NATURAL BIOACTIVE COMPOUNDS AS PLANT GROWTH REGULATORS

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Polyphenolic compounds are biosynthesized in plant as secondary metabolites, having an important role in sensorial and nutritional quality of fruits, vegetables and plants. In this work, the phenolic extracts were obtained from three different sources: Asclepias syriaca, grape seeds and spruce bark. The efficiency of different extraction techniques of polyphenols, such as heat extraction and ultrasound assisted extraction was studied and the influence of extracts was followed in the case of Phaseouls vulgaris seed germination tests. Radicles elongation and fresh biomass were determinate. Polyphenols may act as biostimulators or growth inhibitors in plants depending on the extract concentration applied.

***Key words**: polyphenols, seed germination,bioregulators*

Phenolic compounds, ubiquitous in plants are of considerable interest and have received more and more attention in recent years due to their bioactive functions. Polyphenols are among the most studied phytochemicals mainly because of their antioxidant activity[1]. At the same time, phenolics compounds may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light [3,4].

The aim of this study was to establish the influence of different aqueous extractions (heating extraction and ultrasound assisted extraction) in the case of three different plant materials: spruce bark, *Asclepias syriaca* and grape seeds. Then, the influence of polyphenolic extracts in different concentration was studied on *Phaseolus vulgaris* seeds in germination tests. Biometric mesured parameters were radicle length and fresh mass. The regulator function of bioactive compounds was correlated with the total phenolic content determined by Folin Ciocalteu method and with raw materials used for extraction. Depending on concentrations, the same bioactive compounds may act as stimulators as well as inhibitors in plant growth according to former studies [5,6].

MATERIAL AND METHOD

Heating extraction and ultrasonic-assisted extraction

Ground dried material (spruce bark, grape seeds and *Asclepias syriaca*) with 0.5,0.2 and 1 mm particle size were extracted with 125 mL water in water bath at 80-90°C respectively in ultrasonic bath at 70 °C for 45 min. The extraction was repeated three times and extracts were cumulated to a volume of 500 mL using distilled water. Ultrasonication was carried out using an ultrasonic processor (Bandelin Sonorex) at a frequency of 35 kHz. All experiments were performed on samples of 5, 10 and 20 g raw material dispersed in 125 mL of water with periodical stirring.

Dry matter and ash content

Dry matter content in the extracts was determined by evaporation of 25 mL extract on water bath and drying at 105°C till constant mass according to the Polish Standard PN–90/A75101/03, using a porcelain crucible. After that the crucible was placed into a muffle furnace at 600 C for 6 hours to establish ash and organic matter content.

Total phenol content analysis

Total phenol content (TPC) were estimated colourimetrically using the Folin-Ciocalteu method [2]

Plant material and seed germination

The biotest was performed on bean (*Phaseolus vulgaris*) seeds. Five seeds were washed first with tap water and then placed on Petri dishes (12x12 cm). Each dish contained paper filter saturated with 10mL of extract solution obtained from different biomass sources with different concentrations. Reference samples were prepared in the same conditions in the presence of distilled water. The germination tests were perfomed in standard conditions at 27°C.Five repetitions for each experiment were done. After three days of incubation, fresh mass and radicle length of each seedling were measured.

RESULTS AND DISCUSSIONS

Dry matter content and total phenolic content

The data concerning dry matter content of the extracts are presented in (*tab.1)* and evidences that the values obtained increase proportionally with the raw material quantity subjected to extraction process.

Furthermore, one can observe that the highest values in dry matter content were obtained in the case *Asclepias syriaca* (0.7-3.5 g/L) while spruce bark extracts presents almost the same content in dry matter with grape seeds extracts.

Both used methods allow separation of polyphenolic compounds(*fig.1*) The highest amounts of total phenolic content were identified in the grape seeds extracts. The spruce bark extracts were also contained high amounts of phenolic compounds between 85-190 mg/L. On the other side, the extracts obtained from *Asclepias syriaca* are characterized by the lowest content in phenolic compounds, with concentration values between 37-114 mg/L. The concentration of extracts in polyphenols is correlated with the amount of raw material subjected to extraction process. Total content of polyphenolic compounds shows to be smaller for ultrasound assisted extraction than for heating extraction. These differences may be caused by the distinct extraction conditions (temperature, interface area and contact time)

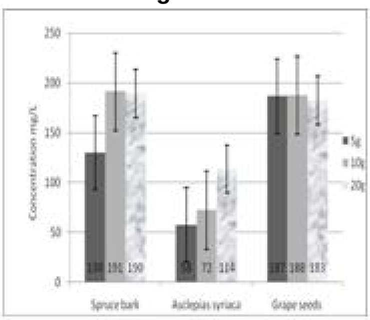
Table 1

Dry matter content for heating extraction and ultraso und assisted extraction

Sample	Dry matter content (g/L extract) for heating extraction	Dry matter content (g/L extract) for ultrasound assisted extraction	Organic matter content (g/L extract) for heating extraction	Organic matter content (g/L extract) for ultrasound assisted extraction
SB5	0.58	0.47	0.57	0.44
SB10	1.00	0.81	0.96	0.76
SB20	1.91	1.56	1.79	1.47
AS5	1.08	0.70	1.00	0.62
AS10	1.85	1.42	1.66	1.28
AS20	3.34	3.53	2.82	3.28
GS5	0.90	0.60	0.71	0.48
GS10	1.83	0.94	1.53	0.74
GS20	2.84	1.35	2.43	1.10

SB5, SB10, SB20 (spruce bark extract obtained from 5,10,20 g raw material),
AS5, AS10, AS20 (*Asclepias syriaca* extract obtained from 5,10, 20g raw material),
GS5, GS10, GS20 (grapes seeds extract obtained from 5,10,20g raw material).

Heating extraction



Ultrasound assisted extraction

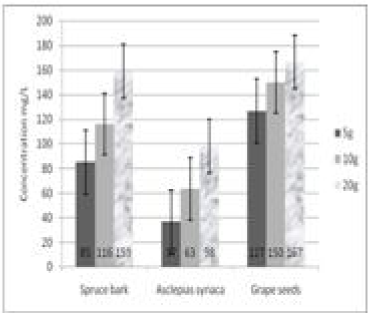


Figure 1 **Total content of phenolic compounds (mg/L extract) for heating extraction and ultrasound assisted extraction**

Polyphenolic extracts as plant regulators in germination tests

Polyphenolic extracts obtained from 5 g of raw material by ultrasonation-assisted extraction stimulate the elongation of radicle comparing with inhibitory effects provided by those obtained in the case of heating aqueous extracts (*fig. 2)* Spruce bark and grape seeds ultrasonation extracts have significant stimulating effects on radicle length. The most inhibitor effect is induce by spruce bark heating extracts. A smaller content of TPC in ultrasonation extraction seems to promote radicle elongation better than heatig extraction. It is known[6] that a higher concentration of the same extracts could be inhibitor even if for a smaller concentration of the same extract present stimulating effects.

Elongation of *Phaseolus vulgaris* radicle is stimulated by the presence of the spruce bark extracts obtained in ultrasonation extraction of 10 g raw material. The other polyphenolic extracts (heating and ultrasonation extraction) seems to have inhibitory effects on radicle length. (*fig 3*). Ultrasonation extract obtained from 10 g spruce bark show a stimulating effects on radicle length which can be correlated with an appropriate concentration of a particular polyphenolic compounds that acted similarly with growth regulators.

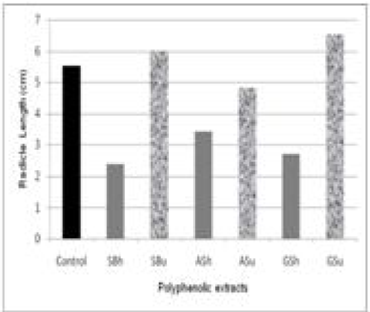


Figure 2 **The influence of polyphenolic extracts obtained from 5g raw material on radicle length.**

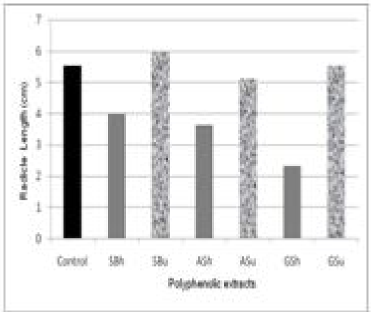


Figure 3 **The influence of polyphenolic extracts obtained from 10g raw material on radicle length**

Extracts, obtained by ultrasonation extraction technique from 20 g of all three raw material, provide a similar effects on radicle elongation as control solution. *Asclepias syriaca* heating extract stimulate the radicle length(*fig.4*). The other two heating extracts obtained from 20 g spruce bark and grape seeds present inhibitory effects on radicle length.even if in the other two concentration of polyphenolic heating and ultrasonation extracts, AS, raw material present inhibitory effects on radicle elongation, in the heating extracts obtained from 20 g raw material, seems to have the optimum concentrations in TPC to stimulate radicle length. The stimulating effects may be also due to the presence of a singular polyphenol compounds with growth tendency.

Weighting the fresh biomass of bean germinated seeds resulted in the presence of polyphenolic extracts obtained from 5 g raw material we conclude that all types of polyphenolic extracts applied in germination test inhibited the fresh biomass accumulation (*fig 5*). The most inhibitory effects is provided by grape seeds heating extracts that can be caused by the highest content of total polyphenolic content, from all the tested extracts.

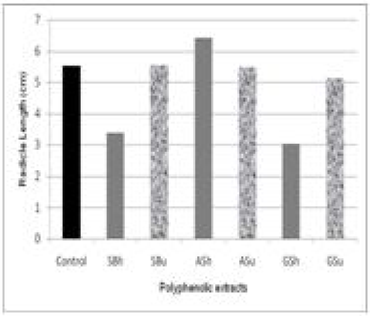


Figure 4 **The influence of polyphenolic extracts obtained from 20g raw material on radicle length**

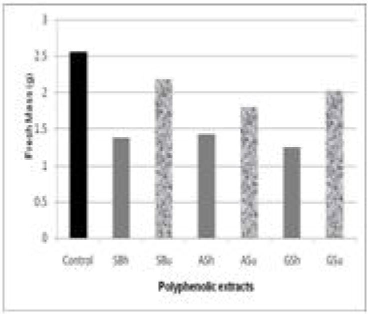


Figure 5 **The influence of polyphenolic extracts obtained from 5g raw material on fresh mass**

Fresh mass of been seed germinated after 72 h it is not eloquent stimulated by the presence of the polyphenolic extracts obtained by ultrasonation extraction of 10 g raw material (*fig.6*).

Polyhenolic heating extract of 10 g grapes seed have pronounced inhibitory effects on the biomass quantity of radicle due to a high concentration of TPC and for the presence of a characteristic phenolic compounds responsible for inhibiting fresh mass bioaccumulation.

None of the polyphenolic extracts obtain by heating or ultrasonation extraction technique has not stimulating effects on fresh biomass quantity (*fig. 7*). Spruce bark and *Asclepias syriaca* ultrasonation extracts presents the same impact on fresh mass of bean seeds as *Asclepia syriaca* heating extracts. The higher concentrations in TPC for spruce bark and *Asclepias syriaca* heating extracts it is the reason for inhibitory effects.

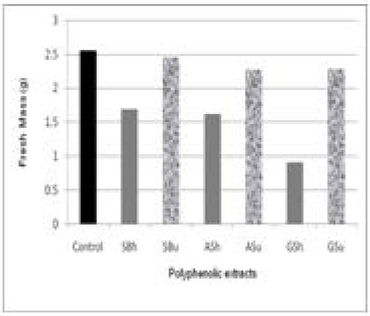


Figure 6 **The influence of polyphenolic extracts obtained from 10g raw material on fresh mass**

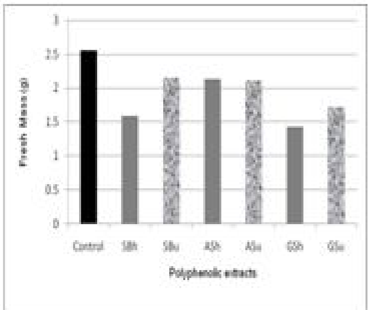


Figure 7 **The influence of polyphenolic extracts obtained from 20g raw material on fresh**

CONCLUSIONS

Concerning the two types of extractions one can conclude that heating extraction is more efficient from the total content of polyphenols point of view. Extraction temperature may have a significant influence in the process, therefore a higher temperature (80-90 °C- heating extraction) increase the yield of extraction comparing with lower temperature (70° C – ultrasound assisted extraction) even with additional ultrasonation.

The extracts separated from the three biomass sources could have inhibitory or stimulatory effects as a function of extraction procedure, concentration and the nature of extracted compounds.

In germination tests, spruce bark and grape seed extracts, obtained by ultrasonation extraction of 5 (85 mg/L TPC for spruce bark and 127 mg/L TPC for grapes seeds extract) and 10 g raw materials (116g/L TPC for spruce bark and 150g/L TPC for grapes seeds extract), stimulate the radicle elongation. This stimulating effect may be correlated with a minor concentrations of TPC in this two extracts, comparing with the other ones.

Stimulatory effects are noticed when the total polyphenolic content present smaller concentrations. Stimulating action it is alsoobserved in a presence of a dominant phenolic compound with similar propretie as growth regulator growth.

Fresh mass of *Phaseolus vulgaris* radicles it is inhibited by all polyphenolic extracts due to the upper concentrations in TPC. It is obvious that the inhibitory effects it is in direct ratio with TPC concentrations.

The total polyphenolic content, in lower concentration in ultrasonation extract provides a less inhibitory effect that the one obtained by heating technique extraction.

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