PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITIES IN SELECTED GREEN MASS OF PROTEINACEOUS PLANTS

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Reactive oxygen species and free radicals have been implicated in a large number of human diseasesof modern society. Then, providing strong antioxidants or free radical scavengers in diet or as a medicine could be advantageous [1,2].

It is well known that the potential sources of natural antioxidant compounds have been searched in several types of plants. [3-5]. Green leaves of plants contain many compounds with antioxidant activity, including ascorbic acidand tocopherols, carotenoids and a variety of antioxidant phytochemicals such as simple phenolics and flavonoids. Phenolic compounds are an integral part of the human diet and could be helpful against cancers, atherosclerosis, ischemia, and inflammatory disease, caused by the exposure to oxidative stress [6].The literature suggests that phenolic compounds of herbs have the ability to scavenge free radicals, and that the factors such as genetic and environmental conditions (growth season and plant maturity) can cause variations in their values [7].

There are many various types of wild edible plants and also leaves of commonly available vegetables, which though underexploited in most cases, possess a tremendous potential to help people to overcome the deadly diseases including cancer, aging, diabetes and atherosclerosis [8,9].Among them, some of the underexploited leaves or the over ground parts of proteinaceous plants are carrot (*Daucus carota L.*), beets (*Beta vulgaris L.*), nettle (*Urtica diocila L.*), purslane (*Portulaca oleracea L.*),and ramsons (*Allium ursinum L.*). They are a rich source of several phytonutrients, and recognized to have significant and wide biological activities.However, no comprehensive literature data on antioxidant activities of all of these plants were available for comparison.

Therefore, the aim of this work was to measure the antioxidant activity of these raw materials and study the correlation between antioxidant activity and the amount of phenolic and flavonoid contents in extractsof green mass of proteinaceous plants.This will help to find new sources of safe and inexpensive natural antioxidants to use them in food or nutraceutical and pharmaceutical preparations to replace synthetic antioxidants.

The separate plant samples were dried using hot air oven at 35°C for 24 h,disintegrated into fine powder and stored in air tight containers for further extract preparation. Ethanolic extracts were prepared using 10 g of sample in 100mLof 96% ethanol under magnetic stirring for 5 hours. After filtration, the extracts were concentrated under reduced pressure on a rotary evaporatorat 40 ºC for further chemical analysis.

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu’s reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.015x + 0.0325$, $R^2 = 0.9999$). The values obtained for the concentration of total phenols are expressed as mg of GAE/g of extract.

The concentration of flavonoids in various plant extracts of green mass of proteinaceousplants was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalent (the standard curve equation: y = 0.0029x + 0.0005, R^2 = 0.9996), mg of RU/g of extract. The results are presented in Table 1.

Table 1 - Summary of total phenolic content and total flavonoid contentin the extractsof green mass of proteinaceousplants(Mean ± standard deviation (n =3))

The raw material	Total Phenolic content(mg of Gallic acid equivalents / g DWE)	Total Flavonoid content(mg of Rutin equivalents / g DWE)
Sugar beet	8,4 ±0,03	8,12 ± 0,24
Red beet	9,0 ± 0,19	6,42 ± 0,80
Carrot	10,2 ± 0,06	9,30 ± 0,99
Ramsons	13,3 ± 0,38	10,48 ± 0,13
Nettle	8,1± 0,43	7,26 ± 0,05
Purslane	11,6 ± 0,11	8,84 ± 0,14

The analysis of the obtained data shows the following facts.

The total polyphenol content in the extracts of green mass of proteinaceous plants oscillated around 8,1–13,3 mg GAE/g with the lowest value for nettle and the highest for ramsons. The total flavonoid concentrations varied from 6,42 mg RE/g for extracts from leaves of red beet to 10,48 mg RE/g for extracts from leaves of ramsons.

Keeping in view the fact thatmany flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables[9-12], the extracts of green mass of proteinaceousplants were also evaluated for antioxidant activity by DPPH method.

The use of DPPH radical has provided a simple and rapid way in determining the antiradical activities of antioxidants. It can accommodate a large number of samples and was sensitive in detecting natural compounds at low concentrations [13]. It has been found that an individual bioactive compound may exhibit better biologicalproperties in the presence of other compounds in the extract, and therefore be morebeneficial than isolated constituents [14]. Therefore, the assays were performed using whole extracts of green mass of proteinaceous plants.

Antioxidant activity of extracts were expressed as percentage of DPPH radicals inhibition(%) and IC_{50} values (μg/ml). The obtained results are shown in Table 2.

It is worthwhile to note that various studies have shown that the antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [15, 16].

Table 2 - Antioxidant activity of green mass of proteinaceousplants by DPPH method(Mean ± standard deviation (n =3))

The raw material	Inhibition, %	IC_{50} (µg*mL-1)
Sugar beet	51,66 ± 2,03	153,45 ± 0,38
Red beet	55,73 ± 1,57	110,31 ± 0,70
Carrot	68,31 ± 3,10	78,14 ± 1,00
Ramsons	89,2 ± 1,13	24,92 ± 0,19
Nettle	44,00 ± 1,49	214,17 ± 0,91
Purslane	71,01 ± 1,81	41,62 ± 0,35
Ascorbic acid (standard)	92,05 ± 0,62	24.32± 0.20

Our researches have shown that the DPPH radical scavenging ability expressed as % ranged from 44,0 % for nettle to 89,2 % for ramsons. Although the leaves were dehydrated (heating), they presented good antioxidant activity showing that the dehydrated product is a source of natural antioxidants.

It has been found that the EC_{50} of ramsons (A.ursinum) extract was similar level with Ascorbic acid, which was used as positive control in this experiment (24,92 and 24.32 µg/ml, respectively).

The correlation between antioxidant activity and TPC had been determined by plotting IC_{50} (µg/ml) against TPC (mg/g) and the results has been graphically presented in fig. 3.

A direct correlation between radical scavenging activity (IC_{50}) and TPC of the samples was observed. The high value of the R^2 coefficient ($y = 7.8476x – 15.943$; $R^2 =0.9606$) suggests virtually full correlation ($0.9 \le R^2 \le 1$), which means that the antioxidant activity of the extracts from green mass of proteinaceous plants mainly (in 96.1%) results from the presence of the phenolic compounds and is proportional to it.

In the present study, the results of the antioxidant activities of the extracts of green mass of proteinaceous plants were also compared to evaluate the possibility of a relationship between their antioxidant activity and total flavonoid contents. However, no exact correlation was observed in between the antioxidant activities of the extracts and their flavonoid contents.

It is worthwhile to note that our results differ from work of Syama et al. [9] for extracts of some of underutilized vegetable leaves. These differences may result from the use of other solvents: we applied ethanol, whereas Syama et al. used methanol.

However, the results of the investigation indicated that the most of analysed proteinaceous plants are rich in phenolic compounds and demonstrated good antioxidant activity. Moreover, the high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity.

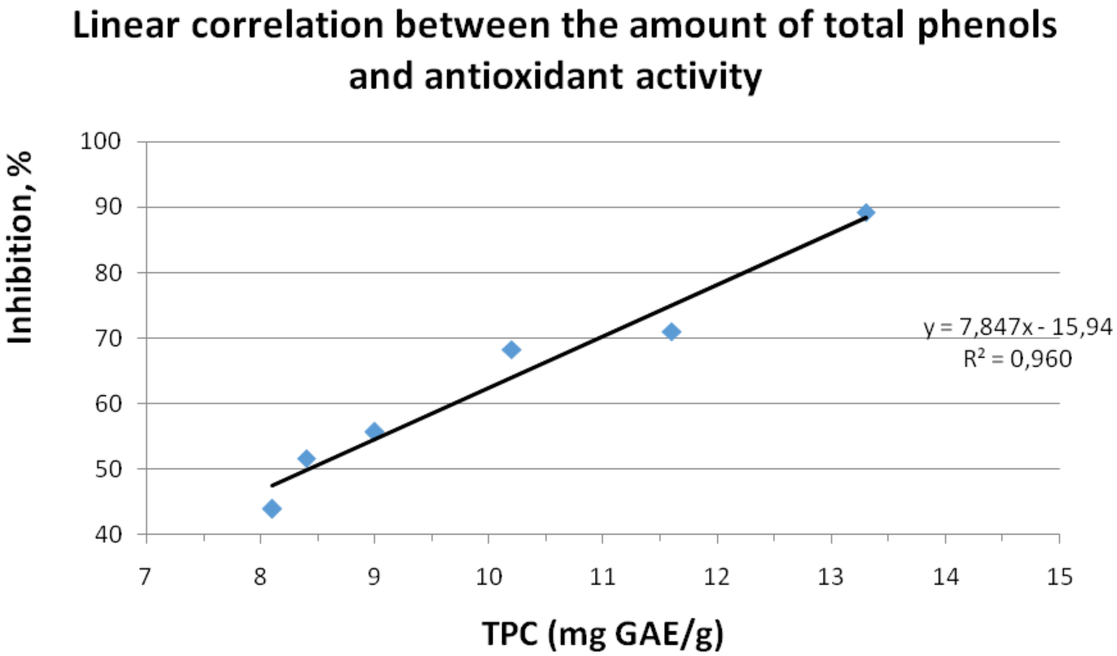

Figure 3. - Linear correlation between the amount of total phenols and antioxidant activity.

Conclusion: The study shows that the green mass of proteinaceous plants containing antioxidants may be of major importance in disease prevention. Therefore, these plants to possess an important potential to be used as functional food ingredient or nutraceutical. The providing data can enrich the existing comprehensive data of free radical scavenging activity of plant materials.

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