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DETERMINATION OF MINERAL CONTENTS AND ANTIOXIDANT ACTIVITY IN SOME PLANTS THAT CONTAIN ALLELOCHEMICALS OF BANAT REGION (WESTERN ROMANIA)

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ABSTRACT
This study was designated to evaluate the correlation between total polyphenols and mineral content with antioxidant activity in various extracts of plants that contain allelochemicals Banat region. The study was carried out to analyze the mineral contents and antioxidant activity of the leaves of *Setaria viridis, Datura stramonium Sorghum halepense and Aristolochia clematitis L.* collected from different Banat zones, using standard method of plants analysis. Among the various macronutrients estimated in the plant samples of different wild leaves plant potassium was present in the highest quantity (16.10–84.40 mg g^{-1}) followed by calcium (5.85–23.70 mg g^{-1}) and sodium (0.95–3.20 mg g^{-1}). Metals, such as iron, zinc, copper, manganese and chromium were analyzed in the different plants. The antioxidant activity of leaves plants was examined.
Phenols content of the extract were measured by FC and $AlCl_3$ assays. The content of total polyphenols in the aqueous methanolic extracts was calculated as GAE and radical scavenging activity was estimated as IC_{50} values using 1, 1–diphenyl–2–picrylhydrazyl (DPPH). The total phenols varied from 17.07±0.13 to 67.26±0.24 mg g^{-1} in the extracts. The highest radical scavenging effect was observed in *Aristolochia clematitis L.* with IC_{50} = 141.04±0.25 µg mL^{-1}. The greater amount of polyphenolic compound leads to more potent radical scavenging effect as shown by *Aristolochia clematitis* L. The methanol extracts of these plants exhibited significant antioxidant activities by different assays and contained significant levels of polyphenols. These results show that methanolic extracts of these plants that contain allelochemicals, could be considered as a natural alternative source for drugs, pharmacology and medicine sectors.
KEY WORDS: metals, leaves plants, antioxidant activity, phenol.

INTRODUCTION
Plants that contain allelochemicals are source of active bioproducts which differ widely in terms of structure and biological properties. Heavy metals are defined as that group of elements that have specific weights higher than about 5g/cm^3. A number of them (Fe, Mn, Mo, Zn, Cu) are essential micronutrients and are required for normal growth and take part in redox reactions, electron transfers and other important metabolic processes in plants that contain allelochemicals. Heavy metals which are considered nonessential (Pb, Cd, Hg etc.) are potentially highly toxic for plants (Sahito *et al*, 2003). In recent studies, with enhanced awareness of the importance of metals on health, an increasing number of reports on the role of metals in plants have been published (Audu *et al*, 2006).

Studies showed that optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper and zinc could reduce individual risk factors, including those related to cardiovascular disease (Jabeen *et al*, 2010). The metals serve as structural components of tissues and function in cellular and basal metabolism and water and acid–base balance (Essiett *et al*, 2011). Heavy metals constitute significant health hazards for man and have become an area of particular concern and priority in environmental research (Khan *et al*, 2008). Metals can be directly taken up by the leaves of plants or they are accumulated in the soil and reach the plants through their roots (Sharma *et al*, 2009). Several studies have been carried out on wild plants (Basgel *et al*, 2006). Most of metals are essential activators for enzyme–catalyzing reactions. Mg (e.g.) plays a structural role in the chloroplast membrane system and may be responsible for colour, taste and smell, and a cofactor for biomolecules synthesis (Karim *et al*, 2008). Fe is an essential activator for enzyme–catalysing reactions involving chlorophyll synthesis and for ferrodoxin nitrate reductase (Nookabkaew *et al*, 2006). K is an essential nutrient and has an important role in the synthesis of amino acids and proteins. Ca and Mg play a significant role in photosynthesis, biomolecules metabolism and binding agents of cell walls. Ca is also the component of bone and assists in teeth development. Zn is an essential micronutrient and is associated with a number of enzymes, especially those for synthesis of RNA. The contents of metals in plants are low and in terms of biological activity they are critical. However, when they are incorporated into mineral complexes, their ability is enhanced (El-Khatib, 2003). Metals such as Cu and Zn are essential for normal plant growth and development since they are constituents of enzymes and other proteins.

All plants produce an amazing diversity of secondary metabolites. One of the important groups of these metabolites is polyphenolic compounds (Lasheen *et al*, 2008). Polyphenols are characterized by at least one aromatic ring (C_6) bearing one or more hydroxyl groups (Saeedeh *et al*, 2007). Phenols are divided into several different groups (Xiaoli *et al*, 2008), distinguished by the number of constitutive carbon atoms in conjunction with the structure of the basic polyphenolic skeleton (simple phenols, benzoic acids, phenylopropanoids and flavonoids) (Zhou *et al*, 2009). Polyphenols have functions in plants (Rodino *et al*, 2011). An enhancement of phenylopropanoid metabolism and the amount of polyphenolic compounds can be observed under different environmental factors and stress conditions (Park *et al*, 2006).

There have been reports of induced accumulation of polyphenolic compounds (Miliauskas *et al*, 2004) and peroxidase activity in plants treated with concentrations of metals. Antioxidant action of polyphenolic compounds is due to their tendency to chelate metals. Polyphenols possess hydroxyl/ carboxyl groups (Loukopoulos, 2005), able to bind particularly iron and copper. The roots plants exposed to metals exude levels of polyphenols (Demiray *et al*, 2009). They may inactivate iron ions by chelating and additionally suppressing the superoxide–driven Fenton reaction, which is believed to be the most important source of reactive oxygen species (Ebrahimzadeh *et al*, 2008).

The aim of this study was to determine the mineral contents of plants that contain allelochemicals and that were conducted to investigate the relationship between effects of total phenols.

The present study aims to evaluate the antioxidant activities of methanol extracts of (bristlegrass, jimson weed, johnsongrass and birthwort) and to determine their total polyphenols contents. The extracts were screened for their possible antioxidant potentials by DPPH free anion radical, power reducing and metal chelating assays.

MATERIALS AND METHODS

Plant materials: The plant were purchased from different locations in the area Banat, on September 2011 and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office under. The plant parts were shed–dried, pulverized and stored in an airtight container. And proximate compositions were carried out in our laboratory (WHO, 2004).

Estimation of minerals in plant material: Plant material was taken in a precleaned and constantly weighed silica crucible and heated in a muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon–free ash was moistened with concentrated sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600°C till the weight of the content was constant (~2–3 h). One gram of sulphated ash obtained above was dissolved in 100 mL of 5% HCl to obtain the solution ready for determination of mineral elements through Atomic Absorption Spectroscopy (AAS). Standard solution of each element was prepared and calibration curves were drawn for each element using AAS (AOAC, 2000).

Extraction of plant material for antioxidant activity: One gram of each plant material was soaked in 20 mL aqueous methanol (20%, v/v) for 18 h at room temperature. The extracts were filtered and diluted to 50 mL and aliquot were analyzed for their total polyphenolic content and their free radical scavenging capacity.

Estimation of total polyphenolic content: The amount of total polyphenolic content of crude extracts was determined according to Folin–Ciocalteu (FG) procedure. 20–100 μL of the tested samples were introduced into test tubes; 1.0 mL of FC reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV–visible PG Instruments UV–VIS spectrophotometer and UV WIN 5.05 software). The total polyphenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram (mg g^{-1}) of extract.

Determination of free radical scavenging activity: The free radical scavenging activity of the plant samples and Butylated Hydroxyl Toluene (BHT) as positive control was determined using the stable radical DPPH (1,1–diphenyl–2–picrylhydrazyl). Aliquots (20–100 μL) of the tested sample were placed in test tubes and 3.9 mL of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. Thirty minutes later, the absorbance was measured at 517 nm (UV–visible spectrophotometer). The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenge (%)=[(Ac–At)/Ac]×100, where, Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration in mg of dry material per ml (mg mL^{-1}) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation. Values are presented as Mean±SE mean of three replicates. The total polyphenolic content and IC_{50} value of each plant material was calculated by using

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Linear Regression analysis (ISO–14502–1:2005; and AOAC 941.15.AOAC, 2003).

RESULTS

The plants identified were green bristlegrass, Jimson weed, Johnsongrass and Birthwort collected from different Banat zones, using standard method of plants analysis. Knowledge of the concentration and type of specific minerals present in plants is often important in the pharmaceutical drugs.

The major physicochemical characteristics of minerals that are used to distinguish them from the surrounding matrix are: their low volatility; their ability to react with specific chemical reagents to give measurable changes; and their unique electromagnetic spectra. The most effective means of determining the type and concentration of specific minerals in pharmaceutical drugs is to use atomic absorption or emission spectroscopy. Instruments based on this principle can be used to quantify the entire range of minerals in drugs, often to concentrations as low as a few ppm. For these reasons they have largely replaced traditional methods of mineral analysis in institutions that can afford to purchase and maintain one, or that routinely analyze large numbers of samples. The results of the trace metal concentrations determined in plant tissue are presented in Table 1. The concentration of metals in plants varied widely in different plants studied. The leaves of all plants that contain allelochemicals contain minerals like sodium, potassium, calcium, manganese, chromium, iron, zinc and copper in varying concentration with potassium having highest concentration and it is shown in Table 1.

Table 1. *Mineral value of plants that contain allelochemicals collected from Banat region*

Minerals (mg g^{-1})	*Setaria viridis*	*Datura stramonium*	*Sorghum halepense*	*Aristolochia clematitis L.*
Na	1.76	2.34	1.34	0.97
K	31.20	49.6	51.3	54.7
Ca	19.3	23.1	22.4	21.2
Mn	0.182	0.132	0.146	0.139
Cu	0.021	0.032	0.034	0.041
Fe	2.21	1.91	2.19	2.21
Cr	0.002	0.005	0.007	0.009
Zn	0.87	0.89	0.85	0.92

Results are the mean values of three replicates of the same sample

Polyphenols are the major compounds with antioxidant activity in plants that contain allelochemicals. Some of the potential health benefits of polypolyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation. As a group, polyphenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases. There was a wide variation in the amount of total polyphenols in the wild leaves plants ranging from 17.07±0.13 to 67.26±0.24 mg g^{-1} (GAE) (Table 2).

Table 2. Total polyphenolic content and free radical scavenging ability in plants that contain allelochemical by the use of a stable DPPH radical (Antioxidant activity expressed as IC_{50})

Samples	Total polyphenolic content of leaves (GAE mg g^{-1} of dry material)	IC_{50} value (IC_{50})
Setaria viridis	31.91±0.84	211.53±0.15
Datura stramonium	54.68±0.32	313.31±0.21
Sorghum halepense	17.07±0.13	229.02±0.13
Aristolochia clematitis L.	67.26±0.24	141.04±0.25
Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean±SEM		

The highest total polyphenolic content was found in the leaves of *Aristolochia clematitis* L. and the lowest in the leaves of *Sorghum halepense*. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. This showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The evaluation of anti–radical properties of wild leaves plants that contain allelochemicals was performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by different wild leaves plants was determined (Table 2). The leaves of *Aristolochia* *clematitis* L. showed the lowest IC_{50} value whereas the leaves of *Datura stramonium* were found to have highest IC_{50} value. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 765 nm which decreases with the scavenging of the proton radicals. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in plant species.

DISCUSSION

The mineral composition in leaves plants that contain allelochemicals, are shown in Table 1. Concentrations of Na were present, ranging from 0.97 mg g^{-1} (*Aristolochia clematitis L.*) to 2.34 mg g^{-1} (*Datura stramonium*). K content was higher in the leaves of *Aristolochia clematitis L.* (54.70 mg g^{-1}) and in the leaves of *Setaria viridis* (31.20 mg g^{-1}). Na and K take part in ionic balance of the human body and maintain tissue excitability. Na plays an important role in the transport of metabolites and K is important for its diuretic nature (Chen *et al*, 2005). The ability of varieties to limit Na transport into the shoots, and thus to reduce Na accumulation in the rapidly growing shoot tissues, is critically important for maintenance of growth rates and protection of the metabolic process in elongating cells from the toxic effects of Na. The ratio of K/Na were significant in the leaves of *Setaria viridis* (17.72), in the leaves of *Datura stramonium* (21.19), *Sorghum halepense* (leaves) (38.28) and *Aristolochia clematitis L.* (leaves) (56.39) and compared with leafy vegetables. Significant and positive linear correlation were found between total polyphenolic content and antioxidant activity ($r^2 = 0.6073$, $p < 0.0001$), indicating that polyphenols were the major antioxidant constituents in the extracts. Ca content was highest in the leaves of *Setaria viridis* (19.3 mg g^{-1}) followed by in the leaves of *Datura stramonium* (23.1 mg g^{-1}), *Sorghum halepense* (22.4 mg g^{-1}) and *Aristolochia clematitis L.* (21.2 mg g^{-1}). Ca constitutes an extracellular fluid; it is also much required for the normal functioning and the regulation of cell permeability (Laladhas, 2010). The sufficient amount of Cu and Zn were present in the leaves of *Setaria viridis, Datura stramonium Sorghum halepense* and *Aristolochia clematitis L*.

Cu is another trace element essential in human body where it exists as an integral part of copper proteins ceruplasmin, the enzyme that catalyzes the oxidation of iron ion. Zn is a component of many metalloenzymes, including some enzymes which play a central role in nucleic acid metabolism. Zn is a membrane stabilizer and a stimulator of the immune response. Its deficiency leads to growth failure and malnutrition (Boonmee *et al*, 2007). The Mn content was higher in the leaves of *Setaria viridis* and appreciable amount of this element were observed in the leaves of *Sorghum halepense*, *Aristolochia clematitis L.*and in the leaves of *Datura stramonium*. This element is very much essential for haemoglobin formation (Kretovich, 2005). Concentration of Fe were present in the leaves of *Setaria viridis*, *Aristolochia clematitis L., Datura stramonium*, and *Sorghum halepense* contain an appreciable amount of this element.

So the metals content of all these plants were similar and comparable to the commercial vegetables. It has been recognized that polyphenolic compounds show antioxidant activity and their effects on human nutrition and health are considerable.

Natural antioxidants have been established to promote health by acting against oxidative stress related diseases such as infections, diabetes, cancer and coronary heart diseases. It has been suggested that antioxidants found in large quantities in in plants that contain allelochemicals. Generally plants antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions (Bucic–Kojic *et al*, 2009). Estimation of total polyphenolic content and DPPH stable free radical method are easy, rapid and sensitive method to evaluate the antioxidant activity of a specific compound or plant extracts (Akowuah *et al*, 2005). There was a wide variation in the amount of total polyphenols in the leaves plants that contain allelochemical ranging from 17.07±0.13 to 67.26±0.24 mg g^{-1} (GAE) (Table 2). The amount of total polyphenolic content of the plant under investigation can be arranged in descending order viz., *Aristolochia clematitis L.* (leaves) > *Setaria viridis* (leaves) > *Datura stramonium* (leaves) > *Sorghum halepense* (leaves). The anti–radical properties of the plant materials were performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by different wild leaves plants was determined (Table 2), a lower value would reflect greater antioxidant activity of the sample. The leaves of *Aristolochia clematitis L.* showed the lowest IC_{50} value (141.04±0.25 µg mL^{-1}) whereas *Datura stramonium* was found to have the highest IC_{50} value (313.31±0.21 µg mL^{-1}). The radical scavenging activity of the plant extracts decreased in the following order: *Aristolochia clematitis L.*> *Setaria viridis* > *Datura stramonium* > *Sorghum halepense*.

CONCLUSION

The result of present study showed that the methanolic extract of *Aristolochia clematitis L.* which contain highest amount of polyphenolic compounds, exhibited the greatest antioxidant activity whereas the leaves of *Sorghum halepense* exhibited minimum radical scavenging activity. The radical scavenging property of *Aristolochia clematitis L.* may is due to the hydroxyl groups existing in the polyphenolic compounds chemical structure that can provide the necessary component as a radical scavenger.

A mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton–type reactions.

Chelating agents may also serve as antioxidants since they reduce redox potential, thereby stabilizing the oxidized forms of metal species. Therefore, the Fe (II) chelating capacities of the extracts were screened. Ferrozine forms complexes with Fe^{2+}. In the presence of chelating agents, complex formation between ferrozine and Fe^{2+} is disrupted, resulting in reduction in the red colour of the complex.

The methanolic extracts of all of the plants that contain allelochemicals under investigation exhibited different extent of antioxidant activity and thus provide a valuable source of nutraceutical supplements. Depending on the values, some plants are more important than some others.

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