red. After water removal, the sections were stained for several tens of seconds (with a few drops of methylene blue) or 1 min (with a few drops of iodine green), washed with 70% ethanol and tap water, and then stained again for several tens of seconds with ruthenium red. Finally, the stained sections were mounted between slides in a few drops of water and analysed with a Novex (Holland) microscope and photographed with a Nikon Coolpix L22.

Statistical analysis

All results were expressed as mean ± standard error, with n = 3. Comparison of the means was performed by the Fisher least significant difference (LSD) test ($p \leq 0.05$) after ANOVA analysis using the program PAST 2.14 (Hammer & Harper, Oslo, Norway). Sampling and chemical analyses were examined in triplicate, both to decrease experimental error and to increase experimental reproducibility.

RESULTS AND DISCUSSION

Polyphenolic Extracts Characterization

Characteristics of polyphenolic extracts obtained in batch extraction were summarized in previous work (Tanase *et al*. 2013). These results showed that the spruce bark polyphenolic extracts contained considerable quantities of aromatic compounds, such as vanillic acid (39.4 mg GAE / 100 g dry bark) and catechin (31 mg GAE / 100 g dry bark). Table 1 summarizes the spruce bark extracts' compositions in terms of organic matter content and total polyphenolic, tannin, flavonoid, and flavonol contents obtained by batch water and ultrasound-assisted extractions.

Table 1. Comparative Analysis of Traditional and Green Extracts' Compositions Used for Sweet Basil Seed Treatment (mg / g dry bark)

Spruce Bark Extract	Organic Content (g/L extract)	TPC (mg GAE/g)	TTC (mg GAE/g)	TFC (mg RE/g)	TFnolC (mg RE/g)
Aqueous extract obtained by batch extraction*	0.42 ± 0.03	5.20 ± 0.45	1.64 ± 0.51	0.22 ± 0.01	0.08 ± 0.01
Aqueous ultrasonic extract	0.72 ± 0.11	6.57 ± 0.70	1.30 ± 0.02	1.18 ± 0.19	0.84 ± 0.06

* Tanase *et al*. (2013); TPC – total polyphenolic content; TTC - total tannin content; TFC – total flavonoid content; TFnolC – total flavonol content, GAE – gallic acid equivalents; RE – rutin equivalents

For further investigation, aqueous extracts were used in four working solutions with different concentrations and polyphenolic contents (Table 2). These solutions were investigated to evaluate to which extent (high or low concentrations) the content of polyphenols could act as a stimulator or inhibitor to basil metabolism and cell division.

Table 2. Spruce Bark Polyphenolic Solutions Investigated as Growth Regulators of Basil Plants

Working Solution	Abbreviation	Concentration (g powder spruce bark/L water)	TPC (mg GAE/mL)
Aqueous extract obtained by batch extraction	SB1	5	0.06 ± 0.01
Aqueous extract obtained by batch extraction	SB2	10	0.13 ± 0.05
Aqueous ultrasonic extract	SB3	5	0.35 ± 0.08
Aqueous ultrasonic extract	SB4	10	0.65 ± 0.04

Elongation of Basil Plants' Vegetative Organs

After 45 days, the resultant basil plants were subjected to biometric measurements to evaluate the influence of phenolic compounds on the elongation of vegetative organs. Figure 1 presents the influence of spruce bark polyphenolic extracts on the vegetative organs' elongation in terms of root and stem lengths. It was observed that the addition of polyphenolic extracts in the growth medium triggered an increase in stem elongation. The most notable observations for stimulation of stem elongation were of SB1 and SB3, with which stem elongation exceeded that of the control (C) by 18.7% and 11.6%, respectively. This result evidenced that spruce bark polyphenolic extracts (obtained by batch water extraction or ultrasound-assisted extraction) with a low concentration (SB1 and SB3) of TPC can effect an increase in stem length. In this case there is no statistical significant difference depending on the extraction method applied.

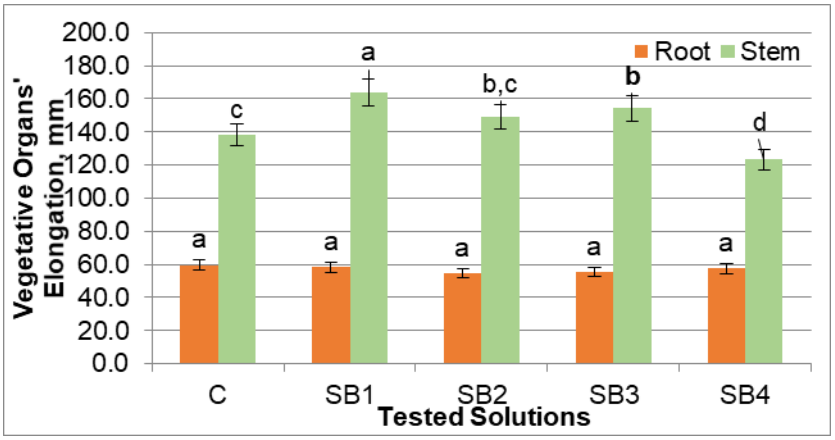

Fig. 1. Influence of spruce bark extracts on vegetative organs' elongation in *Ocimum basilicum* L. Bars show that identical letters are not significantly different at $p \leq 0.05$. Error bars represent the standard deviations of means (n = 3)

These results are in agreement with those of Anghel (2004), in which global polyphenol extract from spruce bark periodically added to the soil of tomato plants (*Lycopersicon esculentum*) determined the doubling of productivity per plant, compared to the control sample. The results obtained from germination tests of rape seeds and of

hydroponic crops highlight the stimulating effect of polyphenol extracts obtained from spruce bark, grape seeds, *Asclepias syriaca*, and chestnuts on the elongation of rape seedlings. Isolated extracts from the chestnuts stimulated the growth and development of rape plantlets' hypocotyls, in direct proportion to the increase of the total concentration of the polyphenol content of the tested extracts (Stingu *et al*. 2009b).

Biomass Accumulation in Basil Plants' Vegetative Organs

For biomass accumulation (Fig. 2), a positive effect of polyphenolic extract treatment was recorded. An increase in the total accumulated amount of biomass was observed for experimental variants with low concentrations of polyphenolic content (SB1 and SB3). The highest value of biomass (1.54 ± 0.06 g dry leaves) was recorded for SB1, where the applied extract concentration was 0.06 mg GAE/mL of polyphenolic content (a result 43% greater than that of the control – 1.08 g). At greater concentration of spruce bark extract (SB4, with 0.65 mg GAE/mL), accumulated biomass is less than the control and other variants. Comparing the two extraction methods, there are statistical significant differences in the vegetative biomass accumulation process. The SB1 extract, obtained by classical extraction, stimulates the process of biomass accumulation in all vegetative organs. The aqueous extract obtained by ultrasound-assisted extraction (SB3) has a lower degree of stimulation comparing with SB1.

Earlier reports have revealed the role of polyphenols in the physiological processes of plants (Stingu *et al*. 2009c; Tanase *et al*. 2015). Thus, it has been hypothesized that these compounds applied to plants as aqueous extracts have an auxinic effect. In low concentrations it manifests as stimulation of plant growth and development, while in high concentrations they inhibit plant growth and development. It seems that the most effective and accessible ways to act on the hormonal balances of plants through bioregulators are those methods related to hormone biosynthesis and the addition of natural or synthetic bioregulators that may have analogous, synergistic, or antagonistic actions to those of natural bioregulators.

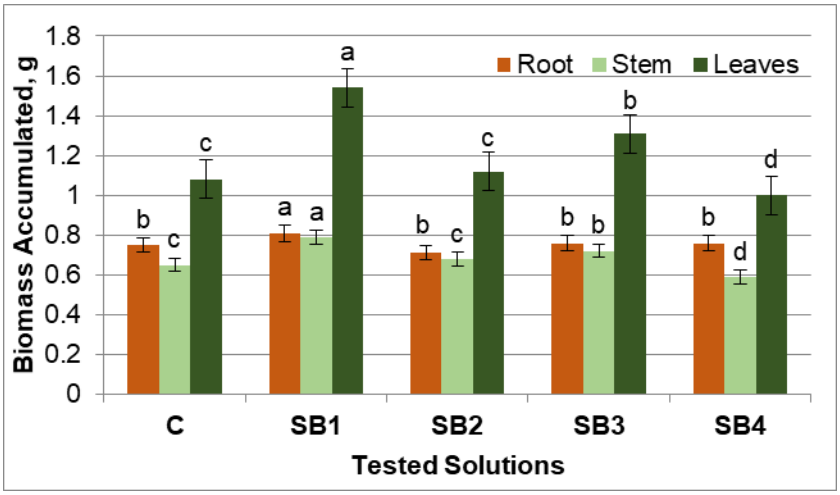

Fig. 2. Influence of spruce bark extracts on biomass accumulation of *Ocimum basilicum* L. Bars show that identical letters are not significantly different at $p \leq 0.05$. Error bars represent the standard deviations of means (n = 3).

Photo-assimilating Pigment Content in Basil Primary Leaves

Table 3 depicts the values of photo-assimilatory pigment content synthesized and measured in the basil leaves. The presence of spruce bark extracts stimulated chlorophyll and carotenoid biosynthesis, especially in the case of SB1, where the highest values for all relevant measures were recorded. Compared with the control sample, the percentages of chlorophyll *a* and chlorophyll *b* synthesized in the basil leaves increased 17.8 ± 1.2% and 12.5 ± 0.5%, respectively, for the SB1 samples. A similar behavior was recorded for the carotenoids' accumulation, with the content 32.9 ± 1.8% greater than in the control samples. Under the polyphenolic extract treatment, amounts of photo-assimilatory pigments showed no significant differences for other experimental variants (SB2, SB3, SB4).

Table 3. Amounts of Photo-assimilatory Pigments Synthesized (µg/g) in *Ocimum basilicum* L. Primary Leaves

Sample	Chl *a*	Chl *b*	Chl *a* + Chl *b*	Chl *a* / Chl *b*	Carotenoids
C	232.5 ± 2.73 ^{d,e}	69.2 ± 0.97 ^{b}	301.7	3.36	52.6 ± 0.43 ^{b}
SB1	273.7 ± 2.12 ^{a}	77.8 ± 0.83 ^{a}	351.5	3.52	69.9 ± 0.89 ^{a}
SB2	234.6 ± 1.75 ^{d,e}	70.0 ± 0.77 ^{b}	304.6	3.35	54.6 ± 0.78 ^{b}
SB3	239.9 ± 2.25 ^{c}	69.2 ± 0.97 ^{b}	309.1	3.46	57.8 ± 0.49 ^{b}
SB4	230.8 ± 2.22 ^{e}	71.1 ± 0.91 ^{b}	301.9	3.25	55.8 ± 0.47 ^{b}

Different letters within columns indicate significant differences ($p \leq 0.05$).

Mitotic Index Determination and Chromosomal Aberrations

The mitotic index is one of the most important elements in cell survival and multiplication, providing information on the influences of some xenobiotic agents (polyphenols) on mitotic division (Popa *et al*. 2008). It can be defined as the ratio (expressed as a percentage) of the number of cells in division to the total number of analyzed cells. Table 4 presents the frequency of the cellular mitotic phases, as well as the mitotic index, recorded in basil root apex, analyzed after applying to the seeds the spruce bark polyphenolic extract treatments.

Table 4. Frequency of Cellular Mitotic Division Phases in Root Apex Cells of *Ocimum basilicum* L. under the Influence of Tested Solution

Sample	Total Cells	Cells in Division	Prophase (%)	Metaphase (%)	Anaphase (%)	Mitotic Index (%)	Telophase (%)
Control	6608	369 ± 1.03 ^{d,e}	38.75 ± 0.39 ^{b}	26.02 ± 0.05 ^{a}	12.20 ± 0.02 ^{c}	5.58 ± 0.01 ^{c}	23.04 ± 0.05 ^{c}
SB1	5501	399 ± 1.12 ^{c,d}	42.36 ± 0.23 ^{a}	22.31 ± 0.04 ^{c}	13.53 ± 0.03 ^{b}	7.25 ± 0.01 ^{a}	21.80 ± 0.03 ^{d}
SB2	5756	365 ± 1.09 ^{e}	36.16 ± 0.32 ^{b,c}	24.66 ± 0.05 ^{b}	9.86 ± 0.04 ^{e}	6.34 ± 0.00 ^{b}	29.32 ± 0.06 ^{a}
SB3	8126	593 ± 0.81 ^{a}	33.39 ± 0.12 ^{c}	26.48 ± 0.07 ^{a}	12.98 ± 0.07 ^{c}	7.30 ± 0.01 ^{a}	27.15 ± 0.08 ^{b}
SB4	8098	443 ± 0.74 ^{c}	37.92 ± 0.11 ^{b}	22.57 ± 0.12 ^{c}	15.80 ± 0.04 ^{a}	5.47 ± 0.00 ^{c}	23.70 ± 0.09 ^{c}

Different letters within columns indicate significant differences ($p \leq 0.05$).

In all experimental variants, the percentage distribution of the main four mitotic division phases was similar to that of the control sample. The results indicated that a large fraction of cells in division were in the beginning phase (prophase), with the other advanced phases (metaphase, anaphase, and telophase) showing a similar distribution for experimental variants compared with the control (Table 4). The frequency of cells in mitosis was greater than in the control, with a maximum for SB3 at 7.29% (30.6% greater than the control), showing a mild mitogenic effect (Table 4). The large fraction of cells in mitosis registered for SB1 and SB3 is correlated with the elongation of vegetative organs and an increase in the amount of biomass.

Chromosomal aberrations were used as an indicator of the plants' sensitivity, often correlated with changes in the morphological and taxonomic structure, but also with plant sterility degree, both providing quantitative and qualitative information regarding their behavior upon exposure to different mutagens. To see how polyphenolic spruce bark solution affects the mitotic cell division process, the main types of aberrations and their frequency must be determined. This parameter is important, as any chemical or physical agent can exert a clastogenic (able to produce chromosomal breakage) or turbagenic effect that has the ability to induce mitotic or meiotic disturbances without direct DNA damage. More predominant types of chromosome aberrations occurred mainly in ana-telophases and telophases and were recorded for SB2 and SB4 (Table 5). The presence of highest rate of chromosomal aberrations types in this variants, could be correlated with a greater number of cells being in the final division phases and also, with a greater number of aberrant ana-telophases. In the control samples, there where predominant aberration types as single and/or multiple bridges and retarded chromosomes. In the case of spruce bark extracts used as treatment, there could be noticed a significant increase of aberrations types, being noticed ana-telophases (A-T) with bridges, or A-T with late chromosomes and also complex aberrations types as A-T with bridges and retarded and/or expelled chromosomes, or multipolar A-T with bridges.

Table 5. Frequency of Cells with Ana-telophase Chromosomal Aberrations in Basil (*Ocimum basilicum* L.) Root Apex under the Influence of Tested Solution

Sample	Total Cells Analyzed	Cells in Division	Mitotic Index (%)	Anaphase + Telophase (%)	Ana-telophase Aberrations (% from cell in division)
Control	6608	369 ± 1.03 ^{e}	5.58 ± 0.01 ^{c}	35.24 ± 0.31 ^{b}	5.38 ± 0.00 ^{e}
SB1	5501	399 ± 1.12 ^{c,d}	7.25 ± 0.01 ^{a}	35.33 ± 0.34 ^{b}	6.38 ± 0.00 ^{d,e}
SB2	5756	365 ± 1.09 ^{e}	6.34 ± 0.00 ^{b}	39.18 ± 0.65 ^{a}	13.29 ± 0.02 ^{a}
SB3	8126	593 ± 0.81 ^{a}	7.29 ± 0.01 ^{a}	40.13 ± 0.41 ^{a}	6.72 ± 0.01 ^{d,e}
SB4	8098	443 ± 0.74 ^{c}	5.47 ± 0.00 ^{c}	39.50 ± 0.32 ^{a}	14.29 ± 0.02 ^{a}

Different letters within columns indicate significant differences ($p \leq 0.05$).

Given that for SB1 and SB3 the recorded mitotic index is close to that of the control, it could be concluded that low spruce bark extract (obtained by batch water extraction or ultrasound-assisted extraction) concentrations have positive effects on cellular division, which can be attributed to the antioxidant properties of the extracts. Notably, however, the test solutions with high concentrations of polyphenolic extracts (SB2 and SB4) yielded more cells with chromosomal aberrations than in samples with lower concentrations (SB1 and SB3).

Earlier reports provide some information about the effects of polyphenols on cell division. Tanase *et al*. (2015) concluded that spruce bark aqueous extract (130 mg GAE/L concentration) and hemp shive aqueous extract (164 mg GAE/L concentration) increase mitotic index compared with control. At high concentrations of the extracts, there were high numbers of cells with chromosomal aberrations. The mutagenic effects of lignin derivatives were studied on *Vicia sativa*, *Vicia villosa*, and *Pisum sativum* (Grama and Bara 2003). The lignin compounds induced the stimulation of the cell division rate in embryonic peaks, compared to the control sample.

Histo-anatomical Aspects of the Basil Plants

To highlight the effects of spruce bark polyphenolic extracts, some histo-anatomical aspects of the internal structures of the basil plants' primary roots and aerial stems were analyzed. Histo-anatomical analysis of the primary root revealed that the main axis had a few lignified pith in the center (Fig. 3a). The libriform fibers had thick and slightly lignified walls. The radial range was surrounded by a few lignified parenchymatous cells. The phellogen layer formation was not based on the pericycle but rather on the differentiating layers of the cortex. Facing out, there was an exfoliating rhytidome. At the root level, (Fig. 3b), the lignification process (SB2 and SB3) was noticed to be more intense compared with the control (Fig. 3a). This was the only notable difference between the variants and the control. Probably, there was an intensification of the lignification process induced by polyphenols' preoxidation. This effect triggers the locking of vascular bundles, which will ultimately lead to difficulty in the movement of sap in the plant, and thus to the inhibition of its growth and development.

In the case of the aerial vegetative organs' (stem) structure, the epidermis was composed of slightly tangentially elongated isodiametric cells with pericline walls thicker than the others (Fig. 4a). Also, very few stoma cells were observed. There were two types of hairs: tectorial trichomes and secretory hairs (Fig. 5). The cortex was thin and slightly colenchymatic in a hypodermic position and did not have a special type of endoderm.

The cortex cells tended to be tangentially elongated. The cambium activity was initially unequal in the circumference of the organ, producing phloem and xylem in the large bundles; thus, the secondary vascular tissue rings were sinuous during this stage. The secondary xylem ring was almost entirely lignified for SB2 and SB3 plants (composed of vessels, libriform fibers, lignified ligneous parenchymatous cells, and horizontally lignified parenchymatous cells). The large bundles had radial ranges of ligneous vessels separated by uniseriate or pluriseriate areas of parenchymatous cellulosic cells, and the phloem was composed of pierced tubes and adjacent cells (Fig. 5a).

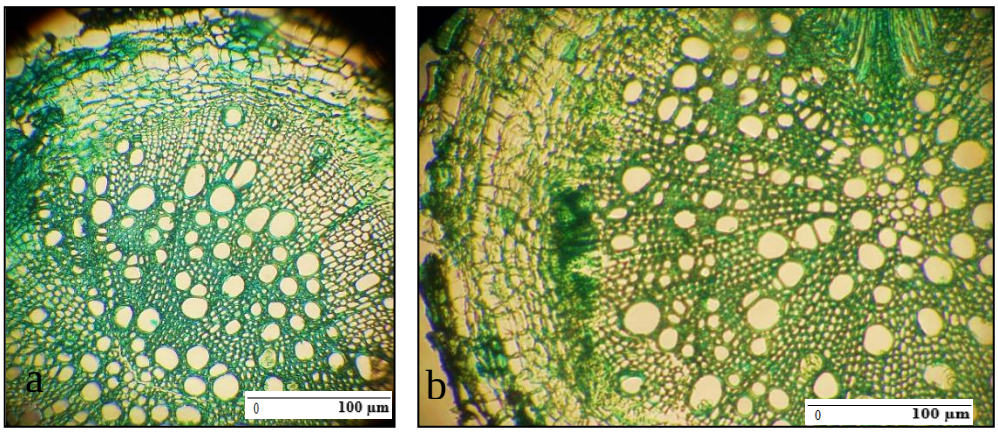

Fig. 3. The internal structure of the main root: a – general aspect (control), b – general aspect (SB2 – central cylinder, cortex, rhizodermis)

Sclerenchyma fibers could be observed at the end of the large phloem vascular bundles that had, in this developing state, less thickened but still cellulosic walls (Fig. 4b and 4c and Fig. 5a). Comparing the two extraction methods, there are no significant differences in the histo-anatomical structure of basil plants.

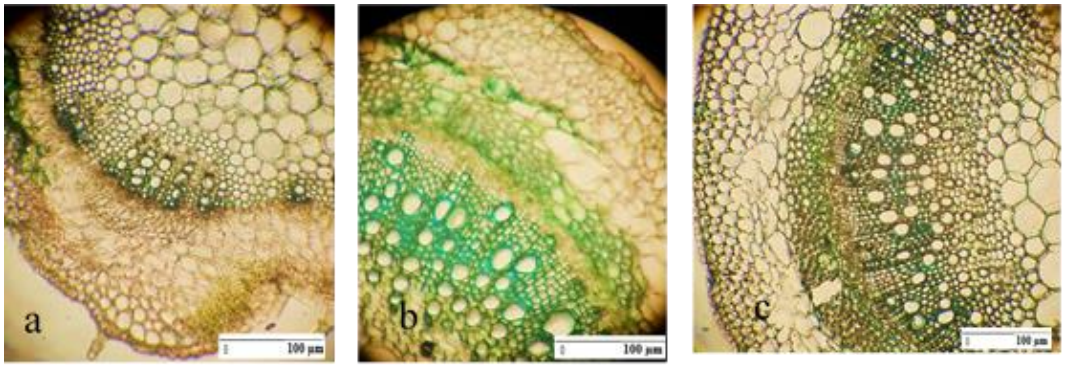

Fig. 4. The internal structure of stem: a – general aspect (control), b – general aspect (SB1), c – general aspect (SB2)

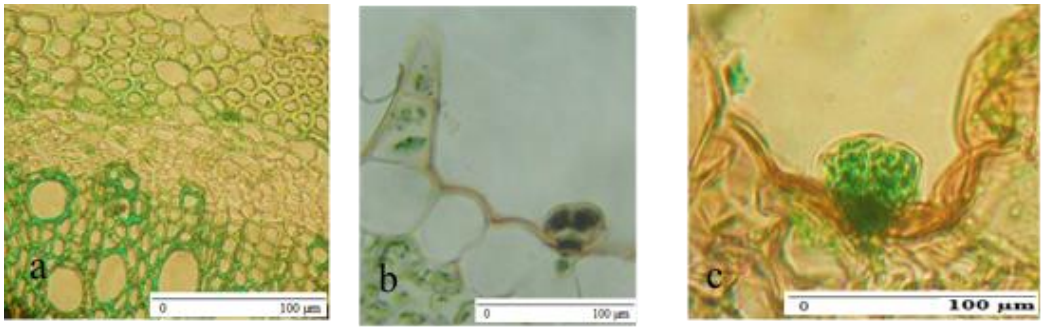

Fig. 5. The internal structure of stem: a – vascular bundles and sclerenchyma fibers (SB1), b – tectorial and secretory hairs (SB1), c – secretory hair (SB4)

The results show that spruce bark crude extracts, which are rich in phenolic compounds, can be used directly or as precursors to bio-based products (*e.g.*,

biofertilizers or plant growth regulators) in biotech crop development. These products can lead to an improvement of the crops' nutrient profile, which is an important aspect for green agriculture. The production process of these extracts involves green extraction techniques and uses renewable materials and/or wastes. In this way, the process can be sustainable, eco-friendly, and eco-efficient. Further studies will take into account the evaluation of chemical composition and the pharmacological activity of the volatile oil obtained from the treated plants.

CONCLUSIONS

1. This work evaluated the effect of spruce bark crude extracts (aqueous extract obtained by batch extraction and aqueous ultrasonic extract) on basil (*Ocimum basilicum* L.) plantlet development and metabolic processes involved in breeding.
2. Spruce bark aqueous ultrasonic extract were analyzed in terms of total polyphenols, tannin, flavonoid, and flavonol content.
3. There was a significantly positive action of the spruce bark polyphenolic extracts on basil root and stem growth, biomass accumulation, and photo-assimilating pigment synthesis.
4. The spruce bark polyphenolic extracts triggered an intensification of metabolic processes and cell division, yielding a high mitotic index and good development of vascular bundles.
5. The lignification process of treated plants with polyphenolic extracts in high concentration was noticed to be more intense compared with the control.
6. Natural phenolic compounds separated from spruce bark wastes can act as stimulating agents (plant activators) on basil plant metabolism and cellular division processes, depending on the concentration.

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