On characterization of some bioactive compounds extracted from *Picea abies* bark

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Abstract

*A hydrophilic extract was obtained by sequential extraction from dry bark of **Picea abies**. This extract was characterized by GC and GC - MS analysis. The chromatographic data evidences that the major components of the spruce bark extract were stilbenes glycosides: isorhapontin, astringin and piceid.*

*The bioactivity of the extract was tested in seed germination of **Lycopersicon esculentum** and **Allium cepa**. The results showed some differences in the biological activity level, depending on concentration and the biological material used.*

Keywords: spruce bark, hydrophilic extractives, stilbenes glycosides, *Lycopersicon esculentum*, *Allium cepa*

Introduction

Bark is an important part of trees and it is produced in large amounts as a result of the processing of logs in the pulp and paper industry. The majority of this raw material is used as fuel, but bark processing is expensive due to transport and maintenance costs [1]. Based on current knowledge about the components of bark, this raw material can be used to separate various extractives with important application potential.

Several studies have been carried out in recent years regarding the influence of spruce extracts on seed germination and plant development. Simionescu Cr. [2] has tested the growth of vegetal tissue culture in the presence of raw extract and the results showed that the spruce bark polyphenols exhibited similar effects to the endogenous hormones auxin and cytokinine. Based on these results, phenolic compounds extracted from spruce bark were tested in standard germination experiments on bean and millet seeds [3] or wheat and rye seeds [4]. The spruce extract had influenced the development phases modifying the medium height and growth speed of wheat plantlets [5]. The data revealed that the biological activity of the phenolic extract varied according to the extraction method, concentration, different chemical modification and the material used.

Taylor et al. [6] studied the effects of the stilbenes and tannin separated from spruce bark on the seed germination and seedling growth of several species. The inhibition of germination and growth was possible by interfering in hormone balance.

This paper presents the results concerning characterization of hydrophilic extract obtained from dry bark of *Picea abies*. At the same time the evaluation of biological activity of the extracted compounds was carried out using seeds of *Lycopersicon esculentum* and *Allium cepa*. The germination capacity of these seeds and characteristics of plantlets were determined as a function of concentration.

Material and methods

Spruce bark extract

The spruce bark, provided by Ambro Suceava, was air-dried and ground in a mill. Sequential extraction was carried out in a Soxhlet apparatus. Lipophilic extractives were first extracted with benzene and then hydrophilic extractives were extracted with methanol. The solution which contained hydrophilic extractives was concentrated through evaporation at room temperature, obtaining a brown-red powder.

Analysis by Gas Chromatograph (GC) and GC – Mass Spectrometry (GC – MS)

After dissolving the spruce bark extract in methanol and evaporation of the solution, the extractives were silylated by addition of 100 μL bis-(trimethylsisyl)-trifluoroacetamide, 30 μL pyridine and 30 μL trimethylchlorosilane. The reaction was completed by keeping the test tubes in an oven at 70°C for 45 minutes.

The hydrophilic extractives were analyzed on a Perkin Elmer Autosytem XL instrument with a 25 m x 0.20 mm i.d. column coated with cross linked methyl polysiloxane (HP – 1) with a film thickness of 0.11 µm. Heneicosanic acid and betulinol were used as internal standards and no corrections factors were used. The column oven parameters were: 120°C, 6°C/min - 300°C (10 min), carrier gas H_2 (20 mL/min), split injector (1:20) 260°C, FID detector 300°C, injection volume 1 µL according to Ekman and Holmbom [7]. All results, given in mg g^{-1}, are calculated on dried extract.

Quantitative distributions of different compounds were determined on o short GC 6 m x 0.53 mm i.d., 0.15 µm HP – 1 column using cholesteryl heptadecanoate as internal standard. The gas chromatograph was a Varian 3400 instrument and the column oven parameters were: 100°C (1.5 min), 12°C/min - 340°C (5 min), gas carrier H_2 (20 mL/min), SPI (Septum-equipped Programmable Injector) 80°C (0.5 min) - 200°C/min - 340°C (18 min), FID detector 340°C, injection volume 0.4 µL.

Identification of individual components were performed by GC – MS analysis of the silylated compounds with a HP 6890 – 5973 GC – quadrupole – MSD instrument, using a similar GC column as above.

Bioassay

Seeds of *Lycopersicon esculentum* M. (cv. ACE 55) and *Allium cepa* L. (cv. Diamant), collected during 2004 and 2005, were obtained from Agrosel. All undersize or damaged seeds were discarded, and the assay seeds were selected for uniformity. For the bioassays, we used Petri dishes in 90 mm diameter with one sheet of filter paper as support. Germination and growth were conducted in aqueous solutions of the global extract at different concentrations, between 1 g/L and 10^{-8} g/L. After the addition of 25 seeds and 5 mL of test solutions, Petri dishes were placed in a thermostat at 25 °C in the dark. Controls tests used the same number of seeds in distilled water. Germination percentage was determined after 5 days according to Macias et al [8]. After growth, plantlets were measured to determine the roots and shoots elongations. In order to determine the dry mass, the plantlets were dried at a temperature of 105^0 C, until they reached constant mass.

In the graphics, data are reported as percentage differences from control. Thus, zero represents the control; positive values represent stimulation of the parameter studied, and negative values represent inhibition. Germination rates of control solution: 76 % onion, 84% tomato. Root lengths of control: 1.09 cm onion, 5.53 cm tomato. Shoot lengths of control: 1.57 cm onion, 2.75 cm tomato.

Results and Discussions

Spruce bark extract composition

The stilbene glycosides and sugars were the main components of the spruce extracts (figure 1, table 1). In addition, small amount of phenolic acids, aromatic compounds with small molecular mass such as p – hydroxyphenyl glycerol and 1 – guaiacylglycerol, free stilbenes and flavonoids were detected in the sample. The spruce extract contained two unknown compounds and the *cis* isomers of stilbene glycosides. The GC analysis on short column quantified 62.29 % of the total extract, while extractives in small amounts and tannins, which have high molecular mass beyond the detection limit of the instrument, represent 37.7 %. Previous analysis on the spruce bark indicated the presence of resveratrol, dihydromyricetin and taxifolin [Hemming J., personal communication], but in this case the compounds are under the limit of detection of the equipment.

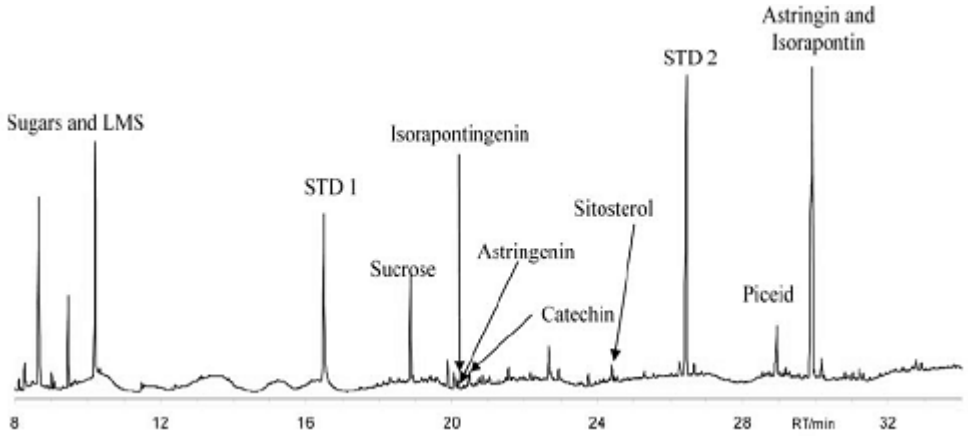

Figure 1. Long column GC chromatogram of hydrophilic extractives from spruce bark. Internal standard: STD 1 = fatty acid 21:0, STD 2 = betulinol (4 mg/g)

Table 1. The hydrophilic extractives from spruce bark

Compounds	mg/g extract
Sugars and LMS compounds	0.39
Sucrose	0.08
Isorhapontigenin	0.002
Astringenin	0.002
Sitosterol	0.002
Piceid	0.01
Stilbene glycosides	0.11
Unknown compounds	0.01

The predominant phenolic compounds from spruce bark were astringin, isorhapontin and piceid (figure 1). Astringin and isorhapontin were quantified as a mixture, because the two compounds have similar chemical structure and the same retention time. The bark of *Picea sitchensis* contained astringin as major stilbene, while isorhapontin and piceid are present in minor amounts [9]. Recent studies on *Picea abies* bark determined that isorhapontin was the major component, while astringin and piceid are found in smaller amounts [Harlamow Reija, personal communication]. The GC data provides information regarding the presence of low molecular size (LMS) compounds, most likely degradation products of tannins. The global extract contained also small amounts of free stilbenes found in outer bark [10].

Bioassay results

The biological activities of the phenolic extract were evaluated in a bioassay on the standard target species. The influence of the spruce extract was pursued through germination capacity of the seeds, dry biomass, roots and shoots lengths (figure 2…5). The results obtained showed that these compounds have different levels of activity depending on the concentrations used.

The phenolic extract had stimulatory effects on the germination capacity of *L. esculentum*, especially at concentrations 10^{-6} g/L and 0.25 g/L (figure 2). The number of *A. cepa* germinated seeds was inhibited in the majority of the tests, but the most active concentration was registered at 10^{-4} g/L. In both cases, the increase of the active compounds concentration induced a reduction of the biological response.

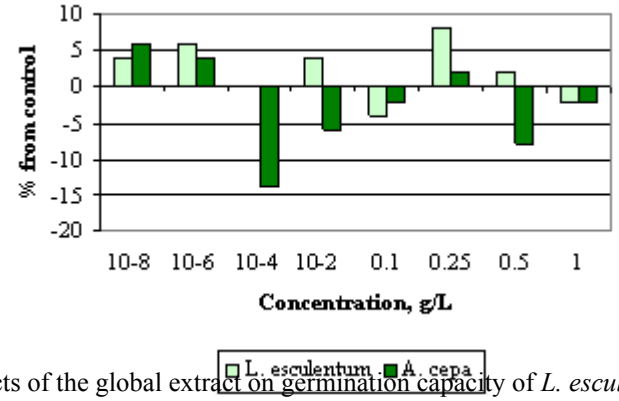

Figure 2. The effects of the global extract on germination capacity of *L. esculentum* and *A. cepa* seeds. The values are presented as percentage differences from control

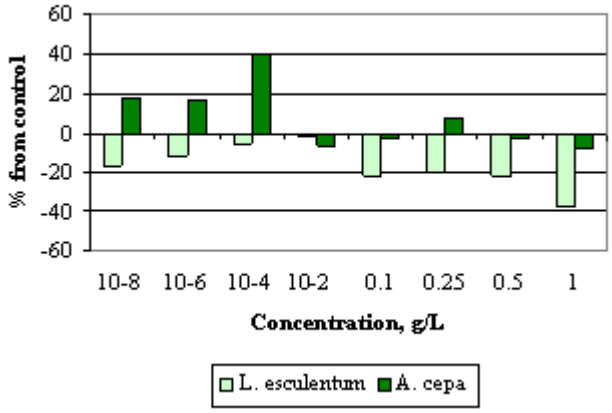

Figure 3. The influences of spruce extract on the roots length of *L. esculentum* and *A. cepa*. The values are presented as percentage differences compared to control

The global extract had strong influences on the plantlets growth at lower concentrations (figure 3). The increase of bioactive compounds concentrations had significant effects on *L. esculentum* and slight effects on *A. cepa*. The shoots length of *L. esculentum* plantlets were inhibited at all the concentrations tested, especially at concentration 1 g/L (figure 4). The presence of spruce bark extract had mainly stimulatory effects on the shoots of *A. cepa*, with a maximum response at concentration 10^{-4} g/L.

The hydrophilic extract increased the quantity of dry biomass for the *L. esculentum* plantlets, especially at lower concentrations (figure 5). The same compounds had slightly inhibitory effects on the dry biomass of *A. cepa* plantlets.

The spruce extract at lower concentrations of 10^{-2} – 10^{-8} g/L had significant effects on germination and development, while the increase of the concentration in bioactive compounds is correlated with inhibition of the studied characteristics.

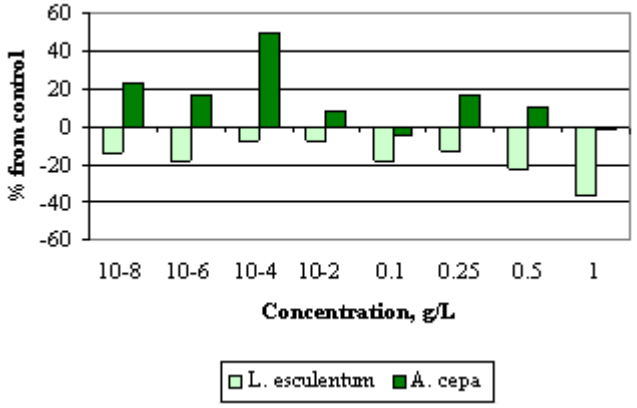

Figure 4. The influences of spruce extract on the shoots length of *L. esculentum* and *A. cepa*. The values are presented as percentage differences compared to control

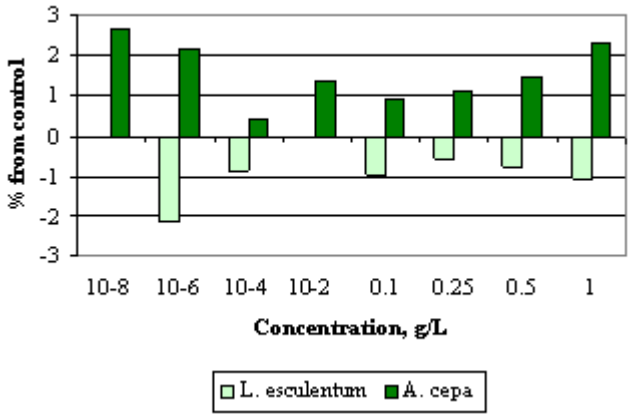

Figure 5. The influences of spruce extract on dry weight of seedlings of *L. esculentum* and *A. cepa*. The values are presented as percentage differences compared to control

Taylor et al [6] determined that stilbenes and tannins from spruce bark influenced the seed germination and seedlings growth by interaction with the hormone balance. Literature data indicate that tannins are capable to inhibit gibberellins – induced growth, acting as inhibitors of proteins that specifically recognizes gibberellin or directly on the gibberellin molecule to render it incapable of promoting growth [11]. We can presume that at higher concentrations, the inhibitory effects were induced by the condensed tannins from spruce bark. At lower concentrations, the germination and plantlets growth may be influenced by the presence of stilbenes glycosides. Experimental results indicated a stimulation of plantlets growth in the presence of extracts with high content of stilbenes glycosides [Bălaş Anca, unpublished data].

Conclusions

Although the biological activity can be explained by the predominant compounds found in the spruce extract, there are still more factors affecting the seed germination and plantlets growth. The processes can be influenced by the synergism of the polyphenols or the compounds in small amounts that strongly contribute to the activity.

It can be concluded that the spruce bark extract is biological active on *L. esculentum* and *A. cepa*. However, it is not certain the influence of every compound from bark, making difficult to elucidate the mechanism in which the growing processes in plants are modified.

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