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THE MEASUREMENT OF ANTIOXIDANT CAPACITY AND POLYPHENOL CONTENT IN SELECTED FOOD SUPPLEMENTS*

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Abstract: Oxidative stress (OS), defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, can result in the development of many serious diseases like diabetes or cancer. Moreover, the role of oxidative stress in the acceleration of the aging process is also confirmed. ROS are constantly produced in the natural biochemical processes, mainly during cellular respiration. Their enhanced production may be the result of e.g., an inappropriate diet high in saturated fats, low in fiber, fruits and vegetables, insufficient physical activity or smoking. To prevent oxidative stress, besides changes in life style, the additional supplementation of antioxidants is proposed. On the Polish market, the number of food supplements with declared antioxidant activity is still increasing. However, their antioxidant properties are rarely confirmed experimentally. The aim of our study was to determine the antioxidant potential of selected dietary supplements available on the market and recommended in chronic fatigue syndrome. The antioxidant potential was measured using four methods: FRAP, ORAC, HORAC, EPR/DPPH. Moreover, the content of polyphenols in the dietary supplements was also determined.

Keywords: FRAP, ORAC, HORAC, EPR/DPPH, pharmaceuticals, antioxidant capacity

Oxidative stress (OS) is a homeostatic imbalance between the natural antioxidant defenses and the production of reactive oxygen species (ROS) (1). ROS are produced in the natural biochemical processes, but in excessive amounts and without sufficient antioxidant defenses what can cause damage to all components of cells: lipids, proteins and DNA (2). Several epidemiological studies have shown that degenerative diseases, including cancer, cardiovascular, neurodegenerative diseases and immune dysfunction, which are associated with increased ROS activity lead to the OS (3-5). Recent studies report that high consumption of antioxidant food products like vegetables, fruits and beverages, for example, tea, wine and cocoa, can reduce the oxidative stress and reduce the risk of chronic diseases (6). These food products are a rich source of antioxidant compounds like vitamins (C and E), selenium and carotenoids, such as β-carotene, lycopene, lutein and polyphenols (7).

Inappropriate lifestyle, exposure to environmental pollutants, toxins, tobacco smoke, artificial chemicals and a diet poor in antioxidants may induce an abnormal increase of ROS production and/or a decrease in antioxidant defenses that could alter an homeostatic balance state and cause OS. Healthy lifestyle associated with low exposure to toxins and a healthful, balanced diet supplies the body with sufficient nutrients, e.g., phytochemicals, and leads to the lower risk for many diseases (8). Supplements with compounds such as vitamins, minerals, essential fatty acids, phytochemicals can enrich the body's internal environment to fortify cellular protection, regeneration and support detoxification processes (9).

There are a number of commercial dietary supplements based on fruits and vegetables with the declared antioxidant activity, which can be defined as a protection against OS (10). As manufacturers declare, dietary supplements contain compounds with proven antioxidant properties, which help in maintaining good health, delay the aging process and reduce the risk of many diseases. However, there are also dietary supplements without declared antioxidant

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properties which are used in the prevention and treatment of many diseases like, for example, digestion.

Dietary supplements are prepared from different parts of plants, have different chemical compositions and different concentrations of active compounds, therefore they vary widely in their antioxidant properties. Furthermore, most manufacturers offer residual information about dietary supplements composition and their labels include no data on their antioxidant activity. Because the dietary supplements are used by man as a source of natural antioxidants, their antioxidant properties should be known, standardized and controlled. The standardization of antioxidant supplements would allow to recommend efficacious doses and to ensure that these products may have a beneficial effect. To date, there are only a limited number of studies on this subject (11, 12).

On the Polish market there are many dietary supplements with declared antioxidant activity and many with potential antioxidant activity. However, their antioxidant properties are rarely confirmed experimentally. In the literature there are many analytical methods for determining antioxidant properties of foods and ingredients of different plants. The objective of this work was to evaluate the antioxidant activity *in vitro* of commercial dietary supplements using different methods: FRAP (Ferric Reducing Ability of Plasma), ORAC (Oxygen Radical Absorbance Capacity), HORAC (Hydroxyl Radical Averting Capacity) and DPPH-EPR (diphenyl-1-picrylhydrazyl radical scavenging with the use of electron paramagnetic spectroscopy) assays. The total content of polyphenols, as the most frequently occurring phytochemicals in the dietary supplements, was also determined.

EXPERIMENTAL

Dietary supplements

Dietary supplements were a kind gift from Gama-Tech. Since they are mainly a mixture of various herbal extracts or other components, their detailed composition as indicated by the manufacturer is presented in Table 1.

Sample preparation

To 500 mg of each supplement 10 mL of ethanol-water (1 : 1, v/v) mixture was added. After 40 min of shaking, the samples were centrifuged and the supernatants were taken for analysis.

Antioxidant activity determination

ORAC assay

The ORAC-fluorescein (ORAC-FL) assay was based on the procedure of Ou et al. (13). All solutions used were prepared daily in PBS (phosphate-buffered saline), pH 7.4. The samples solutions were further diluted with PBS. For measurements, 70 µL of PBS, 30 µL of PBS diluted sample or, in case of a blank, 30 µL of PBS buffer and 100 µL of 112 nM sodium fluorescein solution were mixed in a well and thermostated for 15 min at 37°C. Then, 100 µL of 48 mM AAPH solution was added and fluorescence was measured every 60 s for 90 min with F-7000 Fluorescence Spectrophotometer (Hitachi) equipped with a Micro Plate Reader accessory. The excitation wavelength was 485 nm, the emission wavelength was 520 nm. ORAC values in Trolox equivalents (TE [µmol/L g]) were calculated using the standard curves, prepared in parallel with measurements, with Trolox concentration in the range 6.25-100 µM. All experiments were performed in triplicate.

HORAC assay

The HORAC assay was performed according to Ou et al. (14), using F-7000 Fluorescence Spectrophotometer (Hitachi) equipped with a Micro Plate Reader accessory with excitation wavelength set at 485 nm and the emission wavelength set as 520 nm. To each well 70 µL of PBS and 30 µL of a PBS-diluted sample (2-10-fold) or standard solution was pipetted, then 100 µL of 112 nM fluorescein solution was added and all reagents were mixed thoroughly. In the next step, 50 µL of 0.165 µM hydrogen peroxide solution was added, the reagents were mixed well and then 50 µL of the cobalt solution (17.9 mg of cobalt chloride and 23 mg of picolinic acid per 10 mL) was added, and fluorescence was measured every minute for 35 min. Phosphate buffer was used as a blank, and gallic acid concentrations of 800, 600, 400, 200, and 100 µM were used as standards.

FRAP assay

The FRAP assay was performed based on the procedure of Benzi et al. (15). FRAP reagent was prepared by mixing 10 mM 2,4,6-tri[2-pyridyl-s-triazine] (TPTZ), 20 mM ferric chloride and 300 mM (pH = 3.6) acetate buffer in 1 : 1 : 10, v/v/v ratio. Ten µL of sample or standard solution was added to 200 µL of FRAP reagents, the mixture was kept at 37°C and the absorbance reading at 593 nm (Synergy™ Mx microplate reader, Biotec) was taken after 4 min. Where appropriate, the samples were diluted using Millipore water. Standard solutions of ferrous sulfate in the 100-1000 µM range were used for the standard curve preparation (C_{Fe} = -65 + 693 A, where C_{Fe} – ferrous sulfate concentra-

Table 1. Composition of the commercial antioxidant supplements and the other studied samples.

Dietary supplements/ products	Composition of the supplement/product	Recommended daily dose	Declared properties
Acerola extract, powder	Powdered acerola (*Malpighia punicifolia*) extract	No manufacturer recommendation, in practice average dose: 70 mg/day	Antioxidant properties
ALFA AKTIV	Blackcurrant and aronia extract with honey: Vitamin C (996 mg/2 vials), Vitamin B_3 (4.13/2 vials), Selenium (9.04 µg/2 vials), Iodine (23.76 mg/2 vials), polyphenols (8.24 mg/2 vials)	4 vials/day (80 g)	Antioxidant properites
Antiox	Pressed grape extract (150 mg/capsule), *Ginkgo biloba* extract (26.5 mg/capsule), yeast rich in selenium (50 µg/capsule), ZnO (15 mg/capsule), Vitamin C (65 mg/capsule), Vitamin E (10 mg/capsule), β-carotene (5 mg/capsule)	2 capsules/day (780 mg/day)	Antioxidant properties Stimulates immunity and resistivity of the organism.
Chinese Yam	Powdered Chinese Yam (*Dioscorea batatas*)	No specification, in Traditional Chinese Medicine (TCM) minimal recommended dose is 10 g/day	Stimulates immunity and resistivity of the organism.
Detox+	*Uncaria tomentosa (*cv. *Vilcacora)* extract (380 mg/capsule)	1 capsule/day (387.5 mg)	Antioxidant properties
Duolife (Day)	Extract of: acai berry, aloe vera, elderberry, wild rose, grenade, hawthorn, medicago, raspberry noni - indian mulberry ginseng, cranberry	1 vial/day (25 g)	Antioxidant properties. Stimulates immunity and resistivity of the organism.
Duolife (Night)	Extract of: beetroot, goji berries, mulberry - morus alba, milk thistle, nettle	1 vial/day (25 g)	Antioxidant properties. Stimulates immunity and resistivity of the organism.
Floradix	Water solutions (54%) of: carrots, nettle, spinach, couch grass root, fennel, ocean algae, African mallow flower; fruit juice concentrates are 29.4% (pear, grape, black currant, cherry, apples, oranges, beets, lemon yeast, honey, wild rose), Vitamin C (13 mg/ vial), Vitamin B6 (0.4 (mg/vial), Vitamin B12 (0.6 mg/vial), Iron (7.5 mg/vial)	2 vials/day 20 g)	Supplements the daily diet with iron and vitamins. Recommended in order to improve the health and well-being.
Floradix Ochrona Jelit	Water extract of turmeric, peppermint leaves, artichoke leaf, rosemary leaf (8.4 mL/ vial), apple-plum extract (4.5 g/vial), dry extract of turmeric (76 mg/vial), magnesium (125 mg/vial), Vitamin C (13 mg/vial), Thiamine (0.8 mg/vial), Riboflavin (0.9 mg/vial), Vitamin B6 (0.4 mg/vial), Vitamin B12 (0.6 mg/vial), Iron (7.5 mg/vial)	1 vial/day (20 g)	Helps to maintain healthy intestines and proper digestion.
Gano Excel Cordyceps	Powdered mycelium of *Cordyceps sinensis*	2 capsules/day (900 mg)	Improves and strengthens the immunization system. Improves the health of the respiratory system and pulmonary function.

Table 1. Cont.

Dietary supplements/ products	Composition of the supplement/product	Recommended daily dose	Declared properties
Gano Excel Ganoderma	Powdered spores of the Reishi mushroom (*Ganoderma lucidum*m) (275 mg/1 capsule)	2-4 capsules/day (550-1100 mg)	Stimulates immunity and resistivy of the organism
Gano Exel Excellium	Powdered mycelium of the Reishi mushroom (*Ganoderma lucidum*m) (425 mg/1 capsule)	2-4 capsules/day (850-1700 mg)	Helps to support general well being and nurtures the body's natural defenses
ImmunoBooster	Pomegranate extract with ellagic acid (40 mg/2 capsules), acerola extract with natural Vitamin C (80 mg/2 capsules) red raspberry, graviola and arnica extract, blueberry leaf extract	2 capsules/day (120 mg)	Antioxidant properties
Long Energy	Dry extract of *Rhodiola rosea L.* (200 mg/2 tablets), *Cola nitida (Vent.) Schott. et Endl.* (200 mg/2 tablets), powdered *Panax ginseng C.A. Meyer* (120 mg/2 tablets), dry extract of *Ginkgo biloba L.* (90 mg/2 tablets), yeast rich in selenium (105 µg/2 tablets), coenzyme Q10 (30 mg/2 tablets)	2 tablets/day (641 mg)	Antioxidant properties
Mistify	Acai berry extract (*Euterpe oleracea*) (1.60 g/vial), red grape extract (*Vitis vinifera)* (1.86 g/vial), Concorde grape extract (*Vitis labrusca*) (1.86 g/vial), Highbush blueberry extract (*Vaccinum corymbosum*) (1.25 g/vial), red raspberry extract (*Rubus ideaus*) (1.10 g/vial), blueberry extract (*Vaccinium angustifolium*) (0.40 g/vial), cranberry extract (*Vaccinium macrocarpon*) (0.04 g/vial), Goji berries extract (*Licium barbarum*) (0.028 g/vial), pomegranate extract (*Punica granatum*) (0.006 g/vial), Green tea leave extract (*Camelia sinensis*) (0.005 g/vial)	2 vials/day (60 g)	Antioxidant properties
MonaVie	Acai berry juice 25%, concentrated fruit juice (apple, grape, pear, pineapple, cranberry, passion fruit, elderberry, prune, kiwi, blueberry, blackberry, wolfberry, cherry, pomegranate, count), fruit pulp (acerola , pear, banana, blueberry, black)	60-120 mL/day (60-120 g)	Antioxidant properties
Pau D'Arco	*Tabebula impetignos* powdered bark (500 mg/capsule)	4 capsules/day (2000 mg)	Antioxidant properites
PhytoC	Extract of: acerola, elderberry, lemon juice, wild rose	1 vial/day (30 g)	Strengthens natural resistance of organism.
Phytolife	Sodium – cooper chlorophyllin salt (22.32 mg/3 vials), *Mentha piperita* oil (48.12 mg/3 vials)	3 vials/day (22.5 g)	Antioxidant properties
PhytoMan	Extract of: aloe vera; fragrant cinnamon, wolfberry scarlet; turmeric long, multiflower; okra, chamomile, Chinese astragalus; ginseng	1 vial/day (30 g)	Increases fertility. Reduce the effects of andropause.
ReishiMax	*Reishi* mushroom extract (*Ganoderma lucidum*) (500 mg/capsule) with triterpenes (6%) and polysaccharides (13,5%)	2 capsules/day (1000 mg)	Stimulates immunity and resistivity of the organism

Table 1. Cont.

Dietary supplements/ products	Composition of the supplement/product	Recommended daily dose	Declared properties
SCleanUp	*Tamarindus indica L.* fruit extract (480 mg/vial), *Camellia sinensis L.* leave extract (120 mg/vial), *Cynara scolymus L.* extract (100 mg/vial), *Betula pendula Roth.* extract (100 mg/vial), *Filipendula ulmaria (L.) Maxim.* extract (50 mg/ vial), *Taraxacum officinale Weber* extract (50 mg/vial), *Foeniculum vulgare Miller* (50 mg/vial), *Arctium lappa L.* (25 mg/ vial), *Viola tricolor L.* (25 mg/ vial)	1 vial/day (12 g)	Digestive problems, detoxifies organism.
Super Digestion	Wine extract of: *Cynara scolymus* (450 mg/ vial), *Foeniculum vulgare* (225 mg/ vial), *Mentha piperita* (150 mg/ vial), *Melissa officinalis* (150 mg/ vial), *Angelica archangelica* (150 mg/ vial), *Carum carvi* (90 mg/vial), *Rosmarinus officinalis* (90 mg/ vial), *Taraxacum officinale* (75 mg/ vial), *Cinnamomum aromaticum Nees* (75 mg/ vial), *Gentiana lutea L.* (45 mg/ vial)	2 vials/day (30 g)	Digestive problems
Sweetacertabs	Powdered acerola fruit *(Malpighia punicifolia)* (244.8 mg/tablet), powdered blackcurrant fruit (23 mg/tablet) with Vitamin C (60 mg/tablet) natural flavor of raspberries (3.8 mg/tablet)	1 tablet/day (742 mg)	Antioxidant properites
Wilcashi Forte	*Vilcacora (Uncaria tomentosa)* shredded bark (100 mg/1 capsule), spores of the Reishi mushroom (*Ganoderma lucidum*m) (100 mg/L capsule)	4 capsules/day (800 mg)	Body health improvement. Stimulates immunity and resistivity of the organism
Wild rose juice	Wild rose (*Rosa canina*) extract	200 mL	Antioxidant properties
Xyliacertabs	Powdered acerola extract (240 mg/1 tablet), powdered blackcurrant (23 mg/1 tablet) with natural Vitamin C (60 mg/1 tablet), natural aroma of raspberries (4 mg/1 tablet)	1 tablet/day (742.5 mg)	Antioxidant properties

tion in µM, A – absorbance at 593 nm). Results were reported as mmol of Fe^{2+} per 1 g of supplement. All experiments were performed in triplicate.

***DPPH-EPR* assay**

For DPPH-EPR test, 50 or 100 µL of sample solution was mixed with 1 mL of acetone solution of DPPH (2.5 mM). After 2 min, the EPR spectra were recorded. The DPPH samples with demineralized water in place of a sample solution were used as intensity standards. The intensity was taken as the double integral of the spectra. Results were expressed as Trolox equivalents (µmol of TE per 1 g of the supplement) with the use of standard curve ($I_{EPR} = 5100\ c_{Tr} - 178.5$, where I_{EPR} – intensity of EPR signal, c_{Tr} – Trolox concentration in µmol/L). All experiments were performed in triplicate.

EPR measurements were performed on a EPR/SE/X (9.3 GHz) (Radiopan), with following parameters: central field 334 mT, sweep range 10 mT, sweep time 60 s, diode current 60%, attenuation 10 dB, modulation amplitude 0.2 mT, phase 90°.

Total polyphenols content determination

Polyphenols content was determined by modified Folin-Ciocalteu colorimetric method (16). Briefly, to 100 µL of Millipore water first 25 µL of sample, then 50 µL of working Folin-Ciocalteu reagent were added. After 3 min at room tempera-

ture, 120 µL of 20% sodium carbonate was added, and the reaction mixture was incubated for 30 min at 37°C. The absorbance at 765 nm was measured using microplate reader Synergy Mx (Biotec) and compared to a gallic acid calibration curve (C_{GA} = -30 + 187.2 A, where C_{GA} is gallic acid concentration in mg/L, A – absorbance at 765 nm). The results were expressed as gallic acid equivalents (GAE [mg/g]). All experiments were performed in triplicate.

Statistical analysis

All the results of antioxidant activity and antioxidants content determination (DPPH, ORAC, HORAC, FRAP and total phenolics) are presented as the mean ± SD. Statistical analyses were performed using STATISTICA (StatSoft Inc., USA). The number of variables (FRAP, ORAC, HORAC, DPPH-EPR, total phenolics) was reduced to two factors using factor analysis. The dietary supplements were described using two dimensional data (factor 1, factor 2) and grouped using Ward's hierarchical clustering method (agglomerative method).

RESULTS

The results of antioxidant activity determination by different methods and total polyphenol content of studied samples are presented in Table 2.

Using the ORAC assay, the hierarchy of antioxidant capacity ranging from 124 ± 20 to 21940 ± 420 µmol TE/g was obtained. The highest value was obtained for acerola extract, followed by Long Energy, and only for these samples were ORAC values similar to hydrophilic ORAC values obtained for dietary supplements based on extracts with well established antioxidant properties (12). Another eleven samples exhibited ORAC values over 1000 µmol TE/g (about half the ORAC average value of recommended five fruit and vegetable servings per day), which can be taken as a confirmation of their strong antioxidant properties (in order of decreasing values: ReishiMax, Vitamin C, Antiox, Immonobooster, Wilcashi Forte, Detox +, Duo Live (Day), Phyto C, Pau D' Arco, Duo Live (Night), Sweetacertabs).

The HORAC values ranged from 27.0 ± 6.3 µmol GAE/g for Duo Live (Day) to 2670 ± 510 mg GAE/g for Long Energy. The highest values were on the same level as those obtained by Anthony and Saleh (17) for sylimarin, which is also a dietary supplement with polyphenols as its main components. The majority of samples had HORAC results below 200 mg GAE/g, with only 12 of 29 samples that gave higher results (in decreasing order: Long Energy, ReishiMax, Wilcashi Forte, Antiox, Detox+, acerola extract, Pau D'Arco, Immonobooster, Gano Excel Cordyceps, CleanUp, wild rose juice, Duo Live (Night)). Among these 12 samples four had no declared antioxidant properties (ReishiMax, Wilcashi Forte, Gano Excel Cordyceps, CleanUp), but the producers declared immunostimulating or detoxifying action.

In FRAP assay the highest value was obtained for vitamin C (3602 ± 92 µmol Fe/g), and among supplements for Xyliacertabs (2468.8 ± 9.6 µmol Fe/g), and the lowest for PhytoMan (0.234 ± 0.028 µmol Fe/g). According to the results of this test, the supplements can be divided into two groups: the group with low activity (from 0.234 ± 0.028 to 81.74 ± 0.24 µmol Fe/g) and the group with high activity (from 307.0 ± 8.3 to 3602 ± 92 µmol Fe/g). However, the supplements with declared antioxidant activity were uniformly divided between both groups. On the other hand, among supplements without this declaration only the ReishiMax and Wilcashi Forte preparations had high FRAP value.

The best DPPH scavenger among studied supplements was acerola extract (535 ± 51 µmol TE/g), although its result was about 5 times lower than the result of vitamin C (2650 ± 140 µmol TE/g). The lowest value obtained in this test (10.90 ± 0.55 µmol TE/g for chinese yam) was about 25 times lower than the vitamin C result. It was the only antioxidant assay in which all studied supplements gave lower results than the standard antioxidant, i.e., vitamin C. Total polyphenols content of studied dietary supplements was in the range of 0.776-2255 µmol GAE /g. Similarly to the FRAP assay results, two groups of high and low polyphenol content could be seen (the low-polyphenol group with results in the range from 0.776 ± 0.041 to 44.4 ± 1.2 µmol GAE /g, with the lowest value for Floradix Ochrona Jelit, and the high-polyphenol group with results in the range from 115.0 ± 1.8 to 1198 ± 21 µmol GAE /g, with the highest value for acerola extract). Also in this case the high-polyphenol group is composed mainly of dietary supplements with declared antioxidant activity. It should be noted, however, that in the case of preparations containing vitamin C (as illustrated by the high value obtained for pure vitamin C (2255 ± 76 µmol GAE /g)) or reducing sugars the result of this test is influenced by these compounds.

Spearman's correlation coefficients between different antioxidant activity tests and total polyphenol content are shown in Table 3, and between different antioxidant activity tests results in Table 4. As can be seen, the strongest correlation was between

FRAP test results and polyphenol content, followed by ORAC test results and Folin-Ciocalteu assay. On the other hand, the HORAC test gave results that correlated the weakest with other antioxidant activity tests as well as with total polyphenol content.

In factor analysis, the first factor was due to FRAP, DPPH-EPR and Folin-Ciocalteu assays results, and explained 50% of the variability among studied samples, while the second factor was composed of ORAC and HORAC values and explained 29% of variability. The cluster analysis showed some grouping of the samples (Fig. 1a). As can be seen in Figure 1b, when data are grouped to two clusters, the average values for all assays for one cluster are higher than for the other cluster, i.e., both factors were responsible for this grouping. The majority of samples belonging to the first group were supplements with declared antioxidant properties. When the division of the samples into three clusters was done (Fig. 1c), one cluster consisted only of vitamin C (cluster 1). The other two clusters differed by the average values for all assays except

Table 2. Antioxidant activity and total polyphenol content (± mean standard deviation) of dietary supplements.

Supplement	ORAC [mmol TE/g]	HORAC [mmol GAE/g]	FRAP [mmol /g]	EPR [mmol TE/g]	Folin-Ciocalteu [µmol GAE /g]
Acerola extract, powder	21940 ± 420	510 ± 140	1270 ± 110	535 ± 51	1198 ± 21
ALFA AKTIV	730 ± 120	99 ± 20	405.0 ± 4.2	101.8 ± 5.1	115.0 ± 1.8
Antiox	3500 ± 130	706 ± 41	2250 ± 130	240 ± 12	798 ± 30
Chinese yam	320 ± 40*)	75.4 ± 7.7	15.86 ± 34	10.90 ± 0.55	14.519 ± 0.077
Detox +	2490 ± 160	690 ± 19	307.0 ± 8.3	67.3 ± 3.4	168.7 ± 7.1
Duo Live (Day)	1950 ± 480	27.0 ± 6.3	8.81 ± 0.53	168 ± 27	10.21 ± 0.38
Duo Live (Night)	1199 ± 92	217.4 ± 2.1	7.31 ± 0.35	140 ± 35	8.0 ± 0.5
Floradix Ochrona Jelit	380 ± 40	106 ± 17	16.5 ± 2.6	59 ± 3	12.70 ± 0.95
Floradix	401 ± 25	92 ±10	14.12 ± 0.17	340 ± 17	12.67 ± 0.32
Gano Excel Cordyceps	124 ± 20	335 ± 130	24.52 ± 0.13	29.9 ± 1.5	25.8 ± 1.8
Gano Excel Ganoderma	835 ± 67	131 ± 23	39.80 ± 0.11	92 ± 5	38.6 ± 2.2
Gano Excel Excellium	500 ± 51	93 ± 14	8.59 ± 0.11	86.5 ± 4.4	14.46 ± 0.71
Immunobooster	3490 ± 160	461 ± 62	1460 ± 130	108.9 ± 5.5	327.8 ± 5.9
Long Energy	10000 ± 1000	2670 ± 510	700 ± 100	105.4 ± 5.3	294.7 ± 7.7
Mistify	425 ± 12	137 ± 34	12.42 ± 0.18	30.8 ± 1.6	8.78 ± 0.36
MonaVie	284 ± 32	102.5 ± 3.5	22.34 ± 0.16	59 ± 3	7.28 ± 0.53
Pau D'Arco	1478 ± 83	505 ± 5	81.74 ± 0.24	31.6 ± 1.6	44.4 ± 1.2
Phyto C	1820 ± 320	136 ± 21	31.6 ± 4.0	320 ± 45	36.01 ± 0.79
Phytolife	391 ± 23	151 ± 31	4.066 ± 0.035	30.2 ± 1.6	2.23 ± 0.21
PhytoMan	683 ± 92	100 ± 21	0.234 ± 0.028	185 ± 22	0.776 ± 0.041
ReishiMax	5850 ± 410	2070 ± 270	336.6 ± 2.2	98 ± 5	218.6 ± 3.8
SCleanUp	947 ± 43	306 ± 17	54 ± 28	17.6 ± 0.9	38.0 ± 1.5
Super Digestion	498 ± 57	136 ± 25	28.4 ± 0.3	30.6 ± 1.6	13.81 ± 0.83
Sweetacertabs	1160 ± 150	116 ± 44	2210 ± 150	162.4 ± 8.2	381.5 ± 7.7
Wilcashi Forte	3350 ± 240	1230 ± 140	390 ± 4	41.7 ± 2.1	179.9 ± 8.3
Wild rose juice	448 ± 40	240 ± 40	52.1 ± 4.8	243 ± 45	43.4 ± 2.2
Xyliacertabs	829 ± 89	109.5 ± 5	2468.8 ± 9.6	152 ± 8	487.1 ± 5.9
Vitamin C	5400 ± 600	66.5 ± 4.4	3602 ± 92	2650 ± 140	2255 ± 76

Table 3. Spearman's correlation coefficients between different antioxidant activity tests and total polyphenol content.

Test	Spearman's correlation coefficient	Significance level
ORAC	0.69	0.00004
HORAC	0.38	0.04477
FRAP	0.96	< 0.00001
EPR	0.41	0.02553

Table 4. Spearman's correlation coefficients between antioxidant activity obtained in different assays.

Test	Spearman's correlation coefficient	Significance level
ORAC - HORAC	0.48	0.00780
ORAC - FRAP	0.62	0.00030
ORAC – EPR	0.49	0.00748
HORAC - FRAP	-	**0.06426**
HORAC - EPR	-	**0.45417**
FRAP - EPR	0.38	0.04491

diphenyl-1-picrylhydrazyl assay, though the difference of average result of polyphenol content determination was smaller than for ORAC and HORAC.

DISCUSSION

The ORAC and the HORAC assays, which are based on a hydrogen atom transfer (HAT) reaction and – in case of HORAC assay – also on the chelation of transition metals, an assay measuring electron transfer/reducing capacities, namely the ferric reducing antioxidant power (FRAP) and free radical scavenging properties by the diphenyl-1-picrylhydrazyl (DPPH) radical assays with the use of EPR spectroscopy, were used to investigate the antioxidant properties of hydrophilic fraction of 27 popular dietary supplements present on the Polish market, recommended for the general improvement of health and condition. Vitamin C was used as a standard, as a well-established antioxidant. It is also one of the antioxidants most abundant in the human diet and most popular as a dietary supplements component.

All of the studied dietary supplements exhibited some antioxidant properties, however, there was a large diversity among them. In all tests the values differed by orders of magnitude, with the biggest differences obtained in FRAP assay. Only two supplements gave high results in all antioxidant assays used as well as in Folin-Ciocalteu assay, namely acerola extract and Antiox preparation. This can be easily understood since acerola is an acknowledged rich source of vitamin C, it is also rich in phenolic compounds (18, 19). The declared composition of Antiox preparation also implies high antioxidants content, as it contains grape seed extract of well-established antioxidant properties (20) and is enriched with pure antioxidants (vitamin C, vitamin E and β-carotene).

Besides these two best antioxidant supplements, there were some preparations that gave high results only in some tests, among them Long Energy (high results in all tests except DPPH-EPR test, the highest HORAC value), ReishiMax (as Long Energy, high results in all tests except DPPH-EPR test, the second high HORAC value), Immunobooster (high ORAC, FRAP and total polyphenols content values, low HORAC and DPPH-EPR values) and Wilcashi Forte (high ORAC and HORAC, quite high FRAP value, low DPPH-EPR and polyphenol content). As can be seen, three out of four had high HORAC value. This was due probably to the presence of substantial amounts of substances other than polyphenols, as the correlation of HORAC with polyphenol content was the weakest among all antioxidant assays used. The possible candidates are polysaccharides from Reishi mushrooms extract (ReishiMax) or U. tomentosa bark and Reishi mushroom spores powder (Wilcashi Forte) or ginseng and

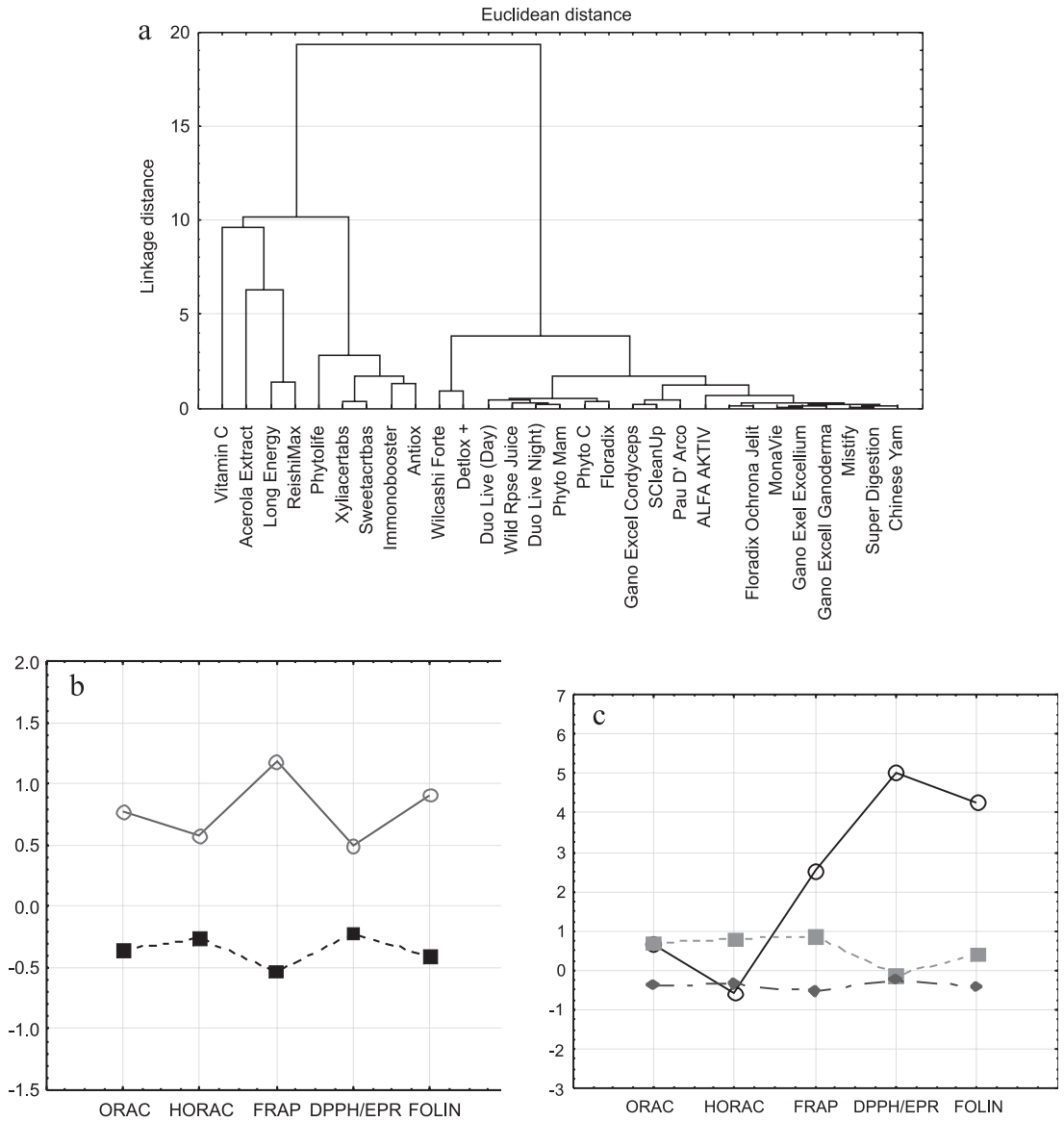

Figure 1. Hierarchical tree diagram (Ward's method) for dietary supplements with antioxidant properties (a), cluster profile plot for two clusters (standardized data) (b), cluster profile plot for three clusters (standardized data) (c)

Gingko biloba extract (Long Energy), which have chelating and antioxidant properties (21, 22), though their antioxidant activity towards DPPH radical is moderate. This would explain also the low values obtained in DPPH-EPR test. Also the fact that the only supplement with low HORAC value in this group was Immunobooster, which did not contain any source of polysaccharides, can be taken as the confirmation of this hypothesis.

Some of the supplements with antioxidant properties as a main or even the only declared action gave relatively low results in all antioxidant activity assays. The examples are AlfaAktiv, Mistify, wild rose juice, MonaVie. It should be noted, though, that these supplements are in the form of solutions, and their recommended daily dose is much higher in mass units than the daily dose of supplements in the form of tablets or capsules. Therefore, if this dose difference is taken into account, these liquid supplements can be treated as a source of antioxidants in the diet. The results of Ward's hierarchical clustering after recalculating the obtained results for the recommended daily dose of each supplement are shown in Figure 2 – different grouping than in the

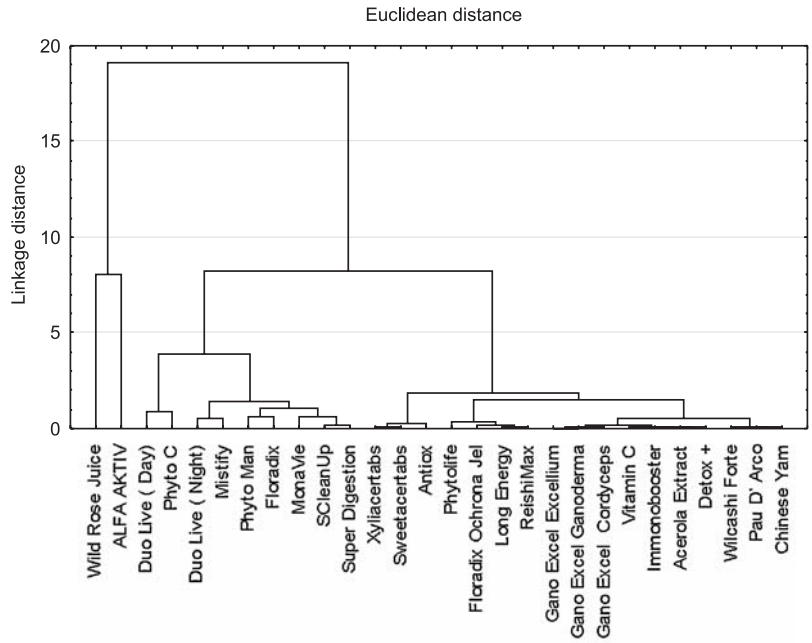

Figure 2. Hierarchical tree diagram (Ward's method) for dietary supplements with antioxidant properties after taking into consideration the daily dose

case of values taken for mass unit can be seen. Most notably, almost all supplements in the form of solution constitute the first three groups when the segregation into four clusters is done (the first two groups consisting of one preparation each), thus the serving form seems an important factor in analyzing antioxidant properties of food supplements and should be considered when choosing the best supplement.

The relatively weak correlations between different methods can be explained by different mechanisms underlying each method. Although ORAC and HORAC assays are both based on the HAT mechanism, in HORAC assay also the chelating properties of compounds such as polyphenols play an important role. Since the hydroxyl radicals are produced through a Fenton-like reaction, the metal complexation results in the prevention of their formation, not only their scavenging (14). Similarly, in the FRAP assay also the chelation of ferric/ferrous ions can influence the obtained results.

CONCLUSION

The determination of the antioxidant capacity of a series of dietary supplements available on the Polish market showed large diversity among the studied samples, confirming the need for devising control procedures for such preparations. However, the order of antioxidant activity of studied supplements depended on the method used. So, it should be stressed that it is important to run multiple antioxidant assays in order to get a better estimate of antioxidant capacity of dietary supplements, especially when comparing supplements with very diverse composition. It is also worth noting that when recommending a supplement with optimal antioxidant properties, the serving form should also be regarded.

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