ANTIOXIDANT ACTIVITY OF EXTRACTS FROM PLANTS GROWING IN UKRAINE

Viktoriia IVANOVA - Irina YASINSKA

Abstract:

Raw materials of some plants growing in Ukraine – the roots of Arctium lappa L., Glycyrrhíza glabra L., Sanguisorba officinalis L., the fruits of Rosa cinnamomea L.- were been used to investigation of its antioxidant properties. The total phenolic content of prepared aqueous extracts was determined using Folin-Ciocalteu reagent; it ranged from 43.2±2.09 to 98.5±4.01 mg GAE per 100 ml of the extract. The highest vitamin C content was found in the extracts from the rose hips and the great burnet roots - 8.94±0.234 and 7,68±0.341 mg / 100 ml of extract, respectively. Free radical scavenging capacity of the extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). It ranged from 0.60± 0.0178 to 8.45± 0.26 mM of the ascorbic acid equivalent (AAE) antioxidant capacity per 100 ml of the extract. The highest antioxidant activity was determined for the extract of great burnet roots.

The results showed that tested extracts possess potent antioxidant activity and may be considered as natural source of antioxidants.

Key words: antioxidants, DPPH, extracts, phenolic compounds, unconventional plant raw materials

The interest in the preventive properties of dietary chemicals has increased in recent years. Nowadays, busy lifestyles are driving the development of functional foods that enriched with health-promoting (functional, bioactive) ingredients. The greatest attention is given to the content of the antioxidants in foods. Their presence can prevent free radical oxidation of biological structures, helps to reduce the risk of certain diseases (etc. inflammatory, cardiovascular, tumor), slowing the aging process.

Medicinal plants have been shown to be an important source of bioactive compounds (Rao et al., 2002). Their antioxidative effect is mainly due to phenolic components - secondary metabolites and potent free radical scavengers that posses a wide spectrum of biological activities (antiapoptosis, antiaging, antitumor and so on) (Fresco et al., 2006; Seifried at al, 2007). In particular, the secondary metabolites of plants are the major source of drugs, with approximately half of all modern pharmaceuticals is originating from natural plant products. Some bioactive substances of polyphenol-rich plant materials, such as green tea, berries, thyme, have been made into functional foods or supplements.

Extracts from plant origin have been used for centuries in traditional medicine and in nutrition. Active substances from medicinal and aromatic plants possess protective effects against endo- and endogenous agents and could be used for prevention of pathological process in human body.

Our goal was to investigate the phenolic content and free radical scavenging capacity of extracts from selected plants growing in Ukraine.

Materials and Methods

Plant material: Fresh plant raw materials were collected in Kiev region (Ukraine) during summer and autumn months of 2011. They were dried at 40 ^{0}C, crushed into pieces of 1-3 mm and used for extraction.

Extract preparation: The aqueous extracts were prepared at the temperature 90°C with shaking at 130 rpm during the time of 30 min. The total phenolic content was measured every 10 min up to the time when the system reaches the steady condition. The steady condition was determined by the immutability of the measured parameters within 10 min. The extracts were filtered through a filter paper.

All reagents were of analytical grade.

Determination of total phenolics content: The total phenolic content of the extracts was determined using a Folin-Ciocalteu assay (Sun et al., 2007). To 0.1 ml of the extract or ultra pure water or gallic acid standard solution (0-0.2 mg/ml) was added 1 ml ultra pure water, 0,1 ml Folin-Ciocalteu reagent and left standing at room temperature for 5 min. Than 2 ml of 2% sodium carbonate was added to the mixture and incubated at room temperature for 20 min. The absorbance was measured at 765 nm, using spectrophotometer BioMate 5 (Thermo electron corporation, USA). The total phenolic content was expressed as mg Gallic Acid equivalents (GAE) per 100 ml of the plant extract. All samples were analysed in triplicate and averaged.

Determination of total flavonoids content: Aluminium chloride spectrophotometric method was used for total content of flavonoids determination (Lin et al., 2007). Diluted extract (0.1 ml) was mixed with 1.5 ml of distilled water and 0.2 ml $NaNO_2$ (5%) and kept at room temperature for 5 min. Aluminium chloride solution (0.2 ml, 10%) was added, the mixture was shaken and incubated for 6 min. And finally sodium hydroxide solution (2 ml, 1 M) and 10 ml of distilled water were added to mixture, shaken vigorously and incubated for 15 min. The absorbance was measured at 510 nm, using spectrophotometer BioMate 5 (USA). The total flavonoid content was expressed as mg quercetin or rutin equivalents per 100 ml of the extract. All samples were analysed in triplicate and averaged.

Determination of ascorbic acid: Ascorbic acid content was determined by colorimetric assay (Guide, 2004).

DPPH assay: The free radical scavenging activity of the extracts, based on the activity of the stable 1,1-diphenyl-2-2-picrylhydrazyl (DPPH, Sigma-Aldrich, USA) free radical, was determined by a method described by Brand-Williams et al., 1995.

Briefly, alcoholic solution of DPPH (1.8 ml of 0.04 mg/ml) was added to 0.2 ml of the samples containing different concentrations of extracts originating from plant materials. The samples were incubated in the dark place at room temperature for 30 min. The decrease in absorbance was measured at 517 nm by UV-VIS spectrophotometer for all samples. The absorbance of the DPPH radical without antioxidant (with 0.2 ml of pure ethanol) was measured as the control (Ac). All determinations were performed in triplicate. The percentage of inhibition of the DPPH radical by the samples was calculated according to the equation:

$$\% \text{ Inhibition} = [(Ac-As)/Ac]\ 100,$$

where: Ac - absorbance control or blank, As - absorbance with sample or standard remaining DPPH radical after reaction with antioxidant.

Blank samples contained 1.8 ml ethanol and 0.2 ml of various concentrations of plant extract; control sample contained 1.8 ml of 0.04 mM DPPH and 0.2 ml ethanol. The concentration of the samples, the control, and the empty samples were measured in comparison with ethanol. The synthetic antioxidant ascorbic acid was used as positive control. The antioxidant activity was expressed as mM of the ascorbic acid equivalent (AAE) antioxidant capacity per 100 ml of the extract.

Statistical evaluation of results: The experimental results were expressed as mean ± standard deviation of three replicates. Where applicable, the data were subjected to one-way

analysis of variance (ANOVA) and the differences among samples were determined using the statistical analysis system Statgraphics. P-value of < 0.05 was regarded as significant.

Results and discursion

Four plants (*Arctium lappa L., Glycyrrhíza glabra L., Sanguisorba officinalis L., Rosa cinnamomea L.)* were used to extraction of bioactive water soluble components. The main criteria in choosing of plants were (i) the presence of active compounds significant amounts, (ii) the original organoleptic properties, (iii) a wide area certain specie distribution in Ukraine (sufficiency of raw materials), (iiii) easiness of processing.

The optimal conditions for extraction of biological compounds from all plants were experimentally determined. Under these conditions the yields of the extractions were 3.2-4.0%.

Phenolic compounds are an essential part of the human diet; its have the considerable interest due to their antioxidant properties. These chemicals posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from a simple phenolic molecule to a complex high-molecular weight polymer (Heim et al., 2002).

The total phenolic content in the extracts is shown on Fig.1. It ranged from 43.2±2.09 to 98.5±4.01 mg GAE per 100 ml of the extract and the highest showed in the extract from great burnet. The phenolic content in extracts increased in the order: great burnet roots > rose hips, burdock roots > licorice roots.

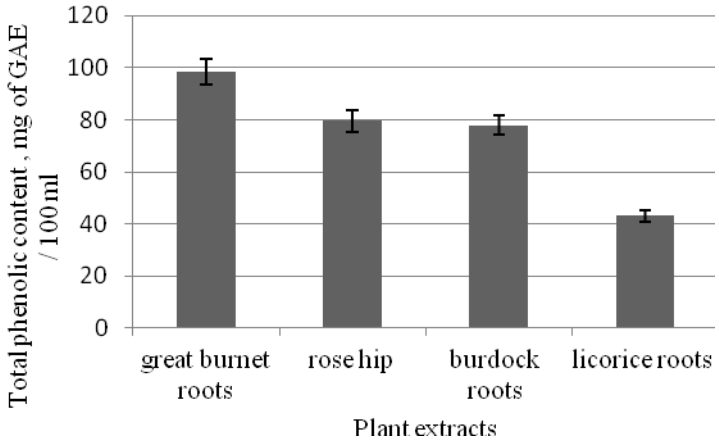

Fig. 1: Total phenolic content in analyzed extracts. Results are expressed as mg gallic acid equivalents (GAE) per 100 ml of the plant extract

Many previous studies have reported that polyphenols of different plants and herbs are beneficial to animal and human health (Lobanov et al., 2004, Triantaphyllou et al., 2001, Kulisic et al, 2006). The group of flavonoids is the most numerous among the phenolics. They have been strongly implicated to the protective effect of the foods.

The absorption spectra of the experimental extracts, the solutions of rutin and quercetin with aluminum chloride are presented on Fig.2.

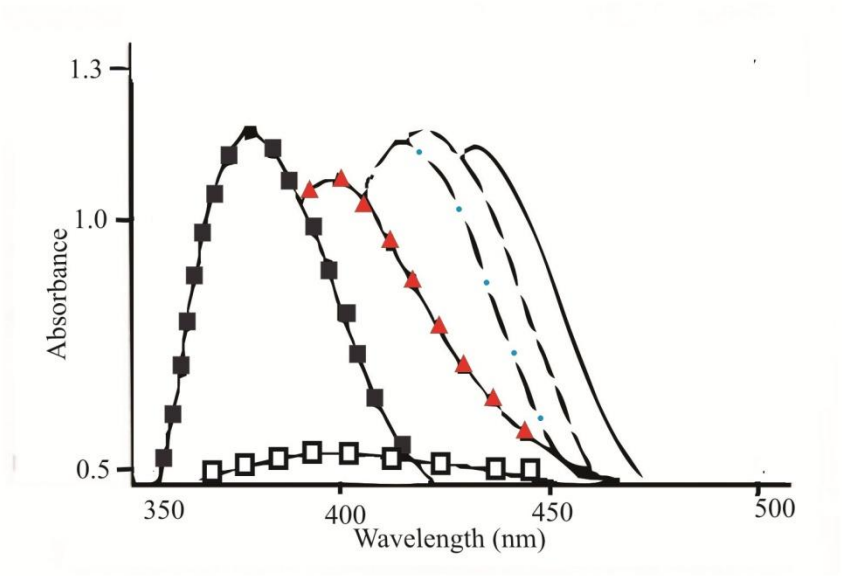

Fig.2: The absorption spectra of $AlCl_3$ complexes of extracts flavonoids and the solutions of quercetin and rutin: — quercetin – – rutin*;* the extracts from - · - licorice roots *(1:2)* burnet roots*;* burdock root *(1:4);* rose hips.

The absorption maxima of the extracts do not coincide with the peaks of quercetin and rutin, probably due to the other flavonoids predominance, so it isn't advisable to recalculate flavonoids content on rutin or quercetin equivalents. Only maximum absorption of the extract from licorice root to close the maximum of rutin. The content of flavonoids in it was 42±0.67 mg of RE / 100 ml of extract.

The ascorbic acid levels were determined in the studied samples. The highest vitamin C content was found in the extracts from hips and burnet roots - 8.94±0.234 mg and 7,68±0.341 mg/100 ml of extract, respectively. In extracts from licorice roots and burdock roots the ascorbic acid levels was too low.

The extracts were investigated for their possible antioxidant activity. Several types of free radicals, such as $OH\cdot$, O_2^-, $LOO\cdot$ with different reactivity, can be formed during the process of lipid oxidation. Relatively stabile DPPH radical has been widely used to determine the ability of biological compounds to act as free radicals scavengers (Jun et al., 2012, Akinmoladun et al., 2010, Niciforovic et al., 2010). It was found that the radical-scavenging activities of all the extracts increased with increasing concentration of bioactive compounds.

Free radical scavenging capacities of the extracts, measured by the DPPH assay, are shown in Table 1 and expressed as concentrations in terms of ascorbic acid equivalents.

Table 1: Antioxidant capacity of the extracts

Plant extract from	Antiradical activity of the extract, mM ascorbic acid equivalents
Licorice roots	0,6±0,0178
Burdock roots	3,775±0,155
Rose hips	3,225±0,112
Great burnet roots	8,45±0,26

Phenolic and polyphenolic compounds are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Scieber et al., 2001). The correlation between the total phenol contents and antioxidant activity has

been widely studied in different foods such as fruit and vegetables (Gao et al., 2008; Jayaprakasha et al., 2008). In our study phenolic content was correlated to antiradical activity, whereas flavonoid content and such activity showed a relatively weak correlation (data not shown).

Conclusion

The results indicate that all extracts from selected plant materials have potent antioxidant activity, and the extract from roots of great burnet was stronger than other.

This investigation suggested that the plant extracts from roots of burdock, licorice, great burnet and rose hips have been shown to possess health-promoting properties and could be considered as a potential source of natural antioxidants for food industry. Those extracts can be used is for the development of new functional ingredients with antioxidant properties. However, more detailed studies on the chemical compositions of those extracts, as well as studies using other models such as lipid peroxidation and *in vivo* assays, are essential to characterize them as biological antioxidants.

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Assoc.Prof. Viktoriia Ivanova, PhD., Mgr. Irina Yasinska

National University of Food Technologies, Faculty of Health-Improving Products Technology and Food Expertise, Vladimirskaya Str., 68, 01033 Kiev, Ukraine