ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SOME NATURAL POLYPHENOLS

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The present study provides information on separation and identification of natural bioactive compounds from different vegetal materials with potential applications as antibacterials and plant growth modulators. Natural extracts were obtained by alcoholic and water extraction from spruce bark, grape seeds, *Crataegus monogyna* (hawthorn) and *Asclepias syriaca* (milkweed).
HPLC-DAD analysis revealed that gallic acid (12.54 mg/100 g) and catechin (85.52 mg/100 g) were the most abundant compounds in hawthorn and grape seed extracts, vanillic acid (71.9 mg/100 g) was found in high concentrations in spruce bark wood, while milkweed extracts were characterized by the presence of hydroxycinnamic acids and flavonoids. The DPPH assay indicated that the highest radical scavenging activity was registered by grape seeds (EC_{50} = 45.75 µg) and hawthorn (EC_{50} = 17.8 µg) alcoholic extracts. The same extracts exhibited antibacterial properties on *Staphylococcus aureus*, while the samples obtained from spruce bark and milkweed extracts inhibited the development of *Escherichia coli* and *Pseudomonas aeruginosa* species. Furthermore, the toxicity of the natural extracts was evaluated by their application in germination tests of *Phaseolus vulgaris*. The obtained results demonstrated that a high concentration of polyphenols has an inhibitory effect on plant development, while lower concentrations determine a considerable stimulation of plantlet elongation.

Keywords: polyphenols, extraction, characterization, HPLC, radical scavenging, antibacterial activity, seed germination

INTRODUCTION

Polyphenolic compounds are a wide spread group of secondary metabolites found in all plants and represent the most desirable phytochemicals due to their valuable properties and potential use as additives in food industry, cosmetics, medicine, and agriculture.[1,2]

In plants, polyphenols play an important role in various defense reactions to protect them against abiotic stresses like UV light or biotic stresses, such as predators and pathogen attacks, being also involved in plant growth and development cycle, as they have been shown to regulate the transport of polar auxins.[3] The stimulatory or inhibitory effect on plant development depends on the concentration of polyphenolic compounds and plant species. Different activities of polyphenolic products can be similar to those of growth hormones (auxins, cytokines).[4,5]

Polyphenols have been demonstrated to act as antioxidants and free radical scavengers. The interest in the physiological role of bioactive compounds present in plants has increased dramatically over the last decade, particularly in relation to human health. The antioxidant effects of polyphenols are considered to be derived from the scavenging action of their structures on oxygen containing free radicals. The *in vitro* antioxidant effectiveness of polyphenols is essentially the result of the ease with which an H

atom from aromatic hydroxyl is donated to a free radical and of the ability of the aromatic structure to support an impaired electron due to delocalization of the π-electron system. In this study, for characterizing the antioxidant activity of naturally occurring phenolic compounds, a method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reactive free radical was used. Now this method is recognized as giving the opportunity to search for radical scavenging ability depending on the electronic structure of polyphenols.

The pharmacological effects exerted by polyphenols on the human body are thought to be strongly related with their high antioxidant capacity,[6,7,8] which may be responsible for the anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial properties.[9,10,11]

Infectious diseases caused by bacteria resistant to multiple drugs have become one of the most serious problems nowadays. Although the use of antibiotics has greatly reduced the incidence of infectious diseases, it has also led to the appearance of drug-resistant bacteria.[12] Various plant phenolics, including phenolic acids, flavonoids and tannins known to be synthesized by plants in response to microbial infection have been shown to possess a broad spectrum of antibacterial effects against a wide array of microorganisms and a number of effective drugs have been created against them.[13,14]

Polyphenols not only exhibit independent antibacterial effects, but also can suppress the antibiotic resistance of pathogen microorganisms or act synergistically in combination with conventional antimicrobial agents like antibiotics.[10,12,13] Moreover, herbal preparations are comparatively cheaper, have lesser side effects and can supplement other medical systems for the treatment of diseases caused by pathogen microorganisms.

Our special interest is in the polyphenols extracted from spruce bark and grape seeds, which constitute abundant and inexpensive residual sources resulting annually from vineyards and pulp and paper companies. In addition, the potential use of spruce bark and grape seed extracts as dietary supplements and alternative medical products[15,16] justify our interest in these materials. The other two vegetal materials used in this study are *Crataegus monogyna* and *Asclepias syriaca*, which are known for their complex chemical composition, health benefits and potential use in different fields.[17,18,19]

This paper presents our recent results, revealing that several types of phenolics extracted from different raw materials are characterized by multifunctional activities, having a significant antioxidant capacity, being effective against Gram-positive and Gram-negative pathogen bacteria and also influencing the growth of plants.

EXPERIMENTAL

Plant material

Aerial parts of *Asclepias syriaca* were collected during the blossoming stage, from an Agricultural Research Station, where the plant was cultivated as an energy crop (1998), as a part of a research program of "Gheorghe Asachi" Technical University of Iasi, Natural and Synthetic Polymer Department. *Crataegus monogyna* fruits were collected from Cucuteni, Iasi County, spruce wood bark was provided by a Romanian pulp and paper company and Merlot grape seeds were obtained from Panciu, Vrancea vineyard.

All the raw materials were air-dried, ground in an electric mill (RETSH GRINDOMIX, GM 200) and reduced to a fine powder of 0.5 mm.

Extraction procedures

The extraction of phenolic compounds was carried out on the defatted (hexane extraction) samples, using either 80% ethanol, or water as solvents. The alcoholic extractions were carried out in a Soxhlet apparatus, during 8 hours at 70 °C. The extracts were then concentrated to 50 mL in a rotary evaporator at 40 °C. These concentrated extracts were tested for antimicrobial activity.

The fractionation of the concentrated extracts was achieved using successive liquid-liquid extractions with ethyl acetate. The organic phases were evaporated to dryness, diluted in methanol and followed by HPLC determination.

The aqueous extraction was performed on 20 g of dried material using 125 mL of distilled water at 70 °C for 45 min. The extraction was repeated three times and the extracts were cumulated and concentrated under vacuum. The fractionation by liquid-liquid extraction was also carried out on the concentrated extracts prior to HPLC analysis.

Spectrophotometric determinations

The total phenolic content of the plant extracts was determined using FCR (Folin-Ciocalteu's Reagent) using a previously developed method.[7] Total phenolic content is expressed as the number of equivalents of gallic acid (GAE).

The total content of tannins was determined using a method based on the precipitation of tannins with casein. The tannins content was determined using the FC method and expressed as the difference between

the initial content of polyphenols and the content after the precipitation with casein.[20]

The contents of flavonoids and flavonols were determined by the aluminum chloride method, using rutin as a reference compound, according to a previously developed modification of the method.[20,21] The results were expressed as mg rutin equivalents (RE). The anthocyanins content was determined using the pH differential method.[22]

HPLC analysis

A previously developed reversed-phase high-performance liquid chromatographic technique was used to identify and quantify the phenolic compounds.[7]

Chromatographic analyses were carried out on a Dionex UltiMate 3000, liquid chromatography system, equipped with a photodiode array detector. Eluent (A) was 1% aqueous acetic acid, and eluent (B) 1% acetic acid in methanol, the flow rate was kept constant throughout the analysis, at 1.2 mL/min. Injections were accomplished with a 5 µL fixed loop. The column was an Agilent Zorbax, C18 RP (4.6 x 150 mm, particle size: 5 µm) and the temperature was maintained at 30 °C. For phenolic acids the elution programme used was the following: the percentage of solvent B was increased linearly from 10 to 40% in 40 min and then decreased in one minute to 10% and maintained for 10 min. For quantification, standards for external calibration were used. Chromatograms were monitored at 280 and 320 nm.

For identifying and quantifying the flavonoids, a gradient method was applied. The flow rate was of 1 mL/min and the elution conditions were as follows: 30% B 0-10 min, 30-80% B 20-25 min, 80% B 25-29 min, 80-30% B 29-30 min, 30% B 31-32 min. The standards used for external calibration were rutin, quercetin, apigenin and kaempferol and the chromatograms were recorded at 254 nm, 360 nm and 365 nm.

For all the standard polyphenols and flavonoids, the regression coefficient was higher than 0.997 and the limits of detection were 0.1 mg/L for gallic acid, 1.4 mg/L for catechine and rutin, 0.6 mg/L for vanillic acid, syringic acid, ferulic acid, sinapic acid, 0.3 mg/L for p-coumaric acid, 1.2 mg/L for kaempferol and apigenin and 0.8 mg/L for quercetin.

Radical scavenging activity

The radical scavenging activity of the natural extracts was evaluated by the reduction of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The antioxidant activity of the extracts was expressed as EC_{50}, equivalent amount of an extract that inhibits 50% of the radical. The colorimetric assay was performed according to a modified method.[23]

For the DPPH assay the concentrated extracts (alcoholic and aqueous) were freeze-dried to avoid solvents interferences. Thereafter, different dosages of a 0.5 mg/mL methanolic solution of either water or ethanol freeze-dried extracts (25, 50, 100, 200, 300, 400, 500 µL each) were added in screw capped glass vials containing 2 mL of the DPPH.

All tubes were adjusted to 3.1 mL with MeOH. After a reaction time of 30 min, the absorbance was measured at 517 nm.

The inhibition percentage of the free radical DPPH (%I) was calculated according to the following equation:

$$\%I = \frac{A_0 - A}{A_0} x100$$

where A_0 = absorbance of DPPH, A = absorbance of DPPH + extracts.

Methanolic solutions of ascorbic acid, gallic acid, vanillic acid and catechin were tested as reference antioxidants. The different quantities of the extracts tested, expressed in milligrams, were plotted on a dose-inhibition curve.

Antibacterial activity testing

Before the analysis, bacterial strains were cultured on tryptic soy agar (TSA) pH 7.3, under aerobic conditions for 24 h, at 37 °C, except for the fungal strain C*andida parapsilosis* cultivated on Sabouraud 2% (m/v) – glucose agar for 48 h, at 37 °C. These microbial cultures were used as inocula in order to test the antibacterial activity of some crude herbal extracts.

The antibacterial activity of the extracts was estimated using the disk agar diffusion method, according to the Kirby-Bauer test,[24] one of the most widely spread and frequently used methods, also recommended by the European Pharmacopoeia.[25] Its advantages, compared to the dilution tests, consist in simplicity and facility to use, the direct control of the bacterial culture purity, easiness to read and interpret the results.[14,26]

Briefly, inocula of bacterial cells and yeast-like fungal blastospores were suspended in sterile physiological saline solutions, in sterile tubes, and homogenized on Vortex, until the density of the test suspension matched the turbidity standard, which was equivalent to a concentration of 1.5×10^8/mL (McFarland Standard, GrantBio, UK). Petri dishes (d = 9 cm) with Müller-Hinton agar (pH 7.3) for bacterial species, or Sabouraud-agar (pH 6.5) for yeast-like fungi, were inoculated with 1 mL of microbial suspension spread to ensure complete coverage. The plates were left for 5 min before excess fluid was removed using a sterile pipette. Then, the inoculated Petri dishes were dried at room temperature for a maximum of 20 min. Inoculation was carried out using a Bioquell ATC 1200 N-MK2 biological safety cabinet (UK).

Sterile paper discs impregnated with 20 µL crude herbal extracts were aseptically applied to the surface of each of the inoculated plates in a central position, using a sterile forceps, gently pressing to ensure even contact with the medium surface. Control discs

impregnated with antibacterial substances and blank discs (impregnated with solvent) were also placed into the inoculated Petri dishes using a disc dispenser. Gentamicin, ofloxacin, amikacin, kanamycin, cefuroxim, erythromycin were used as reference antibacterial, and fluconazole and nystatin as reference antifungal substances. The inoculated plates were incubated for 20 h, at 37 °C for bacterial species, and for 48 h, at 37 °C for *C. parapsilosis*. The inhibition zones were expressed in mm, as the diameters of clear zones around the discs. The results of antimicrobial activity were expressed as the mean value of three independent analyses.

Seed germination tests

Germination tests were carried out in Petri dishes, each one containing 5 bean seeds pre-disinfected with 1% NaOCl, for 15 min and 10 mL tested sample solution.

Three different concentrations of polyphenolic extracts (ethanolic and aqueous) expressed as TFC (0.05 mg GAE/L, 0.1 mg GAE/L and 0.2 mg GAE/L) were tested. The reference was tap water. Petri dishes (5/each sample) were kept at dark in a thermostatic chamber (25 °C) for seven days. The experiment was carried out in triplicates and the average values of the parameters were reported.

Pot experiments

Pot tests were carried out under greenhouse conditions using sandy soil. Bean seeds (*Phaseolus vulgaris*) were sown directly into pots after applying 10 mL of the tested solution. Three different concentrations of natural extracts were tested (0.05 g GAE/L. 0.1 g GAE/L, 0.2 g GAE/L). Each treatment was replicated in five pots, and three uniform plants were allowed to grow in each pot, at uniform spacing.

For ten days, the cultivated soils were wetted daily with 15 mL of the tested extracts. After 10 days from the beginning of the experiments, the bean plantlets were separated into roots, stems and primary leaves and measured to evidence the different effects of the natural extracts on the growth and development of the plants. Biometric measurements of plantlet elongation and quantitative determination of biomass fresh weight (FW) were determined.

RESULTS AND DISCUSSION

It is known that the biological properties of natural compounds depend on their composition and concentrations. Therefore, to establish the range of applications of polyphenols, it is necessary to isolate and characterize them from the viewpoints of compositional, structural and biological activities.

Characterization of polyphenolic extracts

Spectrophotometric determinations

The concentrations of different classes of phenolic compounds existing in the extracts separated from different raw materials were determined spectrophotometrically using colorimetric methods based on complex formation, oxidation and pH decreasing. The content of the extracted polyphenols (Table 1) varied depending on the plant material and the extraction agent used. Thus, the highest content of polyphenols was acquired for the grape seed and *Crataegus monogyna* alcoholic extracts (13.68, respectively, 57.22 mg GAE/g). The same samples were also characterized by higher amounts of flavonoids and flavonols, compared to the spruce bark and *Asclepias syriaca* extracts.

HPLC analysis

The major compounds identified in almost all the samples in different ranges of concentration were gallic acid and catechin. Hence, the highest content of these polyphenols was registered in the case of grape seed and *Crataegus monogyna* extracts. As expected, the concentrations in the alcoholic samples were significantly higher, compared to the aqueous ones. The concentrations calculated based on the calibration curves of the standards are reported in Table 2 and the chromatographic profiles of ethanolic extracts are presented in Figures 1, 2 and 3.

In the spruce bark alcoholic extract, besides the presence of the gallic acid and catechin, vanillic acid was found in high concentration (71.9 mg/g), while the other four phenolic acids (gallic, vanillic, syringic and p-coumaric acids) were identified in *Asclepias syriaca* extracts.

Concerning the four flavonoids surveyed in the natural extracts, the results showed that quercitin was present in all the vegetal materials in different ranges of concentration, starting from 0.14±0.05 mg/100 g in *Asclepias syriaca* extract and up to 2.38±0.1 mg/100 g in the grape seed extract. On the other hand, relatively high amounts of rutin were found in *Crataegus monogyna* (30.32±0.9 mg/100 g) and *Asclepias syriaca* (2.25±0.21) samples.

Table 1
Total content of polyphenols, tannins, flavonoids, flavonols and anthocyanins in aqueous and ethanolic extracts of the vegetal samples

Raw material	Type of extract	Total phenolic content, mg GAE/g	Total tannins content, mg GAE/g	Total flavonoids content, mg RE/g	Flavonols content, mg RE/g	Anthocyanins content
Spruce bark	Aqueous extract	5.17±0.04	1.64±0.03	0.22±0.04	0.081±0.009	-
	Ethanolic extract	13.55±0.11	4.68±0.04	1.01±0.01	0.15±0.02	0.41±0.08
Grape seeds	Aqueous extract	5.06±0.05	1.98±0.06	0.27±0.05	0.071±0.01	0.18±0.05
	Ethanolic extract	13.68±0.14	5.23±0.06	1.79±0.05	0.15±0.07	0.62±0.03
Crataegus monogyna	Aqueous extract	10.32±0.07	3.45±0.08	0.29±0.02	0.13±0.08	0.37±0.03
	Ethanolic extract	57.22±0.23	40.78±0.21	2.02±0.32	0.16±0.08	0.76±0.09
Asclepias syriaca	Aqueous extract	2.87±0.1	1.59±0.01	0.08±0.01	0.09±0.005	-
	Ethanolic extract	11.79±0.18	4.0±0.03	0.70±0.09	0.09±0.007	0.03±0.008

Values are expressed as means ±SD of three replicate analyses

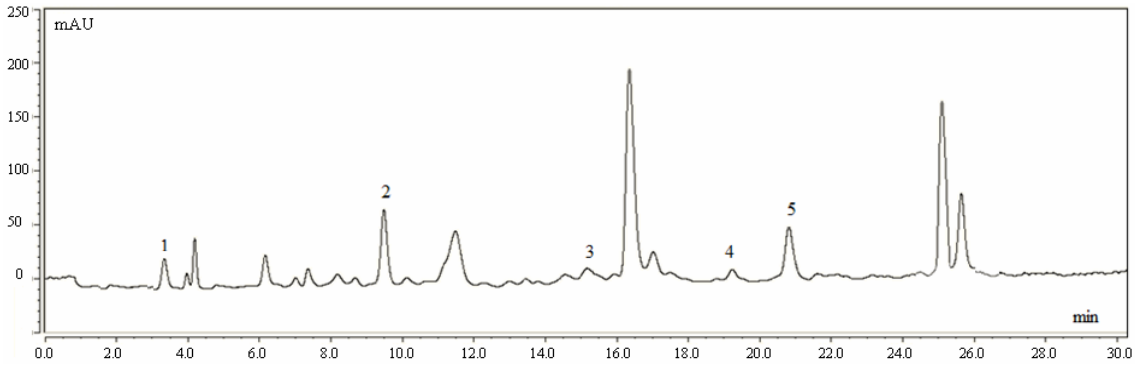


Figure 1: Chromatographic profile of *Crataegus monogyna* ethanolic extract: 1 – gallic acid; 2 – catechin; 3 – syringic acid; 4 – p-coumaric acid; 5 – ferulic acid

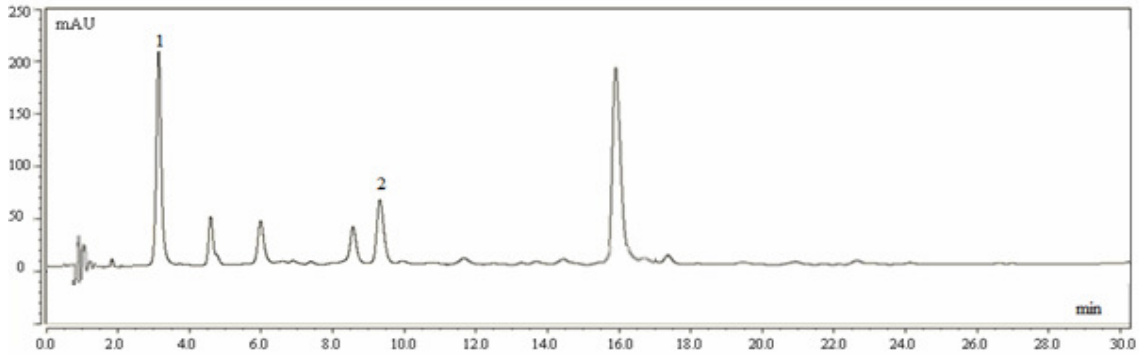


Figure 2: Chromatographic profile of grape seed ethanolic extract: 1 – gallic acid; 2 – catechin

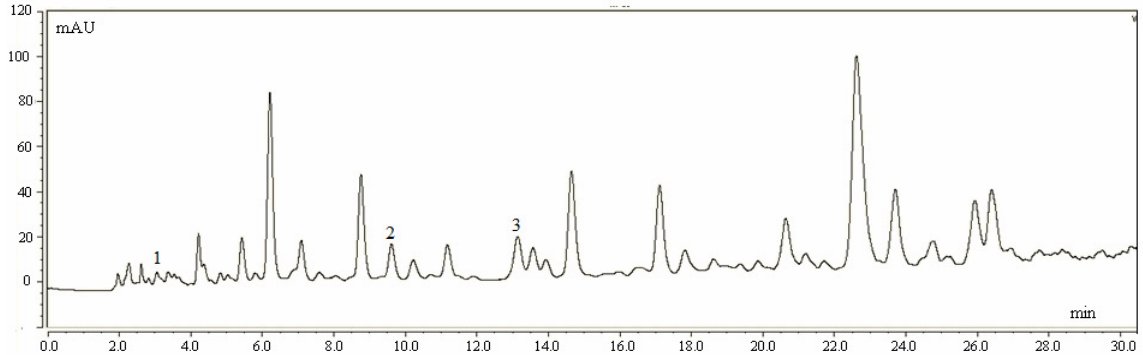


Figure 3: Chromatographic profile of spruce bark ethanolic extract: 1 – gallic acid; 2 – catechin; 3 – vanillic acid

Radical scavenging activity

The radical scavenging activity of the alcoholic and aqueous extracts was compared with that of some standard polyphenols (gallic acid, catechin, vanillic acid) and ascorbic acid, one of the most common antioxidant used in food industry.[27]

A decrease in absorbance was recorded at 517 nm for different quantities of standards and extracts, and the results were plotted on a dose-inhibition curve. The resulting linear calibration curves were used to derive the EC_{50} value, which represents the equivalent amount of an extract that neutralizes 50% of the DPPH radical.

Linear regression showed a good fit to experimental data and high R^2 values were observed for almost all the samples (Table 3).

Figure 4 shows the inhibition, %, of DPPH for different quantities of standard compounds and vegetal extracts.

The complete neutralization of DPPH radicals was obtained for ascorbic acid, gallic acid and catechin, while vanillic acid provided only 80% inhibition.

As concerns the vegetal extracts, significant differences in scavenging activity against the DPPH radical were registered according to their characteristic compounds and the concentrations involved. The alcoholic extracts showed a higher radical scavenging activity, compared to the aqueous ones, but slightly lower, compared to the standard compounds.

The DPPH assay indicated that the grape seed and hawthorn alcoholic extracts had the highest radical scavenging activities, which can be attributed to the considerable amounts of gallic acid and catechin, compounds which exerted a good inhibition of DPPH radicals on their own. The results are strongly correlated with the total phenolic content and HPLC determinations previously reported. Therefore, the antioxidant capacity depends not only on the quantity, but also on the type of polyphenolic compounds present in extracts. The EC_{50} values for the standards and vegetal extracts are reported in Table 3.

Antibacterial activity

The obtained results showed that the spruce bark, *Crataegus monogyna* and grape seed ethanol extracts exerted antibacterial activity (the largest inhibition zones, 12-15 mm) against Gram-positive pathogenic bacteria (*Staphylococcus aureus*). Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) are less susceptible (smaller inhibition zones) to herbal ethanolic extracts like spruce bark and *Asclepias syriaca* and not susceptible to aqueous extracts. Unfortunately, aqueous extracts recorded none or less significant inhibition zones both for bacteria and yeast-like fungi.

The results suggested that the antibacterial activity of ethanolic extracts against Gram-positive pathogenic bacterial strains was comparable to the antimicrobial activity of the reference antibacterial substances. Regarding fungicidal activity, none of the tested extracts was active against *C. parapsilosis.*

Gram-positive bacteria are known to be more sensitive in general than Gram-negative species to the phenolics naturally occurring in plant extracts.[10,11,14] Furthermore, *Escherichia coli* and *Pseudomonas aeruginosa* were previously demonstrated to be the most resistant bacteria to phenolics from coffee and tea extracts.[10]

Table 2
Concentration of phenolic compounds (mg/100 g dried plant) in the investigated samples

Raw material	Type of extract	Gallic acid	Catechin	Vanillic acid	Syringic acid	p-coumaric acid	Ferulic acid	Sinapic acid	Rutin	Quercetin	Kaempferol	Apigenin
Grape seeds	Aqueous extract	6.12±0.2	44.36±0.1	-	-	-	-	-				
	Ethanolic extract	12.54±0.8	63.60±1.7	-	-	-	-	-		2.38±0.1		
Crataegus monogyna	Aqueous extract	-	23.42±0.9	-	2.14±0.09	-	-	-				
	Ethanolic extract	10.98±0.7	89.52±2.1	-	2.95±1.1	3.59±0.4	2.25±1.4	-	30.32±0.9	0.64±0.02		
Spruce bark	Aqueous extract	-	31±1.9	39.4±0.2	-	-	-	-				
	Ethanolic extract	10.2±0.3	71.9±2.7	71.9±0.8	-	-	-	-		1.39±0.08		
Asclepias syriaca	Aqueous extract	-	-	0.87±0.1	0.98±0.09	0.11±0.1	-	-				
	Ethanolic extract	0.65±0.4	-	2.94±1.1	1.94±0.9	0.40±0.09	-	-	2.25±0.21	0.14±0.05	0.17±0.02	

Values are expressed as means ±SD of three replicate analyses

Table 3
EC50 values obtained for the standard compounds and vegetal extracts

Samples	EC50, µg	R^2
Ascorbic acid	4.6±0.87	0.99
Gallic acid	1.7±0.32	0.90
Vanillic acid	41.9±1.14	0.99
Catechin	12.7±0.98	0.99
Grape seeds alcoholic extract	45.75±1.09	0.99
Grape seeds aqueous extract	76.25±1.12	0.99
Spruce bark alcoholic extract	159.15±3.21	0.98
Spruce bark aqueous extract	246.0±5.56	0.99
CM alcoholic extract	17.8±0.98	0.99
CM aqueous extract	28.32±1.02	0.99
AS alcoholic extract	212.45±4.31	0.98
AS aqueous extract	303.1±5.12	0.98

GS – grape seeds; SB – spruce bark; AS – *Asclepias syriaca*; CM – *Crataegus monogyna*;
Values are expressed as means ±SD of three replicate analyses

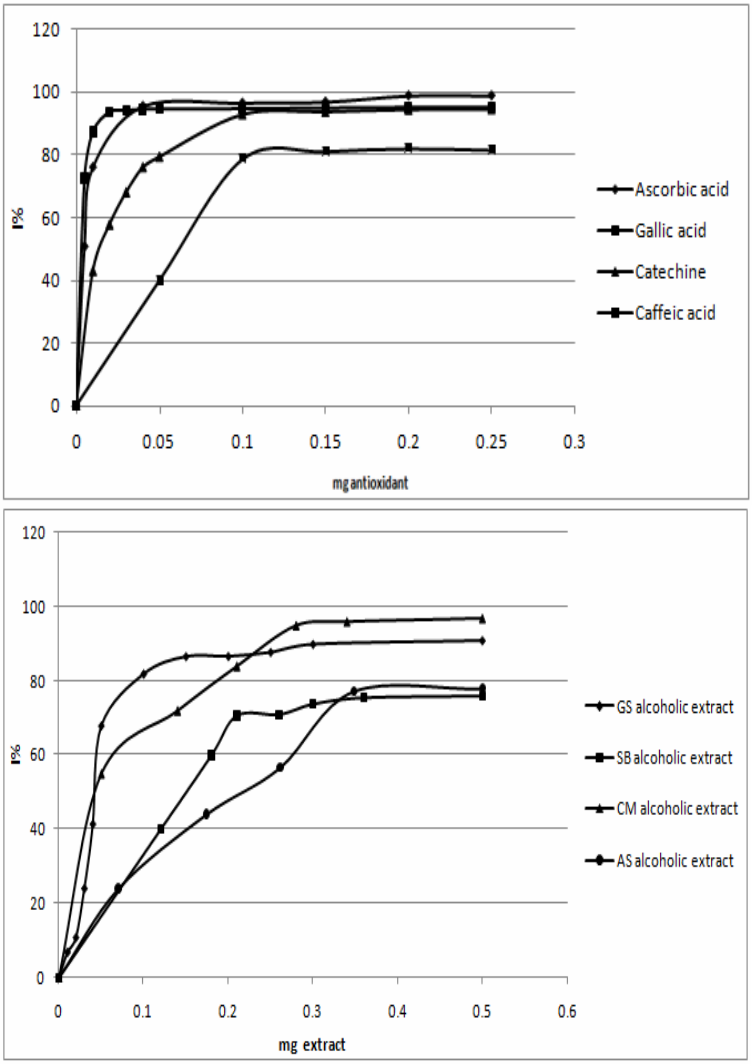


Figure 4: The inhibition effect of standard polyphenols and natural extracts on DPPH reagent

Table 4
Antimicrobial activity of the herbal extracts compared to antibacterial and antifungal reference substances

	Inhibition zones (mm)			
Reference microbial strains	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida parapsilosis*
Asclepias syriaca aqueous extract	-	6	6	-
Asclepias syriaca ethanolic extract	-	12	6	-
Spruce bark aqueous extract	-	-	-	-
Spruce bark ethanolic extract	15	10	10	-
Crataegus monogyna aqueous extract	-	-	-	-
Crataegus monogyna ethanolic extract	15	-	-	-
Grape seed aqueous extract	-	-	-	-
Grape seed ethanolic extract	12	-	-	-
Gentamicin	17	19	ND	ND
Ofloxacin	21	19	22	ND
Amikacin	ND	21	21	ND
Kanamycin	18	-	18	ND
Cefuroxime	26	-	17	ND
Erythromycin	20	10	11	ND
Fluconazole	ND	ND	ND	-
Nystatin	ND	ND	ND	25

ND – not determined

Due to the lipophilic nature of phenolics, they fail to diffuse across the outer membrane, this fact offering a reasonable explanation for their generally reduced activity towards Gram-negative bacteria.

Correlating the antibacterial activity with the antioxidant capacity and the characteristic compounds from each sample, one can affirm that ethanolic extracts from *Crataegus monogyna*, spruce bark and grape seeds, having high amounts of catechin and gallic acid, were the most efficient samples.

As expected, all ethanolic extracts having higher content of total phenolic, compared to aqueous extracts, exerted statistically significant antibacterial activity (inhibition zones between 10-15 mm).

Milkweed extracts characterized by the presence of hydroxycinnamic acids and flavonoids exhibited antibacterial effects on *Pseudomonas aeruginosa* and *Escherichia coli* and the spruce bark alcoholic extracts showed to be more effective with relatively large inhibition zones on *S. aureus*, *P. aeruginosa* and *E. coli*.

Generally, flavonoids are effective compounds on pathogenic bacteria, but in our case due to the reduced concentrations in vegetal extracts, no significant correlation was observed.

Germination tests and pot experiments

The polyphenols are known not only as potent antioxidants, but also as cell regulators. In this process, cell-cell communication and signal transduction is very important and some polyphenols can be considered to be essential for plant life. Thus, the polyphenols, as a function of their structure, can be involved in dividing plant cells, polar transportation of auxins, and enhancing the protection system against ultraviolet rays. At the same time, polyphenols exhibit antibiotic and phytoalexin activities, protecting plants from diseases, or acting directly on the genes required for infection. Therefore, the vegetal systems could play an important role in the study of the biological properties of

polyphenols, using tests of seed germination and plant cultivation.

In this study, the aqueous and alcoholic extracts obtained were applied in different concentrations in seed germination tests and plant growth and development experiments, using *Phaseolus vulgaris* seeds.

Biotests, unlike instrumental (chemical) methods, allow simple and inexpensive estimation of toxicity and stimulatory or inhibitory effects of natural bioactive compounds. These experiments are intended to evaluate the potential use of vegetal extracts as amendments in plant growth and by testing different concentrations of polyphenols, we can also obtain valuable information on the toxicity of the samples.

Considering these aspects, the selected plant for these tests was *Phaseolus vulgaris*, as it is one of the plant species recommended by the US FDA (Food and Drugs Administration) for phytotoxicity tests.[28]

To evaluate the plant response in the presence of phenolic compounds, germination percentage, biometric measurements (shoot and rootlet elongation) and fresh biomass quantity were determined.

The influence of milkweed and grape seed alcoholic extracts on plantlet elongation and fresh biomass accumulation are presented in Figures 5 and 6.

Our experiments revealed that the effect of natural polyphenols is strongly related to the concentration used. In the case of *Asclepias syriaca*, for all the concentrations applied, a generally stimulatory effect was obtained for shoot and rootlet elongation, excepting the concentration of 0.2 g/L, which had an inhibitory effect on shoots' length. The most significant results were registered for the concentration of 0.05 g/L, where the stimulatory effect on rootlets was more than 100%, compared with the control.

For all the concentrations tested, a significant inhibitory effect (50% compared with the control in the case of 0.2 g/L) was registered for the shoot and rootlet fresh biomass accumulation.

The use of hawthorn, spruce bark and grape seed extracts as amendments led to different results. The presence of these extracts in the growth medium had a strong inhibitory effect on shoot and rootlet elongation, especially in high concentrations (0.1 and 0.2 g GAE/L).

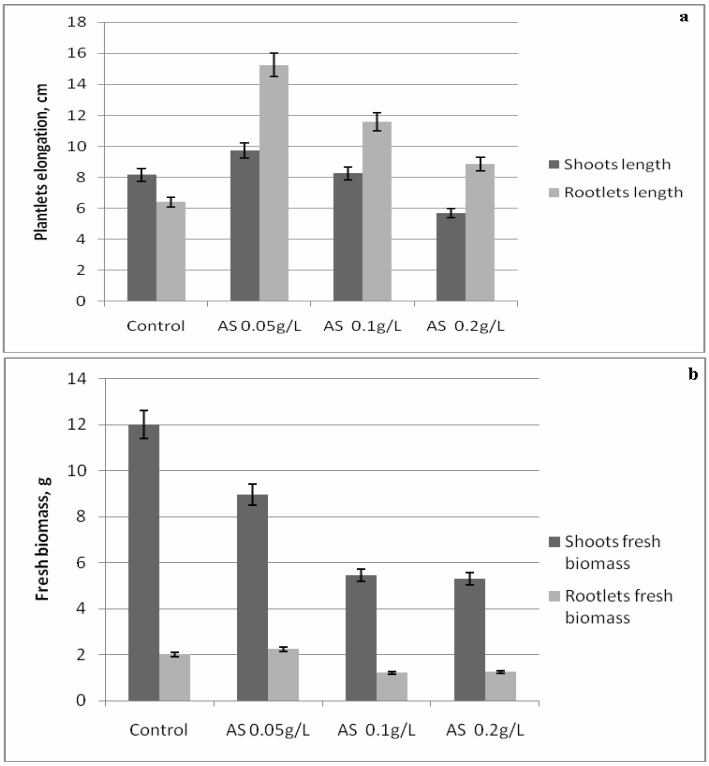


Figure 5: The influence of milkweed alcoholic extracts on plantlet elongation (a) and fresh biomass accumulation (b) (germination tests); AS – alcoholic extract (different concentrations) of *Asclepias syriaca*

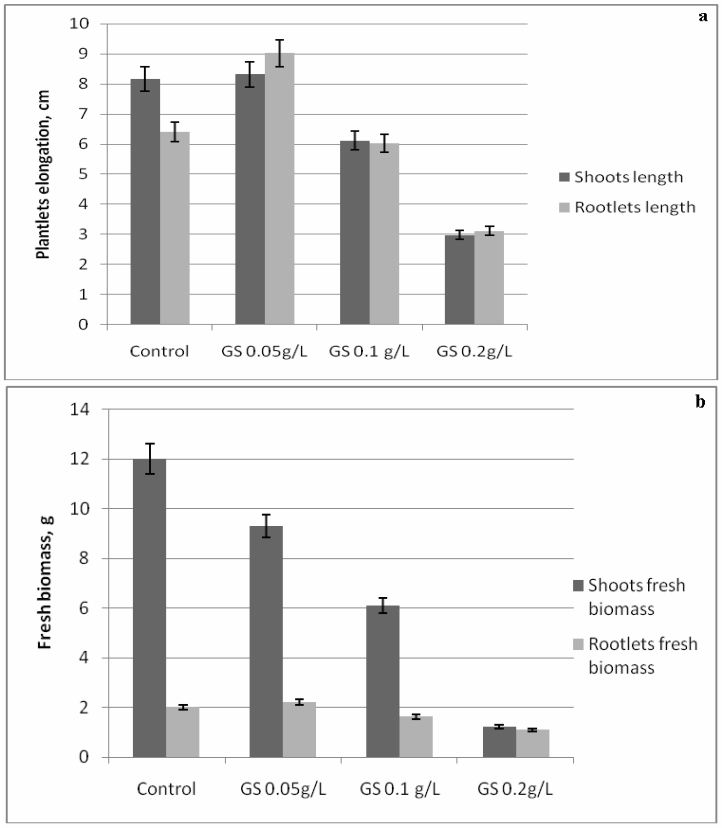
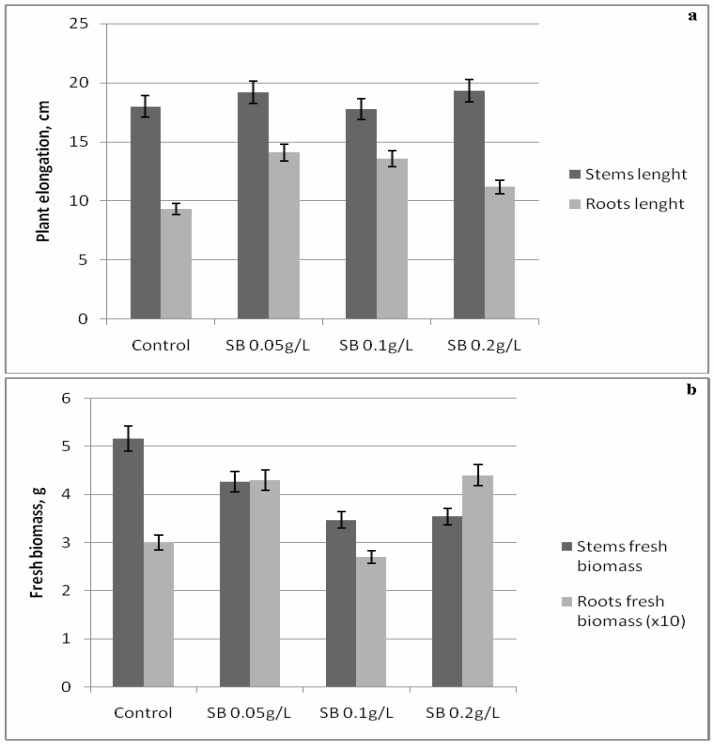


Figure 6: The influence of grape seed alcoholic extracts on plantlet elongation (a) and fresh biomass accumulation (b) (germination tests); GS – alcoholic extract (different concentrations) of grape seeds



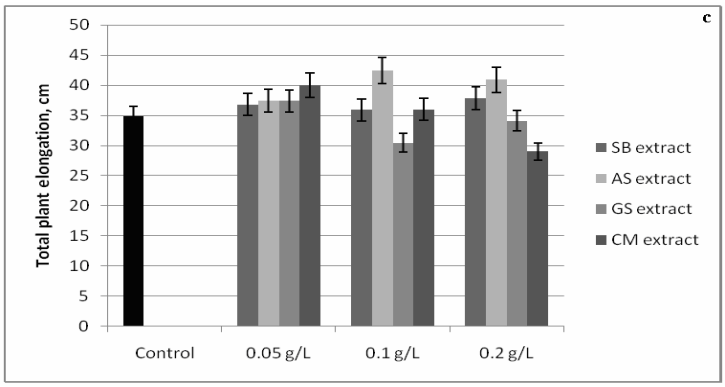


Figure 7: The influence of vegetal extracts on plant elongation (a), fresh biomass accumulation (b) and total plant elongation (c) in pot experiments; SB – alcoholic extract (different concentrations) of spruce bark

On the other hand, the lowest concentration significantly increased the length of both rootlets and shoots. The most relevant results were obtained for hawthorn and spruce bark, where the stimulating effect was approximately 100%, compared with the control sample.

It must be mentioned that from the beginning the highest concentration delayed germination and significantly reduced the final germination percentage (50-60%) recorded after 7 days.

As concerns the biomass accumulation, all the vegetal extracts inhibited the shoot biomasses, while only 0.05 g/L slightly stimulated the accumulation of biomass for the roots.

A similar trend was recorded in the pot experiments treatment (Figure 7), where only the concentration of 0.5 g/L ameliorated plantlet growth (stems, roots, ramifications).

The obtained results confirm that polyphenolic compounds can be considered allelochemicals. It is known that allelochemicals can induce hormetic dose response effects in biological systems. In such cases, low concentrations of polyphenols enhance seed germination and plant growth, whereas higher concentrations usually exert an inhibitory effect.

It must be emphasized that in plantlet development (germination test and pot experiments) under the influence of different vegetal extracts the hormetic effect was found for 0.5 mg GAE/L, while the higher concentrations (0.1 mg GAE/L and 0.2 mg GAE/L) induced a toxic effect especially on rootlets and biomass accumulation. This double response may depend, on a variation in polyphenols uptake by the different plant organs.[29]

The results are explicable considering that besides the endogenous growth inhibitors (abscisic acid), polyphenols can exhibit strong biological inhibitory activity when applied exogenously in high concentrations.[28] We can appreciate that polyphenols can regulate biosynthesis processes in plants, their action depending on the structure, composition of the mixture and concentration. The polyphenols could interfere with the plant hormones, thus influencing either stimulation or inhibition of plant development.

CONCLUSION

This study revealed that the selected raw materials are rich sources of phenolic compounds with high radical scavenging activity, which enables their use in different fields. *Crataegus monogyna* and grape seed extracts showed the highest antioxidant capacity due to the considerable amounts of gallic acid and catechin. At the same time, the ethanolic extracts obtained from *Crataegus monogyna* and grape seeds proved to be effective against Gram-positive pathogens, spruce bark ethanolic extract exerted antibacterial activity against both Gram-positive and Gram-negative bacteria, while *Asclepias syriaca* extracts showed antibacterial activity only against Gram-negative species. Regarding the selectivity of the tested pathogen species towards the vegetal extracts, it would be interesting to mix them and induce a synergetic effect of polyphenols to increase the antibacterial activity.

Regarding the germination tests of *P. vulgaris* and plant development under the influence of natural phenolic compounds, we can affirm that the inhibitory/stimulatory effect was almost similar for all the tested solutions and the growth declined with increasing concentration, indicating a dose-response behaviour. The highest percentage of inhibition was determined in the case of grape seed and *Crataegus monogyna*

extracts (0.2 mg GAE/L), while a significant stimulatory effect was observed for all the extracts at the minimum concentration (0.5 mg GAE/L).

An interpretation of the mechanisms of different effects of the natural extracts and of the individual compounds existing in their composition is now being undertaken. We are seeking for information from studies (using different experimental models) of molecular biology of genomics and proteomics in order to enhance our comprehension of the role of polyphenols at the molecular level.

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