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VALORIZATION OF WASTES FROM FORESTRY INDUSTRY IN THE CULTURE OF SAGE (*SALVIA OFFICINALIS* L.)

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ABSTRACT: This study is focused on the influence of polyphenolic compounds, extracted by hot water extraction and ultrasound assisted extraction, from *Picea abies* bark resulted as a waste from the wood processing industry, on sage (*Salvia officinalis L.*). Sage seeds were treated with different spruce bark polyphenolic extracts that was characterized in terms of the total content of polyphenols, tannins, flavonols, flavonoids and anthocyanins in a previous paper. The comportment of main biosynthesis processes on seeds germination and plantlets development induced by the present of polyphenolic compounds in different concentration was analysed, several parameters being monitored: germination energy and capacity, growth and development of vegetative organs, accumulation of biomass and photoassimilating pigments content. Results showed that aqueous polyphenolic extract obtained has influence on sage plant acting as a stimulating on seed germination energy and capacity, root and stem growth, biomass accumulation and photoassimiling pigments synthesis, in lower concentration.

Keywords: spruce bark, germination, polyphenols, sage, *Salvia officinalis* L.

INTRODUCTION:

Tree bark it is a waste that contains a large fraction of extractives and lignin, which can be utilized as a renewable source of chemicals, particularly aromatic chemicals. The technical routes and technologies on the valorization of tree barks for chemicals and materials, include direct utilization of bark for wooden panels, and extraction for extractives (Almaghrabi, 2012; Le Normand *et al.,* 2014). Softwood bark, especially spruce bark, is regaining interest as a readily available source of valuable precursors and polyphenols (García-Pérez *et al.,* 2012).

In plants, polyphenols play an important role, acting against UV light or predators and pathogen attacks. Applications of polyphenols is an innovative concern in the plant science world, which shows an increasing success, their biostimulative effects as antioxidant agents or plants growth regulators has already gratefully tested on a large group of plants as basil, soybean, sunflower, maize etc. (Talmaciu *et al.,* 2015; Ignat *et al.,* 2009; Tanase *et al.,* 2013).

The present research is concerned with the use of the wastes from the forestry industry to obtain polyphenolic extract and to identify the influence on sage (*Salvia officinalis* L.) germination, growth and development of vegetative organs, accumulation of biomass and photoassimilating pigments content.

MATERIALS AND METHODS:

Biological material

Spruce (*Picea abies*) bark was provided as waste from a wood processing company (Vatra Dornei, Romania). Prior extraction, the spruce bark was air-dried at room temperature (10.44 % humidity) and milled in a GRINDOMIX GM 2000 mill up to a mean particle size diameter of 0.6 mm. The biomass was directly used without any pre-treatments.

Extraction methods

The extracts were obtained by applying traditional extraction methods (batch water extraction) and non-conventional methods (ultrasound assisted extraction).

For batch water extraction, 20 grams of ground dried material with particle size of 0.5-1mm were extracted consecutively for three times with 125 mL distilled water on a water bath, for 45 minutes, at 80°C, and finally, the extracts were cumulated to a final volume of 500 mL using distilled water. Dry matter content in the extracts was determined by evaporation of 25 mL extract on water bath and drying at 105°C, until a constant mass is reached. Folin Ciocalteu (FC) method was used to determine the total polyphenolic content (TPC) in aqueous extract and the concentrations were expressed as the number of equivalents of gallic acid (GAE).

Ultrasound assisted extraction was performed using 5 g of spruce bark, immersed in 125 mL distilled water in an Erlenmeyer flask, under ultrasounds action (using ultrasonic bath, Bandelin Sonorex), at 35 kHz frequency, 70°C and for 15 min. After the operation was repeated four times (until full exhaustion of the spruce bark - colourless extract), the filtration of the extracts was carried out. The obtained filtrate was transferred to a 500 mL volumetric flask and diluted with distilled water. Extracts were stored at -16 °C prior analysis.

Experimental assessment

Sage seeds were from seeds bank at Botanical Garden of University of Medicine and Pharmacy and used after sterilization (immersion in an HCl 20% solution for 2 min and well washed with distilled water).

Germination tests were carried out going through a standard procedure, using increments of 10 Petri dishes

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for each solution studied: SB1 (SBPE with 96 mg GAE/L) and SB2 (SBPE with 191 mg GAE/L).

The seeds germination tests and physiological analyses were performed. The sage (*Salvia officinalis* L.) plants have been cultivated in greenhouse conditions. Sage seeds were directly sown into pots. Each sample was replicated in ten pots and three uniform plants have been spaced evenly in each pot and were allowed to grow. The cultivated soils were wetted daily with 15 mL solutions (tap water for Control sample and spruce bark extract for test solutions) for one week, until germination of the plants was observed. From this point, sage plants were wetted every 3 days with the same amount of tested solutions.

Plant growth and development analysis

To evaluate the influence of polyphenolic extract from spruce bark on plant growth and development, the sage plants were separated into roots, stems and leaves, followed by biometric measurements of plant elongation and quantitative determinations of biomass.

For pigments content quantification, 0.05 g of fresh vegetal material was milled with quartz sand and extracted with acetone (80%). The carotenoids and chlorophyll content (chlorophyll *a* and chlorophyll *b*) were spectrophotometrically determined at specific wavelengths (470, 646 and 663 nm) and quantified using the equations:

chlorophyll a (μg/ mL) = 12.21 (A_{663}) - 2.81 (A_{646})
chlorophyll b (μg/ mL) = 20.31 (A_{646}) - 5.03 (A_{663})
carotenoids (μg/ mL) = (100 · A_{470} - 3.27 [*chl a*] - 104 [*chl b*])/ 22

where: A_{663}, A_{646}, A_{470} represent the specific absorbance read spectrophotometrically; [*chl a*] and [*chl b*] are the *chlorophyll a* and *chlorophyll b* contents. Some research was conducted using CERNESIM infrastructure (CERNESIM – POS CCE-O 2.2.1, SMIS-CSNR 13984-901, No. 257/28.09.2010)

Statistical analysis

All the results are expressed as mean ± standard error where n = 3. Comparison of the means was performed by the Fisher least significant difference (LSD) test (p ≤ 0.05) after ANOVA analysis using program PAST 2.17c. Sampling and chemical analyses were examined in triplicate, in order to decrease the experimental errors and to increase the experimental reproducibility.

RESULTS AND DISCUSSION:

Extract characterization

The spruce bark extract characterization was made in a past work (Tanase *et al.* 2013). Thus, the total polyphenols content of spruce bark aqueous extract was 191 mg GAE/100g. The chromatographic analysis reveald: catechins (31mg GAE/100g plant material), vanillic acid (39.4mg GAE/100g) and galic acid in reduced quantites.

For further investigation, aqueous extracts were used in four working solutions with different concentrations and phenolic content (Table 1).

Tab. 1.
Spruce bark phenolic solutions investigated as growth regulator on sage plants

Working solution	Abbreviation	Concentration (g/L)	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

Sage seed germination tests

The influence of phenolic compounds on *Salvia officinalis* L. seeds germination was evaluated by mathematical estimation of seeds germination energy and capacity. Taking into account the recorded values of these two parameters, a positive influence on both germination process was observed in case of seeds treatment with spruce bark extracts, compared with the control sample (Fig. 1). Higher values were obtained when seeds were treated with lower concentration of polyphenolic extracts (SB1). The maximum stimulation percentages for germination capacity (19.7 ± 2.2 %) were attended in case of seeds treatment with the hot water extract by a 0.06 mg GAE/ mL phenolic content (SB1). Although, it should be noticed that a good simulative effect on germination process, compared with the control was also induced by the aqueous ultrasound extract, used in lower concentration (SB3), but an inhibitory effect was recorded further with increasing the TPC concentrations (SB2 and SB4).

Obtained results are sustained by our previous studies, which shown also the stimulative action of phenolic compounds from spruce bark, as well from another plant materials as chestnuts shell, grape seeds or *Asclepias syriaca*, on seed germination process Balas *et al.*, 2007; Ignat *et al.*, 2009; Tanase *et al.*, 2013). Although, an important factor that should be taken into account is the used concentration, at higher content of phenolic compounds the inhibitory effect being more visible.

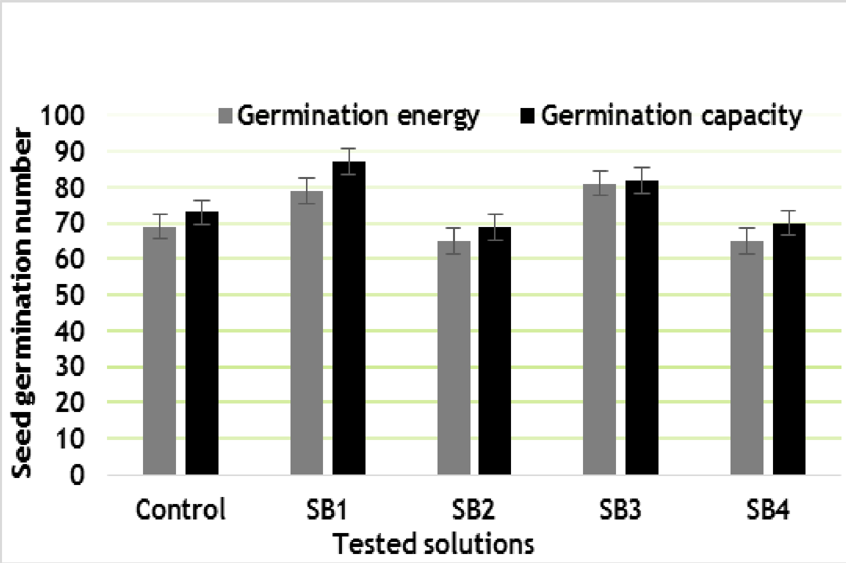

Fig. 1. Influence of spruce bark extracts on *Salvia officinalis* L. seed germination energy and capacity. Error bars represent the standard deviation of means (n = 3).

Elongation sage vegetative organs

After 60 days, the resulted sage plants were subjected to biometric measurements to evaluate the influence of phenolic compounds, on the elongation of the vegetative organs. Figure 2 presents the influence of spruce bark phenolic extracts on the vegetative organs elongation, in terms of roots and stems length. The most important observation about stimulating the development of stem is found for SB1 and SB3 variants, were the percentage of stimulation compared with control is 48 % and 44% respectively. This results evidenced that the spruce bark polyphenolic extracts (obtained by batch water extraction and with ultrasound) with a low concentration (SB1 and SB3) in TPC, determines an increase in stem length. The same pattern is for root.

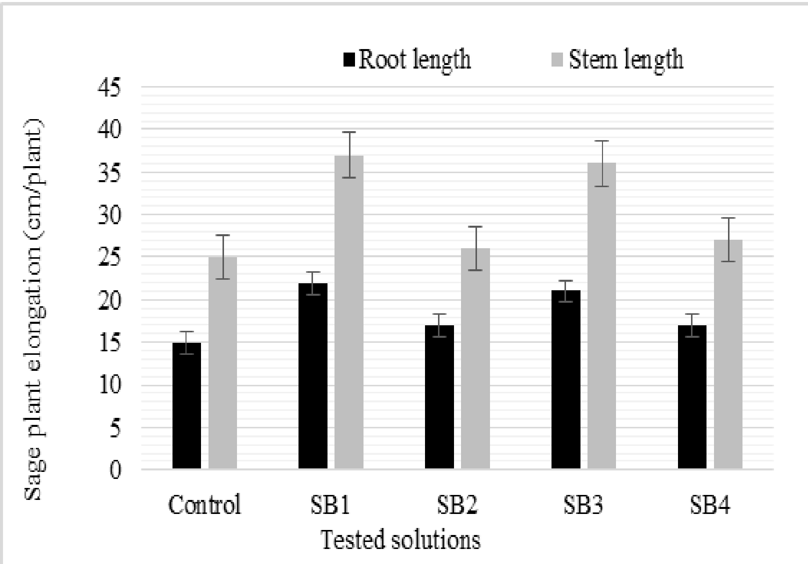

Fig. 2. Influence of spruce bark extracts on vegetative organs elongation of *Salvia officinalis* L. Error bars represent the standard deviation of means (*n = 3*).

Biomass accumulation

Regarding the biomass accumulation (Fig. 3), a positive effect of the plants treatment with phenolics extracts was recorded. An increase in the total accumulated amount of biomass was observed for all experiments. The highest value of 4.7 ± 0.06 g total biomass, was recorded for SB1 version, where the applied extract concentration was 0.06 mg GAE/mL of phenolic content (with 67.8% higher comparing with control – 2.8 g). At higher concentration of the spruce bark extract (SB2 and SB4), biomass accumulated value is compared to control.

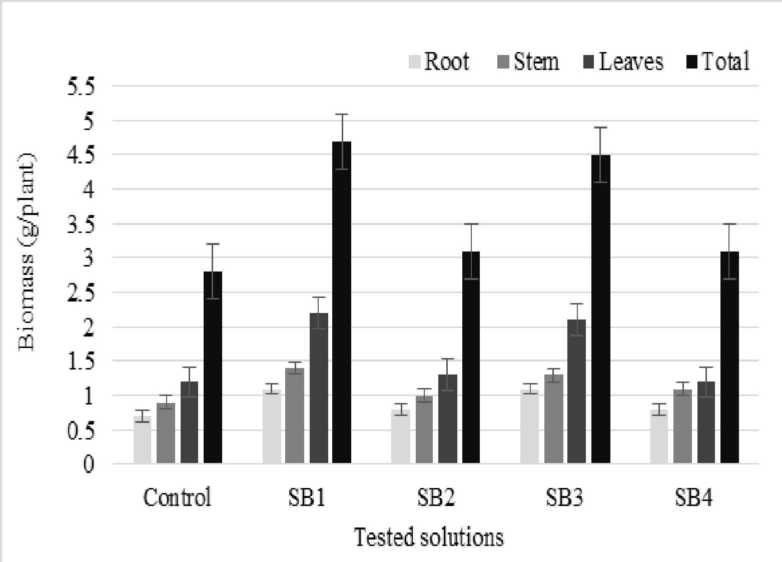

Fig. 3. Influence of spruce bark extracts on biomass accumulation of *Salvia officinalis* L. Error bars represent the standard deviation of means ($n = 3$).

Photoassimilating pigments content

Table 3 depicts the obtained values of photo-assimilatory pigments content synthesized and measured in the sage leaves. The presence of spruce bark extracts stimulated the chlorophylls and carotenoids pigments biosynthesis, especially in case of SB1 variant, where the highest values of all indicators were recorded.

Table 3.
The amount of photo-assimilatory pigments synthesized in *Salvia officinalis* L. primary leaves

	***Chl a* µg/g**	***Chl b* µg/g**	***Chl a+b* µg/g**	***Chl a/b* µg/g**	***Carotens* µg/g**
C	245.4±1.73[d,e]	71.1±0.87[b]	316.5	3.445	55.2±0.53[b,c]
SB1	287.7±2.22[a]	79.9±0.53[a]	367.6	3.6	68.4±0.59[a]
SB2	224.4±1.74[f]	68.2±0.67[c]	292.6	3.29	58.6±0.88[b]
SB3	265.8±2.15[b,c]	71.4±0.97[b]	337.2	3.722	59.1±0.59[b]
SB4	234.5±2.12[e,f]	72.1±0.81[b]	306.6	3.252	55.3±0.47[b,c]

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

Compared with the control sample, the percentages of chlorophyll *a* and chlorophyll *b*, synthesized in the sage leaves, increased with 17.23 ± 0.8 %, respectively 12.37 ± 0.4 % in case of SB1 variant. A similar behaviour was recorded for the carotenoids accumulation, where the content was 23.91 ± 1.4 % higher in comparison to the control samples. Under the phenolic extracts treatment, for amount of photo-assimilatory pigments no significant differences were reported between another experimental variants (SB2, SB4)

CONCLUSIONS:

Results showed that aqueous polyphenolic extract obtained has influence on sage plant acting as a stimulating on seed germination energy and capacity, root and stem growth, biomass accumulation and photoassimiling pigments synthesis, in lower concentration. Results indicated that natural phenolic compounds, separated from spruce bark wastes can act as stimulating agents (plant activator) on sage plant metabolism depending on the used concentrations. Considering the obtained data, we sustain that spruce bark extracts, can be use directly or as precursors to biobased products (bio-fertilizers, bio-insecticides, plant growth regulators) in biotech crops development. A direction of research emerges is evaluation of chemical composition of the volatile oil obtained from the treated plants and the testing of pharmacological activity of the volatile oil obtained from the plants treated with natural polyphenolic extracts.

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