ROCZN. PZH 2010, 61, Nr 2, 151 - 154

EVALUATION OF SELECTED FOOD SUPPLEMENTS CONTAINING ANTIOXIDANTS

OCENA WYBRANYCH SUPLEMENTÓW DIETY ZAWIERAJĄCYCH ANTYOKSYDANTY

Katarzyna Łastawska

Department of Physical Chemistry, Faculty of Pharmacy
Medical University, Warsaw

Key words*: food supplement, antioxidants, green tea extract, chokeberry extract, cranberry extract*
Słowa kluczowe: *suplement diety, antyoksydanty, wyciąg z zielonej herbaty, wyciąg z aronii, wyciąg z żurawiny*

ABSTRACT

Seven commercial food supplements present on the Polish market were evaluated for their in vitro antioxidant capacity. The selected products were in the form of hard gelatin capsules. They contained the extracts from chokeberry, cranberry, blueberry and green tea. The mixture of vitamins and minerals as well as the product containing vitamin C in substantial dose were included into comparison. The products were examined using three methods in order to evaluate their antioxidant capacity: electron paramagnetic resonance (EPR), Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbing antioxidant capacity (ORAC) assays. Total polyphenolic content was determined according to Folin-Ciocalteu method. The results were calculated per capsule. All studied preparations showed antioxidant properties and may provide substantial antioxidant protection. The in vitro antioxidant capacity varied considerably and was associated with the content of polyphenols in the capsule. The supplement with 250 mg of green tea extract was the most potent antioxidant in all assays. Nevertheless it must be remembered that the amounts of extracts were different in encapsulated products. As the quality of extracts and their properties are miscellaneous, there is a need to standardize dietary antioxidant supplements with respect to their antioxidant capacity if effective doses are to be recommended.

STRESZCZENIE

Zbadano zdolność antyoksydacyjną siedmiu suplementów diety obecnych na polskim rynku. Wybrane produkty miały formę twardych kapsułek żelatynowych. Zawierały wyciągi z zielonej herbaty, aronii, żurawiny i czarnej jagody. Dla porównania do badania włączono też preparat będący mieszaniną witamin i minerałów oraz preparat z dużą dawką witaminy C. Produkty testowano wobec trzech różnych wolnych rodników, stosując fluorymetrię (metoda ORAC), spektroskopię UV-VIS (metoda TEAC) oraz spektroskopię elektronowego rezonansu paramagnetycznego (EPR). Całkowitą zawartość polifenoli oznaczono metodą Folin-Ciocalteu. Wyniki podano w przeliczeniu na kapsułkę produktu. Wszystkie badane preparaty wykazały właściwości antyoksydacyjne i mogą zapewnić znaczącą ochronę antyoksydacyjną. Zdolność antyoksydacyjna poszczególnych produktów była różna i proporcjonalna do zawartości polifenoli w kapsułce. Największą pojemność antyoksydacyjną miał produkt z wyciągiem z zielonej herbaty (250 mg ekstraktu). Niemniej trzeba pamiętać, że zawartość ekstraktów w produktach była różna. Jako że jakość i własności poszczególnych ekstraktów są zróżnicowane potrzebne jest standaryzowanie suplementów diety z antyoksydantami ze względu na ich zdolność antyoksydacyjną. Pozwoli to zalecać odpowiednie dawki antyoksydantów.

INTRODUCTION

Increasing experimental, clinical and epidemiological evidence shows the involvement of oxidative stress in a variety of diseases and aging. At the molecular levels aging is characterized by the progressive accumulation of molecular damage caused by free radicals and reactive oxygen species, generated by environmental and metabolic factors. The radicals attack lipids, sugars, proteins and nucleic acids and induce their oxidation. Consequently, the role of antioxidants, which suppress such oxidative damage, has received

Adres do korespondencji: Katarzyna Łastawska, Department of Physical Chemistry, Faculty of Pharmacy, Warsaw, Medical University of Warsaw, 02-097 Warszawa, Banacha 1, Poland
tel. 48 22 57 20 950, fax 48 22 57 20 951, e-mail: katarzyna.lastawska@wum.edu.pl

increased attention. Antioxidants are necessary: (1) to slow down the radical-caused processes of aging, (2) to prevent degenerative diseases (e.g. cardiovascular diseases, neurodegenerative disorders, rheumatoid arthritis, cancer), and (3) to support antioxidant defense and maintain the redox homeostasis.

The intake of antioxidants is often inadequate due to bad dietary habits. For this reason, there is a need to supplement dietary intake, using fortified foods or nutritional supplements. Plant foods and natural plant extracts are the excellent sources of compounds with antioxidant and anti-radical properties. The most important representatives of antioxidant phytochemicals are polyphenols e.g. flavonols, anthocyanins, catechins and tannins, hydroxycinnamic acids, abundant in fruits, vegetables and herbs. Antioxidant-rich extracts obtained from berries (cranberry, bilberry, chokeberry), as well as the extract from green tea leaves are used in food supplements. The properties of such preparations ought to be evaluated in order to confirm product efficacy. Different methods are used to evaluate the *in vitro* antioxidant capacities of bioactive compounds, each having its advantages and disadvantages [5]. The relative antioxidant activity of a compound may vary according to different testing methods. Dietary supplements composed of natural antioxidants and plant extracts are complex and multifunctional, so the measurement of their antioxidant activity by more than one method (based on different antioxidant mechanisms) is recommended.

The main objective of this work was to evaluate the *in vitro* antioxidant capacity for selected food supplements present on the Polish market.

MATERIAL AND METHODS

Dietary supplements in capsules containing black chokeberry, green tea and cranberry extracts (1-5) were selected, all are known as rich sources of polyphenolic compounds. The mixture of vitamins and minerals with Ginkgo biloba extract (6) and vitamin C with bioflavonoids (**7**) were included for comparison.

Diphenylpicrylhydrazyl radical (DPPH) was purchased from Sigma. Trolox was purchased from *Aldrich*. 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), Folin-Ciocalteu's phenol reagent, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from *Fluka*. Gallic acid and uranine were purchased from POCh.

Sample preparation

The content of hard gelatin capsule was dissolved in 96% ethanol and filtered. The stock solution was further diluted according to the requirements of particular analytical method. Three replicate experiments were performed for each sample, and the mean value is presented.

Table 1. Composition of the studied supplements, as indicated by the manufacturer
Skład badanych suplementów podawany przez producenta

Sample	Recommended dose	Composition
1	1-2 capsules/day	Green tea extract (including 30 mg catechin compounds)
2	1-2 capsules/day	Green tea extract 250 mg
3	2 sachets/day	Vaccinium macrocarpon extract 400 mg
4	2-4 capsules/day	Aronia melanocarpa extract 100 mg
5	2 capsules/day	Vaccinium macrocarpon extract 100 mg, Green tea extract 50 mg, Aronia extract 50 mg, Blueberry extract 20 mg
6	1 capsule/day	Vitamins A, D3, E, C, B1, B2, B6, B12, niacin, folic acid, biotin, panthotenic acid (all as 100% RDA), lutein 1 mg; minerals: Mg, Ca, K, Zn, Fe, Mn, Cu, I, Cr, Se, Mo and Ginkgo biloba extract 20 mg
7	1 capsule/day	Vitamin C 500 mg, citrus bioflavonoids 100 mg, Calcium 59 mg

Methods

DPPH assay using EPR spectroscopy

Samples were mixed with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and kept in dark. The radical activity was measured after 60 minutes on a MiniScope MS 200 spectrometer from Magnettech. The radical scavenging activity was expressed by the ratio $(I_0 - I)/I_0$ (I – integral intensity of the signal after addition of the extract, I_0 – the intensity of DPPH signal in control solution).

TEAC assay using UV-Vis spectroscopy

Trolox equivalent antioxidant capacity (TEAC) assay is based on the suppression of the absorbance of the 2,2'-azinobis(3-ethylbenzothiazoline6-sulfonic acid) (ABTS) radical cation by antioxidants in the test sample [6]. Trolox, the water-soluble vitamin E analogue, was used as a standard. The measurements were performed at 734 nm on a UV-Vis scanning spectrophotometer from Shimadzu.

ORAC assay using fluorescence spectrometer

Oxygen radical absorbing antioxidant capacity (ORAC) measures antioxidant scavenging capacity against peroxyl radical induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The protective effect of an antioxidant was measured by assessing the area under the fluorescence decay curve of the sample

as compared to that of the blank. The ORAC assay provides a direct measure of hydrophilic chain-breaking antioxidant capacity against peroxyl radical [4]. Trolox was used as the reference standard. The measurements were performed on Perkin Elmer Fluorescence Spectrometer.

Total polyphenol content

Total polyphenol content was determined according to the Folin-Ciocalteu method, using gallic acid as a standard. The results were expressed as milligrams of gallic acid equivalents (GAE) per capsule. The measurements were performed at 765 nm on a UV-Vis scanning spectrophotometer Shimadzu. It should be noted that this method is not specific for phenolic compounds and may suffer interference from other components (e.g. vitamin C).

RESULTS AND DISCUSSION

The effect of antioxidants from dietary supplements on different radicals was analyzed according to three distinct methods: an ORAC method for superoxide anion radicals (O_2^{*-}), an UV-Vis method using ABTS*- radical, and an EPR method for the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH*). The antioxidant activity of the seven popular supplements was evaluated in TEAC and ORAC assays. In addition, the radical scavenging activity of these preparations against the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was studied using electron paramagnetic resonance (EPR) since this assay is not sensitive to colored (due to the presence of anthocyanins) samples from berries (Fig. 1).

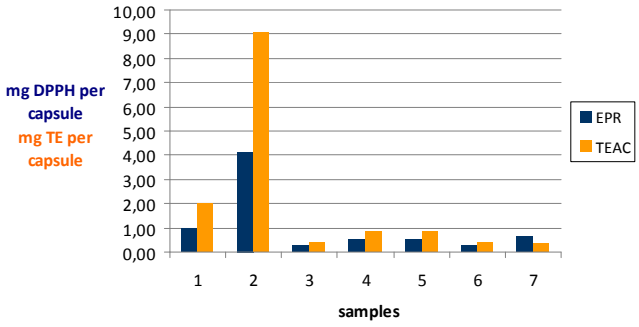

Fig. 1. The EPR and TEAC assays results
Wyniki pomiarów metodami EPR i TEAC

All studied samples (1-7) showed antiradical properties, but their activities differ significantly. The strongest antioxidant activity showed green tea extract [2], and this feature was confirmed in all assays. It is not surprising, because 250 mg of green tea extract contains 55% of epigallocatechin gallate, the strongest antioxidant known (due to the presence of eight phenolic OH groups). Green tea extract is widely used in food supplements. [1, 7]. Monomeric catechins and the fractions of dimeric and trimeric procyanidins were identified in the extracts from berries of Vaccinium species (cranberry, bilberry). Catechin, procyanidins and anthocyanins were good scavengers of free radicals, and more efficient than the respective cranberry fractions. The scavenging effects of tea catechins and their derivatives on DPPH radical were evaluated by EPR [2]. It was suggested that the galloyl moiety attached to flavan-3-ol at C-3 position, as well as the ortho-trihydroxyl group in the B ring, have a strong scavenging ability on the DPPH radical. It elevates the radical scavenging efficiency above that of the ortho-dihydroxyl group; as has been recognized in other flavonoids.

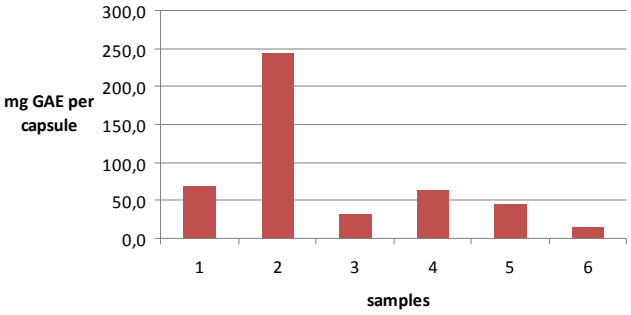

Fig. 2 Total polyphenols content
Całkowita zawartość polifenoli

There was a correlation between the results obtained by three different methods. In all assays the highest antioxidant capacity was observed for 2 (containing 250 mg of tea extract) and the lowest for 7 (vitamin mix). The sequence of supplements results from the content of polyphenols. There is a linear correlation between the antioxidant activity and the total polyphenols content, as illustrated in Figure 3.

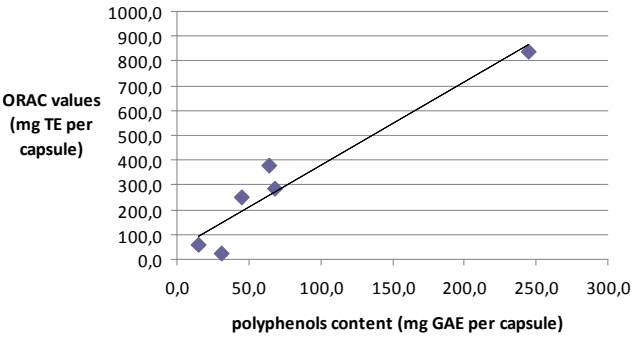

Fig. 3 Antioxidant capacity measured by ORAC versus total polyphenols content
Zdolność antyoksydacyjna mierzona metodą ORAC a całkowita zawartość polifenoli

These results indicate that the supplements and the polyphenols isolated from them exhibited effective radical-scavenging activity, and may be promising agents for scavenging free radicals and preventing diseases associated with the excess of free radicals. The need to standardize dietary antioxidant supplements with respect to their antioxidant capacity is important if effective doses are to be recommended for a person's current oxidative status.

However, the amounts of extracts were different in encapsulated products, so direct comparisons of antioxidant capacity calculated per capsule can be misleading. Additionally, it is important to deliver various antioxidants, e.g. from all types of polyphenols, carotenoids and tocopherols. Dietary supplements with cranberry extract are consumed because of its anti-adhesive properties with respect to bacteria, which may prevent urinary tract infections. Although the antioxidant function is also valuable.

CONCLUSIONS

Using of three different spectroscopic methods and three radicals give better insight into antioxidant and anti-radical properties of the complex mixtures of compounds. Radical scavenging and antioxidant activities of berry fractions were attributable to the composition of polyphenolic compounds (catechins, procyanidins, epigallocatechin derivatives). Further studies are needed to understand the activities of particular polyphenols and also possible synergic and antagonist effects. The supplementation could be better addressed if daily intakes of particular antioxidants were known. Green tea, black chokeberry and cranberry extracts are good sources of compounds with antioxidant activities and should be included in anti-aging diet, for example in the form of food supplements.

REFERENCES

1. *Henning S.M., Fajardo-Lira C., Lee H.W., Youssefian A.A., Go V.L.W., Heber D.*: Catechin Content of 18 Teas and a Green Tea Extract Supplement Correlates With the Antioxidant Capacity. Nutrition and Cancer 45, 2, 226-235.
2. *Nanjo F., Goto K., Seto R., Suzuki M., Sakai M., Hara Y.*: Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radic Biol Med. 1996, 21(6), 895-902.
3. *Opala T., Rzymski P., Pischel I., Wilczak M., Woźniak J.*: Efficacy of 12 weeks supplementation of a botanical extract-based weight loss formula on body weight, body composition and blood chemistry in healthy, overweight subjects-a randomised double-blind placebo-controlled clinical trial. Eur J. Med. Res. 2006, 11(8), 343-50.
4. *Ou B., Hampsch-Woodill M., Prior R.L.*: Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. J. Agric. Food Chem. 2001, 49, 4619-4626.
5. *Prior R.L., Wu X., Schaich K.*: Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements J. Agric. Food Chem. 2005, 53 (10), 4290–4302.
6. *Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C.*: Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Radic. Biol. Med. 1999, 9/10(26), 1231-1237.
7. *Seeram N.P., Henning S.M., Yantao N., Lee R., Scheuller H.S., Heber D.*: Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. J. Agric. Food Chem. 2006, 54,1599-1603.

Otrzymano: 20.03.2009
Zaakceptowano do druku: 05.01.2010