Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

ESTIMATING FREE RADICALS SCAVENGING ACTIVITY OF SOME BERRIES SPECIES

Gabriela LUȚĂ, Florentina ISRAEL-ROMING, Daniela BĂLAN, Evelina GHERGHINA

University of Agronomical Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies, 59, Marasti Blvd., 011464, Bucharest, Romania, www.usamv.ro

Corresponding author Florentina ISRAEL-ROMING: florentinarom@yahoo.com

Abstract
Berries fruits contain phytochemical components with antioxidant activity, such as polyphenols, ascorbic acid, that have possible protective effects on human health. Free radicals can induce changes in different cell biomolecules such as lipids, proteins, and nucleic acids. This oxidative stress is involved in pathogenesis of many human diseases: cancer, cardiovascular diseases, osteoporosis, neurodegenerative processes. The objective of these researches was to estimate and compare the free radicals scavenging activity of total extracts of certain berries fruits species: raspberry (Rubus idaeus), strawberry (Fragaria ananassa), sea-buckthorn (Hippophaea rhamnoides). The evaluation involved determination of total phenols using spectrophotometrical method and of ascorbic acid content by HPLC method. Total antioxidant capacity was determined using the stable free radical diphenylpicrylhydrazyl (DPPH) method and calculating the parameter IC_{50} (the concentration of sample which is required to scavenge 50% of DPPH free radicals). As expected, sea-buckthorn fruits manifested the highest radical scavenging activity expressed as EC_{50} value (512.76 µg/ml). A linear correlation was obtained for total phenols content (Pearson's correlation coefficient -0.945) and for ascorbic acid (-0.8607).

Keywords: *ascorbic acid, berries, scavenging activity, total phenols*

INTRODUCTION

Berries fruits are an important source of active natural biocompounds known to be responsible for free radicals scavenging activity. These fruits contain phytochemical components with antioxidant activity, such as polyphenols, ascorbic acid, that could be involved in preventing the occurrence of oxidative-stress related diseases, caused by the attack of free radicals on key biocomponents like lipids, proteins or nucleic acids (Mayne S.T., 2003; Pisoschi A., 2011). This oxidative stress is involved in pathogenesis of many human diseases: cancer, cardiovascular diseases, osteoporosis, neurodegenerative processes. Vitamin C is an electron donor, and this property accounts for all its known functions. As an electron donor, vitamin C is a potent water-soluble antioxidant in humans. Antioxidant effects of vitamin C have been demonstrated in many experiments *in vitro* (Kelly F.J., 1998; Padayatty S., *et a*l, 2002).
The food industries used some synthetic antioxidants for the protection against the oxidizing agents, but recent researches emphasized their possible toxicity for human health, therefore a preference for antioxidants from natural rather than from synthetic sources have imposed. Considering that, an increasing interest for investigating the antioxidants provided by berries fruits was observed in recent years.
The objective of these researches was to estimate and compare the free radicals scavenging activity (antioxidant activity) of total extracts of certain berries fruits species: raspberry (*Rubus idaeus*), strawberry (*Fragaria ananassa*), sea-buckthorn (*Hippophaea rhamnoides*). The evaluation involved determination of total phenols using spectrophotometrical method and of ascorbic acid content by HPLC method. The total antioxidant capacity was determined using the stable free radical diphenylpicrylhydrazyl (DPPH) method and calculating the parameter EC_{50} (the concentration of sample which is required to scavenge 50% of DPPH free radicals).

MATERIALS AND METHODS

Biological materials. The analyzis were performed on three wild berries fruits species: three samples of raspberry (*Rubus idaeus*), five

samples of strawberry (*Fragaria ananassa*), and two samples of sea-buckthorn (*Hippophaea rhamnoides*) provided by the local market. The determinations were made in triplicate, using fresh fruits. The extractions were conducted according to the protocol used for each determination.

Total phenolic content was performed according to the modified Folin-Ciocalteu assay (Singleton *et al*., 1999). The method consists in chemical reduction of Folin-Ciocalteu reagent (which is a mixture of tungsten and molybdenum oxides) and measuring the intensity of the obtained blue colour at 750 nm. The measurements were achieved with a UV/Visible ThermoSpectronic Helios spectrophotometer. Total phenols values were expressed in terms of gallic acid equivalent (GAE), which is a common reference compound.

The ascorbic acid contentwas determined by HPLC-RP with UV detection. The mobile phase consisted in 0.1% phosphoric acid. Samples were centrifuged at 4,000 rpm for 10 minutes; 1 ml supernatant was ten times diluted with elution solvent. Before injection, samples were filtered using 0.22 µm PTFE filters. Data acquisition and processing were realized with EMPOWER software. Ascorbic acid detection was performed at 210 nm. Calibration curve was achieved using a 0.1 µg/ml standard solution of L-ascorbic acid, in five concentration levels with three injections for each level.

The free radical scavenging activity (total antioxidant capacity) was determined using the stable free radical diphenylpicrylhydrazyl (DPPH) method according to Blois, M.S. (1958) procedure adaptated by Brand-Williams W. (1995) for complexes matrices. Briefly, a 100 µM solution of DPPH in methanol was prepared and 2 ml of this solution was mixed with 1 ml of different concentrations of berries fruits extract in 80% aqueous methanol. After 30 min incubation in dark at room temperature, absorbance was measured at 515 nm. The percentage of the radical scavenging activity (RSA) was calculated as follows:

$$\% \text{ RSA} = (1-[A_{sample}/A_{control\ t=0}])/100$$

DPPH solution in 80% methanol was used as a control. Gluthation at various concentrations (25 to 200 µg/ml) was used as a standard.

The EC_{50} parameter for each sample, defined as the concentration of sample which is required to scavenge 50% of DPPH free radicals, was calculated from the non linear regression curve of Log concentration of the sample extracts (µg/ml) against the percentage of the radical scavenging activity.

The statistical analysis was performed using the one-way Analysis of Variance (ANOVA). Pearson's correlation coefficient (*r*) was used to calculate the relationship between the DPPH and total polyphenol contents and ascorbic acid content of the three berries fruits species.

RESULTS AND DISCUSSIONS

The phenolics constitute a very divers and widespread group of biochemical compounds occurred in natural vegetal sources. Biological effects of polyphenols are attributed to their antioxidant effects, so that their determination is of considerable interest.

The biochemical analysis performed in this study indicated high values of the total phenols content in the tested berries (table 1), well known for their rich content in bioactive compounds (Battino M. *et al*, 2009; Ribera A.E. *et al*., 2010). The results emphasized sea-buckthorn as the richest phenolics source among the tested fruits. The total phenols content in sea-buckthorn fruits was 669.63 mg GAE/100g, which is 1.93 times higher than in strawberry and 1.63 times higher compared to raspberry. Other authors found that the total content of phenols in sea-buckthorn depends on the cultivar and varies from 828.7 to 1099.6 mg/100g (Novruzov E., 2005; Seglina D. *et al*., 2008).

Table 1. The total phenols content in the tested berries

Samples	**Total phenols** (mg GAE/100g fresh weight)
Strawberry	345.34 ± 3.93
Raspberry	410.78 ± 6.39
Sea-buckthorn	669.63 ± 8.18

Regarding the level of the ascorbic acid content (table 2), also sea-buckthorn fruits reached the highest amount (1154.24 mg/100g), which was according with the expectations. The results of ascorbic acid content in sea-buckthorn agree with those reported by Gutzeit D. *et al*. (2008) and Christaki E. (2012).

Table 2. The ascorbic acid content in the tested berries

Samples	Ascorbic acid (mg/100g fresh weight)
Strawberry	64.21 ± 2.18
Raspberry	52.02 ± 1.50
Sea-buckthorn	1154.24 ± 4.15

For estimating the antioxidant capacity of tested berries fruits was used the DPPH method because is adequate for screening antioxidant compounds formed by small molecules (such as phenols or ascorbic acid), considering that the reaction intensity can be measured using a spectrophotometrical method (Nickavar B. *et al*, 2009). The EC_{50} values for every tested fruits were calculated for further comparison.

For this purpose the extracts of selected berries were screened for their possible radical scavenging activity (RSA). Extracts in different concentrations of selected berries exhibited a high antioxidant activity, expressed as percentage of DPPH reduction (figure 1). The measurements indicated the highest antioxidant activity for sea-buckthorn fruits, confirming the expectations due to their rich content in total phenols and ascorbic acid.

The EC_{50} values were calculated for all the tested berries fruits (table 3), showing that sea-buckthorn fruits manifested the highest scavenging activity (512.76 µg/ml).

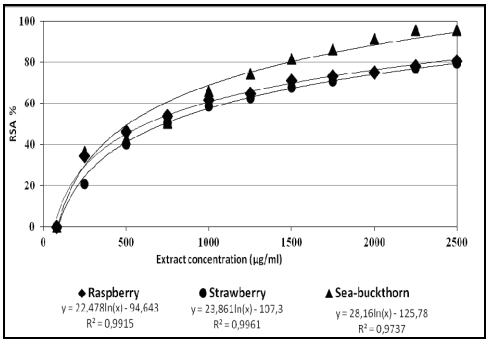

Figure 1. Radical scavenging activity (%) of the tested berries fruits

The extract with the lowest scavenging capacity was strawberry, which required a higher concentration (727.63 µg/ml) to scavenge 50% of DPPH free radicals.

However, this results are significant low compare to the standard (gluthatione) scavenging power (79.66 µg/ml), but we must take into account that, in contrast with the standard, the berries extracts are complex mixtures of numerous compounds with different properties.

Table 3. EC_{50} values of DPPH scavenging activities of the studied berries

Samples	EC_{50} (µg/ml)
Strawberry	727.63
Raspberry	620.05
Sea-buckthorn	512.76
Standard (gluthatione)	79.66

The rich content in phenols and ascorbic acid of the berries may cause the antioxidant properties of these fruits.

The correlation between total phenols content, as well as ascorbic acid content, and the antioxidant activity of certain fruits and vegetables extracts was studied by different authors (Pellegrini N. *et al*, 2003; Franco D. *et al*, 2008; Dvorakova M. *et al*, 2008), which reported an increasing antioxidant activity correlated with the concentration of these active biocompounds.

In the present paper a correlation study was performed between the radical scavenging activity (expressed as EC_{50}) and the content in total phenols and ascorbic acid in order to reveal the contribution of these biochemical compounds to the total antioxidant capacity of the berries fruits (figure 2).

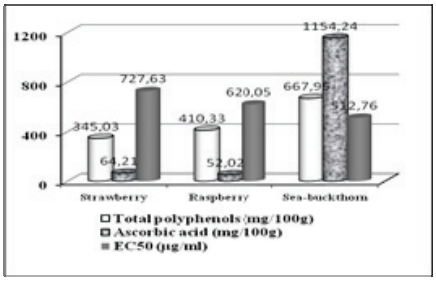

Figure 2. Antioxidants content and radical scavenging activity in berries

A strong linear correlation with Pearson's correlation coefficient - 0.945 was obtained for total phenols content. For ascorbic acid the linear relationship had a lower value, respective - 0.8607.

The obtained correlation coefficients were in accordance with those reported by Rufino M.S.

et al. (2010) and Arancibia-Avila P. et al. (2012)

CONCLUSIONS

Biochemical analysis performed indicated sea-buckthorn fruits as the richest phenolics sources among the tested fruits (669.63 mg GAE/100g).

Sea-buckthorn fruits reached also the highest amount of ascorbic acid content (1154.24 mg/100g).

As expected, sea-buckthorn fruits manifested the highest radical scavenging activity expressed as EC_{50} value (512.76 µg/ml).

The radical scavenging activity reached high values in the extracts rich in total phenols and ascorbic acid, so that a linear correlation was obtained for total phenols content (Pearson's correlation coefficient -0.945) and for ascorbic acid (-0.8607).

REFERENCES

Arancibia-Avila P., Namiesnik J., Toledo F., Werner E., Martinez-Ayala A.L., Rocha-Guzmán N.E., Gallegos-Infante J.A., Gorinstein S., 2012. The influence of different time durations of thermal processing on berries quality, Food Control 26, 587-593.

Battino M., Beekwilder J., Denoyes-Rothan B., Laimer M., McDougall G.M., Mezzetti B., 2009. Bioactive compounds in berries relevant to human health. Nutrition Reviews, Vol. 67(Suppl. 1), S145–S150.

Blois M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181, 1199-1200.

Brand-Williams, W., Cuvelier M. E., Berset C., 1995. Use of free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und Technologie/Food Science and Technology, 28, 25-30.

Christaki E., 2012. *Hippophae Rhamnoides L.* (Sea Buckthorn): a Potential Source of Nutraceuticals. Food and Public Health; 2(3), 69-72.

Dvorakova M., Guido L.F., Dostalek P., Skulikova S., Moreira M., Barros A., 2008. Antioxidant properties of free, soluble ester and insoluble-bound phenolic compounds in different barley varieties and corresponding malts. J. Inst. Brew. 114(1), 27-33.

Franco D., Sineiro J., Rubilar M., Sanchez M., Jerez M., Pinelo M., Costoya M., Nunez M. J., 2008. EJEAFChe, 7(8), 3210-3216.

Gutzeit D., Baleanu G., Winterhalter P., Jerz G., 2008. Vitamin C content in sea buckthorn berries (*Hippophaë rhamnoides L. ssp. rhamnoides*) and related products: a kinetic study on storage stability and the determination of processing effects. J Food Sci., 73(9).

Kelly F.J., 1998. Use of antioxidants in the prevention and treatment of disease. J Int Fed Clin Chem 10 (1), 21–3.

Mayne S.T., 2003. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J. Nutr. 133 Suppl 3, 933S–940S.

Nickavar B., Abolhasani F., 2009. Screening of antioxidant properties of seven *Umbeliferae* fruits from Iran. Pak. J. Pharm. Sci., vol 22, No 1., 30-35.

Novruzov E., 2005. Biochemical Characteristics of Seabuckthorn (*Hippophae rhamnoides L.*) Growing in Azerbaijan. Seabuckthorn (Hippophae L.): A Multipurpose wonder Plant, Vol. II Singh V. (ed.) Daya Publishing House, New Delhi, India, 133–144.

Padayatty S., Katz A., Wang Y., Eck P., Kwon O., Lee J. H., Chen S., Corpe C., Dutta A., Levine M., 2002. Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention, J. Am. Coll. Nutr. 22 (1), 18–35.

Pellegrini N., Serafini M., Colombi B., del Rio D., Salvatore S., Bianchi M., Brighenti F., 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assesed by three different in vitro assays. University of Parma, Italy.

Pisoschi A.M., Negulescu G.P., 2011. Methods for Total Antioxidant Activity Determination: A Review. Biochem & Anal Biochem 1:106.

Ribera A.E, Reyes-Díaz M., Alberdi M., Zuñiga G.E., Mora M.L., 2010. Antioxidant compounds in skin and pulp of fruits change among genotypes and maturity stages in highbush blueberry (*Vaccinium corymbosum*) grown in southern Chile. J. Soil. Sci. Plant Nutr. 10 (4), 509 - 536.

Rufino M.S., Alves R.E., Brito E.S, Jiménez J.P., Saura-Calixto F., Mancini-Filho J., 2010, Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil, Food Chemistry 121, 996–1002.

Seglina D., Karklina D., Gailite I., Ruisa S., Krasnova I., Heidemane G., 2008. Changes of biochemical compounds in seabuckthorn marc during storage. Foodbalt, 109-113.

Singleton V. L., Orthofer R., Lamuela-Raventos R. M., Lester P., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth. Enzymol. 299, 152-178.