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Antioxidant activity of the phenolic compounds of hawthorn, pine and skullcap

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Abstract

The significance of antioxidants in preventive medicine is well known. Increasing interest has been devoted to naturally occurring compounds – polyphenols – because of their beneficial health effects. The subject of this study was to examine the antioxidative activity of polyphenolic preparations containing oligomeric procyanidin from the bark of common pine (*Pinus sylvestris* L.) and hawthorn (*Crataegus oxyacantha* L.) and flavones of skullcap (*Scutellaria baicalensis* Georgi) roots. Multi-constituent mixtures were fractionated, and the antioxidative activity of fractions was tested *in vitro* with linoleic acid oxidation by AAPH-generated radicals. All preparations at 6 and 12 ppm concentrations exhibited protective activity, from 45% to 95% in relation to the control sample. The average activity of preparations was higher than those of their fractions used at the same concentrations, and it was similar to trolox and BHT activity.

Keywords: Natural antioxidants; Antioxidant activity; Procyanidins; Flavones

1. Introduction

Medicinal and spice plants, which are well known for their pharmacological activity, contain many substances that exhibit radical-scavenging properties. Among other substances included in this group are polyphenolic compounds, which are abundant in foods of plant origin. The application of such bioactive plant components may increase the stability of foods and, at the same time, improve their healthy properties associated with anti-cancer, anti-allergic, and anti-inflammatory activities of polyphenols in the human body (Moure et al., 2001; Rice-Evans, Miller, & Paganga, 1996).

Polyphenols are believed to possess the ideal chemical structure for scavenging free radicals. It has been demonstrated that *in vitro* they are more active than are vitamins E and C. The radical-scavenging activity of flavonoids is dependent on their structure and hydroxyl group arrangement. The highest radical scavenging activity is exhibited by compounds that have an *ortho* 3′,4′-dihydroxy structure at ring B (e.g. catechin, quercetin) or hydroxyl groups in position *meta*, e.g. 5,7-dihydroxy at ring A (e.g. kaempferol, apigenin), as well as those that have a double bond between the C2 and C3 and the C3-hydroxyl group at ring C. The activity is also influenced by the flavonoid particle glycosylation (Kondo et al., 2000; Lotito et al., 2000; Meyer, Heinonen, & Frankel, 1998; Moure et al., 2001; Rice-Evans et al., 1996; Rice-Evans, Miller, & Paganga, 1997).

Phenolic compounds may participate in radical-scavenging reactions as donors of electrons of hydroxyl groups to form stable radicals. They delocalise an unpaired electron by reacting with other antioxidants or by binding metals. The antioxidative effect also depends on both the operating environment and the concentration and composition of the antioxidant extract. Many researchers have also found the prooxidative activity of plant extracts at low or, conversely, at high concentrations. Antioxidative effects may be influenced by the temperature and duration of the experiment, as well as by the composition of the antioxidants applied (Frankel, Huang, Aeschbach, & Prior, 1996; Hotta et al.,

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2002; Kondo et al., 2000; Moure et al., 2001; Wanasundara & Shahidi, 1998; Yen & Chen, 1995).

Oligomeric procyanidins (OPCs) are found in many woody plants. The two most common sources of proanthocyanidins are grape seeds (*Vitis vinifera*) and white pine (*Pinus maritima, Pinus pinaster*). OPCs are also abundant in hawthorn (*Crataegus oxyacantha*), as well as in apples, berries, barley, bean hulls, cacao beans, rhubarb, rose hips and sorghum.

Hawthorn (*Crataegus oxyacantha* L.) is a traditional medicinal plant. Considered a "cardiotonic" herb, the hawthorn plant has been used in traditional medicine to treat irregular heartbeat, high blood pressure, chest pain, hardening of the arteries, and congestive heart failure; the antioxidants in hawthorn may help control high blood pressure and high cholesterol. The constituents responsible for the pharmacological effects of hawthorn preparations include flavonoids and oligomeric procyanidins (OPCs). These compounds have lipid-lowering, antioxidant and anti-inflammatory properties, which may protect against myocardial damage and arrhythmias. Procyanidins occur in *Crataegus*, and are primarily composed of (−)epicatechin (Svedstrom et al., 2002; Zhang et al., 2001).

Pine (*Pinus sylvestris* L.) tree bark is also valued medicinally for its rich content of proanthocyanidins. Pine bark extract has been used as a folk medicine and is used as a dietary supplement (Pycnogenol). It has also been shown to be a very powerful antioxidant and free radical-scavenger, even more powerful than either vitamin C or vitamin E. Pine bark extract is used in cardiovascular and heart formulas and has also been shown to have benefits for those with chronic venous insufficiency (Arcangeli, 2000; Petrassi, Mastromarino, & Spartera, 2000). Procyanidins occurring in pine bark consist mainly of the flavan-3-ol units of (+)catechin.

The third plant that we used was skullcap. The dried root of *Scutellaria baicalensis* Georgi is a very old, well-known drug used in traditional Chinese medicine, rich in flavone derivatives ($\sim$200 g kg^{-1}) (Tang & Eisenbrand, 1992).

Over the past years skullcap has been recognised as a mild relaxant that affects the nervous and muscular-skeletal systems. It has often been included in nerve tonics for treatment of mild depression, neurasthenia, hysteria, and nervous tension. It is also a traditional herb for withdrawal from alcohol or other drugs. Skullcap has an antioxidant activity. The main bioactive components of skullcap are baicalin, baicalein, wogonoside, and wogonin.

Searching for new, effective natural antioxidants is an important subject of research. Isolation and purification procedures of single substances are expensive and laborious. Plant extracts, which are considered not to be detrimental to human health and widely used in natural (traditional) medicine, could be utilized as food antioxidants (Amarowicz, Pegg, Kolodziejczyk, & Oszmiański, 2004; Gao, Huang, Yang, & Xu, 1999; Tang & Eisenbrand, 1992).

The purposes of this study were to compare the radical-scavenging activities of polyphenolic preparations containing different groups of polyphenols, from various materials with single fractions obtained from them, in model solutions and to elucidate, whether it is possible to use extracts consisting of compound mixtures instead of isolated substances.

2. Materials and methods

2.1. Plant material

The following materials were selected: hawthorn (*Crataegus oxyacantha*) and common pine (*Pinus sylvestris*), both rich in catechins and skullcap (*Scutellaria baicalensis* Georgi), containing flavones.

Testing materials included: (1) procyanidin preparations [derivatives of (−)epicatechin], obtained from the bark of hawthorn (*Crataegus oxyacantha*) by extraction from ground raw material with acetone solution and then, after its removal, by further extraction with ethyl acetate (Oszmiański, 1996); (2) procyanidin preparations [derivatives of (+) catechin], obtained (Oszmiański, 1996) from the bark of common pine (*Pinus sylvestris*); (3) flavone preparations obtained from the roots of skullcap (*Scutellaria baicalensis* Georgi) by methanol extraction (at 60 °C) from ground raw material (Amarowicz et al., 2004).

Concentrations of the phenolic compounds used in the study on the level of 3, 6 and 12 ppm (0.003; 0.006 and 0.012 g/kg) were rather smaller than doses recommended for antioxidant substances in food products, in most cases – 0.02–0.1 g/kg (Directive No 95/2/EC, 1995).

2.2. Chemicals

2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), linoleic acid, (+)catechin, and (−)epicatechin were obtained from Sigma (Poland, Poznań), baicalin and wogonin were obtained from Nacalai Tesque (Japan, Kyoto). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was obtained from Fluka (Poland, Poznań); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was from Aldrich (Poland, Poznań). Ammonium thiocyanate, polyoxyethylenesorbitan monolaurate (Tween-20), butylated hydroxytoluene (BHT), methanol, acetonitrile, formic acid and isopropanol were from Merck (Poland, Warsaw). All reagents were of the highest grade available.

Procyanidin and flavone standards were obtained by the method described earlier (Amarowicz et al., 2004; Oszmiański & Bourzeix, 1995).

2.3. Fractionation of polyphenolic preparations by LC

2.3.1. Isolation of procyanidins of hawthorn and pine

A weighed amount of procyanidin preparation was dissolved in methanol and placed in a column approximately 25 cm high and 5 cm in diameter, filled with fractogel

(TOYOPEARL HW-40S, TOSOH – Japan), and connected to an ISCO 229 UV/VIS (Lincoln NE, USA) detector. Elution was performed with methanol at a flow rate approximately 1 ml/min. Four fractions were obtained, which were evaporated to dryness under lowered pressure, and identified (Oszmiański & Bourzeix, 1995).

The determination of procyanidin content was performed by high performance liquid chromatography with a WatersTM 486 Tunable Absorbance Detector, equipped with a Waters 600 E Multisolwent C-R6A (Waters Associates, Milford, MA, USA,) reagent mixing system and computer data acquisition system, Star Chromatography Workstation (Varian).

Procyanidins were determined on the RP-18 column Chromolith RP-18e 100-4.6 (Merck, Darmstadt, Germany). As eluents 2.5% formic acid (Solution A), and 80% solution of acetonitrile in 2.5% formic acid (Reagent B) were used at a flow rate of 2 ml/ min. The elution profile was 0–10 min 0–10% B in A, 10–30 min 10–20% B in A, 30–35 min 20–40% B in A, 35–40 min 40–100% B, and 40–45 min 100–0% B. The analysis was performed by recording a chromatogram on the absorption detector at 280 nm wavelength.

One of the hawthorn fractions containing two procyanidins was additionally separated on a minicolumn, SEP-PAK C-18 (Waters Millipore USA). The powder was dissolved in water and injected into a cartridge (preconditioned by sequentially passing 10 ml methanol and distilled water). Then 5 ml of acetonitrile in water (15:85 v/v) were passed through the cartridge to elute the first fraction, and the next 5 ml of methanol to elute the second fraction; compounds were identified by comparing retention times with standards and, additionally, the composition of fractions was confirmed by the thiolysis method (Oszmiański & Bourzeix, 1995).

2.3.2. Isolation of skullcap flavones

A weighed amount of flavone preparation was dissolved in 25% methanol and placed in a column approximately 25 cm high and 5 cm in diameter, filled with Sephadex LH-20 and connected to an ISCO 229 UV/VIS (Lincoln NE, USA) detector. Elution was performed with 40% isopropanol solution in 1% acetic acid at a flow rate of approximately 1 ml/min. The solvents were evaporated under lowered pressure. Fractions were concentrated under vacuum to dryness and then identified. The identification of phenolic compounds was performed by the HPLC-DAD method, on the basis of retention times and standard spectra. A sample for analysis was prepared by dissolving a weighed amount of the product in methanol. It was then subjected to a 10 min ultrasonic treatment in an ultrasonic washer (UNITRA UM-4, Poland) for accurate dissolution. The content was centrifuged at 12,000 rpm for 10 min. With the methanol extract prepared in this way, an HPLC analysis of flavone compounds was performed. A Polymer Laboratories PLRP-S 100 Å (5 μm) column was used for the analysis. Eluents used were 80% solution of acetonitrile in 4.5% formic acid (Reagent A), and 4.5% formic acid (Reagent B), at a flow rate of 1 ml/ min. The elution profile was 0–7 min 0–85% B in A, 7–15 min 85–0% B in A, and 15–21 min 0–100% B. Recording was carried out at 280 nm.

2.4. Methods

2.4.1. AAPH assay

The experiment relies on linoleic acid oxidation by radicals generated by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), added to the solution. The peroxides formed from linoleic acid were determined by the thiocyanate method. The experiment was performed according to the methodology given by Foti, Piattelli, Baratta, and Ruberto (1996) and Haraguchi, Hashimoto, and Yagi (1992). Prior to performing the determination, the following reagents were prepared: 0.05 M Tris–HCl buffer, pH 7.4, containing 0.3% of Tween-20; 0.07 M AAPH solution, in buffer, 0.026 M linoleic acid solution in buffer, trolox, BHT or phenolic compounds (powder) dissolved in an amount of 1 mg in 1 ml of buffer.

Test samples were prepared by mixing 4 ml of linoleic acid solution with 0–0.05 ml solution of phenolic compounds or their mixtures and by supplementing the solution volume to 4.2 ml with Tris–HCl buffer. All samples were incubated at 50 °C for 10 min. Then 0.1 ml of AAPH was added, and the samples were vortexed and kept in a water bath at 50 °C with no light exposure for 45 min. In solutions thus prepared, polyphenol concentrations were 0–12 ppm (parts per million). At the beginning of each analysis and every 15 min thereafter, samples were mixed (VORTEX, Genie 2, Bochemia, N.Y. USA), and 0.1 ml portions were taken from each bottle and placed in three test tubes. The formed peroxides were determined by the thiocyanate method; absorbance was measured at 500 nm (Spectrophotometer Shimadzu UV–Vis, Model 2401 PC, Japan). The results were given as the percentage of inhibition (H) of the radical reaction by sample with the polyphenols (ΔAbs_p) in relation to the control sample, without polyphenols (ΔAbs_c).

$$H[\%] = 100 \times \left(1 - \frac{\Delta Abs_p}{\Delta Abs_c}\right)$$

2.4.2. Peroxide value determination by the thiocyanate method

The peroxide value was determined using the thiocyanate method of Haraguchi et al. (1992) by placing 100 μl of a sample into a test tube containing 9.7 ml of 75% ethanol. Then 0.1 ml of 30% ammonium thiocyanate solution was added, as well as 0.1 ml of $FeCl_3$ (20 mM in 3.5% HCl). The solution was mixed and, exactly after 3 min, the solution absorbance was measured at 500 nm in a spectrophotometer SHIMADZU UV-2401 PC (Japan).

2.5. Statistical anaysis

All experiments were carried out in duplicate, and analyses were carried out in triplicate.

The experimental results are expressed as means ± SE. The results were processed using STATISTICA v.5.1 The data were subjected to analysis of variance (ANOVA) and the significances of differences between sample means were calculated by Duncan's multiple range test. P values <0.05 were regarded as significant.

3. Results

3.1. Composition of polyphenolic preparations

The compositions of pine and hawthorn procyanidins are much simpler than those of other plants, for example grapes. Their composition makes them easier to fractionate.

Four main fractions were isolated from the bark of hawthorn preparation, which were then identified as (−)epicatechin (10.1%), procyanidin B_2 (24.2%), and procyanidins $B_4 + B_5$, which were further separated into B_4 (14.0%) and B_5, procyanidin C_1(16.1%) (Fig. 1).

The following compounds were isolated from the pine bark preparation and identified: (+)catechin (3.3%), procyanidin B_3(19.4%), and procyanidin C_2(16.1%).

Baicalin was the main constituent (98%) of the skullcap root preparations (Fig. 2). After the baicalin separation, the residue contained: baicalein (20.2%), wogonin (8.5%), and wogonoside (5.4%), all of which were isolated from the residue.The purity of fractions was above 90% (Amarowicz et al., 2004; Oszmiański & Bourzeix, 1995).

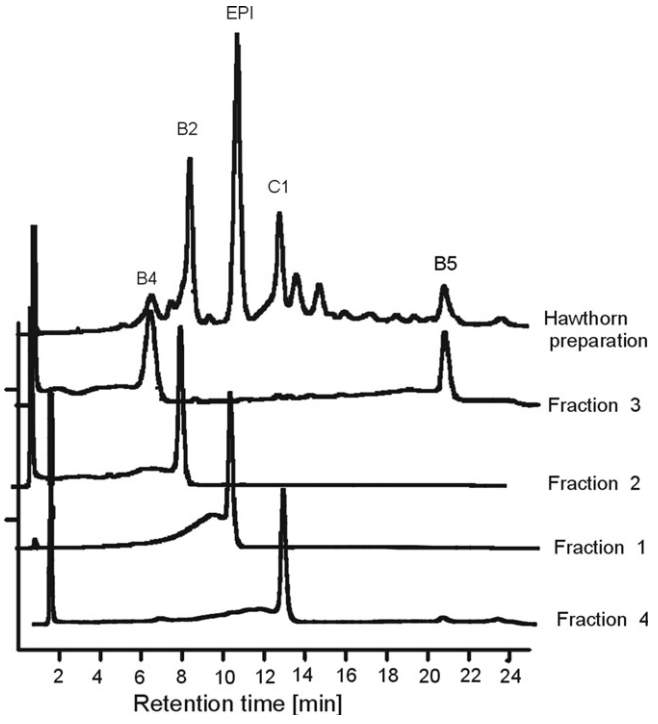

Fig. 1. HPLC chromatograms (280 nm) of hawthorn preparation and fractions Fr.1 – (−) epicatechin, Fr.2 – procyanidin B2, Fr.3 – procyanidins B4 and B5, Fr, 4 – procyanidin C1.

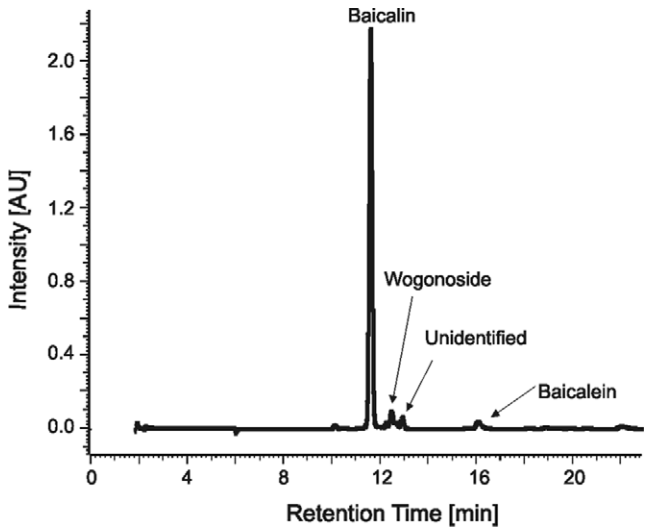

Fig. 2. Chromatogram HPLC (280 nm) of skullcap flavones preparation.

3.2. Activity of polyphenolic preparations

Preparations, added to the linoleic acid solution at 6 and 12 ppm concentrations, exhibited protective activity. In all samples containing polyphenols, linoleic acid was oxidized more slowly than it was in those samples with no preparation added. Antioxidative activity, expressed as a percentage inhibition of oxidation in relation to the control sample, was from 45% to 95% Fig. 3.

During the 45 min of the reaction, the average inhibition of the peroxide radical formation was more than 85%. The pine preparation at 12 ppm concentration exhibited signif-

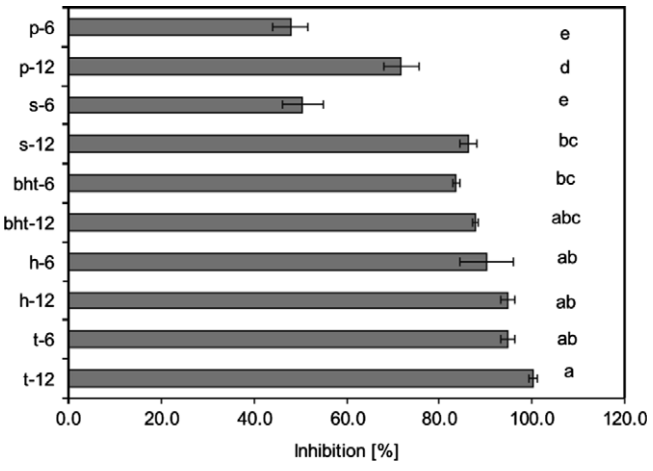

Fig. 3. Inhibition of linoleic acid oxidation [%] by BHT, Trolox (t) and phenolic preparations from pine (p), hawthron (h) and skullcap (s) applied at 12 ppm and 6 ppm. Results are means ± SE. Statistically homogeneous (P value $\leqslant 0.05$) groups are designated with the same letters.

icantly weaker activity than did hawthorn and skullcap preparations – approximately 70%. However, the average antioxidative effect in samples with skullcap or pine at 6 ppm concentration was at a level of 45%.

3.3. Antioxidative activity of pine fractions

The following three fractions were isolated from the bark of pine: monomer, (+)catechin, a dimer of catechin, procyanidin B_3, and a trimer, procyanidin C_2. Under the experimental conditions, the pine preparation exhibited weak antioxidative properties, despite the fact that it contained compounds of the flavan-3-ol group Fig. 4. The activities of procyanidin B_3 and C_2 fractions were statistically different and nearly twice as great as that of the preparation applied in an analogical concentration.

The activity of samples decreased during the course of the experiment; the decrease was slightly more in solutions with the lower antioxidant concentration.

3.4. Antioxidative activity of hawthorn fractions

Fig. 5 shows the comparison of average linoleic acid oxidation inhibition by polyphenols present in model solutions. The solutions contained the hawthorn preparation or fractions obtained from that preparation by the LC method. The following five fractions were isolated from the bark of hawthorn: a monomer, (−)epicatechin, dimers of (−)epicatechin, procyanidins B2, B4, B5 and a trimer, procyanidin C1.

When applied individually, the fractions of hawthorn polyphenols exhibited similar or weaker protective effects on linoleic acid than did the preparation of the compound mixture. It was only in the solution containing hawthorn (−)epicatechin, applied at 3 ppm concentration, that linoleic acid oxidation was determined to be greater than in the solution containing the 3 ppm hawthorn preparation. During the 45-min incubation period of model solutions containing 6 ppm of polyphenols, the decrease in activity

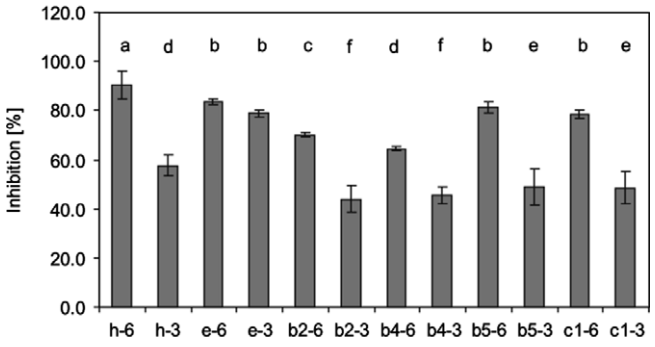

Fig. 5. Average inhibition [%] of linoleic acid oxidation by hawthorn bark preparation (h) and its fractions: (−)epicatechin (e), procyanidin B2 (b2), procyanidin B4 (b4), procyanidin B5 (b5), and procyanidin Cl (cl), applied at 3 and 6 ppm concentrations. Results are means ± SE. Statistically homogeneous groups (P value $\leqslant 0.05$) are designated with the same letters.

was low and ranged between 2, 4 and 6% (for hawthorn (−)epicatechin, procyanidins B_4 and B_2 respectively) to 13–17% (for C_1 and B_5). In solutions with lower antioxidant concentrations (3 ppm) the decrease in activity was much larger – from 10 to 50% (for hawthorn (−)epicatechin and procyanidin B_5, respectively).

3.5. Antioxidative activity of skullcap fractions

The following four fractions from the roots of skullcap were examined: flavones; baicalein and wogonin, and glucuronide; baicalin and glucoside; wogonoside. When applied individually, the fractions of skullcap polyphenols exhibited similar (baicalin was not significantly different) or weaker (statistically different, $P > 0.05$) protective effects on linoleic acid than did the mixture of compounds. Faster oxidation, particularly at an early part of the period, was observed in the solution containing skullcap preparation at 3 ppm concentration. That phenomenon was not observed in other samples. As expected, the major part of the flavone preparation activity came from baicalin, the main constituent of the preparation. The other constituents even reduced the activity, particularly at lower concentra-

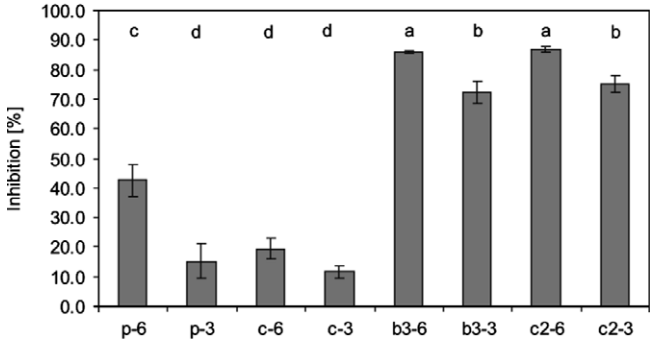

Fig. 4. Average inhibition [%] of linoleic acid oxidation by pind bark preparation (p) and its isolated fractions: (+)catechin (c), procyanidin B3 (b3) and procyanidin C2 (c2) applied at 3 and 6 ppm concentrations. Results are means ± SE. Statistically homogeneous groups (P value $\leqslant 0.05$) are designated with the same letters.

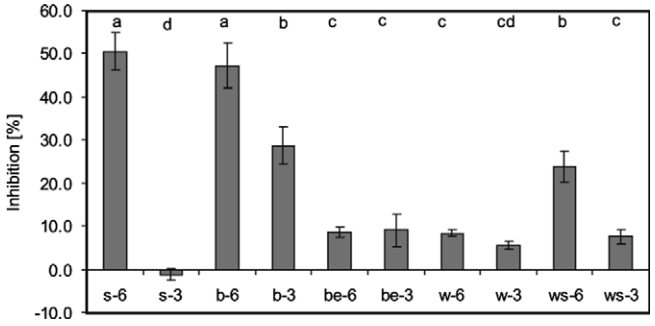

Fig. 6. Average inhibition [%] of linoleic acid oxidation by phenolic compounds from roots of skullcap (s): baicalin (b), baicalein (be), wogonin (w) and woggonoside (ws), used at 3 and 6 ppm concentrations. Results are means ± SE. Statitically homogeneous groups (P value $\leqslant 0.05$) are designated with the same letters.

tions. When used individually, each constituent did not show any notable activity. The effectiveness of flavones of skullcap was lower at lower concentrations; in particular the skullcap preparation used at 3 ppm concentration (Fig. 6) accelerated the oxidation process. Furthermore, the antioxidative effect was much weaker than that in solutions containing hawthorn procyanidins.

4. Discussion

We have demonstrated that preparations containing different phenolic compounds showed significant antioxidant activity. It was found that the average antioxidative activity of hawthorn and skullcap preparations containing compound mixtures was higher than the activity of individual constituents of those mixtures at those same concentrations. For example, the hawthorn procyanidin preparation applied at 6 ppm concentration inhibited linoleic acid oxidation by on average, 90%. However, (−)epicatechin, isolated from that preparation and used at the same concentration, exhibited approximately 84% activity, and the antioxidative effect of procyanidin B2, also applied at 6 ppm concentration, was approximately 70%. Similar results were observed in the preparation of skullcap flavones.

During the experiment, the effectiveness of preparations decreased by 3–40% (data not shown); however, a few percentage points of increase in the antioxidative effect were observed in some samples (hawthorn at 6 and 12 ppm, skullcap at 12 ppm). According to Kondo et al. (2000), during radical reactions of procyanidin B_2 in an environment containing AAPH or DPPH, through the abstraction of hydrogen at carbon C-2 and then through the quinone structure, procyanidins A_2 may be formed, which also exhibit radical- scavenging activity. This may explain the stability of activity or its increase in the course of the experiment. In the studies by Frankel et al. (1996) and Salah et al. (1995), it has been demonstrated that the stability of radical-scavenging activity depends on the antioxidant composition.

(Ariga, Koshiyama, & Fukushima, 1988) and (Ariga & Hamano, 1990), in their studies on the activity of procyanidins B_1 and B_2, demonstrated, similarly to our studies, that the activity of dimers was higher than that of the monomer. Guo, Zhao, and Packer (1999) have reported that, among the Pine bark procyanidins, only the radical formed from procyanidin B_3 is relatively stable and it may contribute to most of the radical-scavenging activity. They explain this by the formation of the intramolecular hydrogen bond between O^* in position 4′ of the upper molecule and the OH group of second ring B, which causes the delocalisation of electron spin density in the π orbital of ring B, containing unpaired electrons, and stabilization of the structure. They explain this, in the first place, by the presence of a great number of hydroxyl groups in the molecule, and additionally by the impact of these groups on solubility in the water environment – procyanidin B_3 dissolves better in water than does catechin, which makes it an effective hydrogen donor.

Foti et al. (1996) have explained that the differences in activity among and within various classes of polyphenols result from their chemical structure and individual ability to transfer a hydrogen atom to a radical, as well as from the system in which an experiment is carried out (Foti et al, 1996; Wang & Helliwell, 2000). A similar phenomenon has been observed by many researchers. The phenomenon of synergism or antagonism is explained by the differences in paths of activity of single compounds. There is no single compound able to react with all kinds of radicals, or which could optimally act on lipid oxidation products; hence, the mixtures of antioxidants acting on various radicals and oxidation products may be more efficient than are individual compounds (Gao et al., 1999; Hanasaki, Ogawa, & Fukui, 1994; Saucier & Waterhouse, 1999). Researchers in many studies (Rice-Evans et al., 1997; Frankel et al., 1996; Salah et al., 1995) have demonstrated synergism, among extract components of tea and wine, in antioxidative activity. As in this study, it has been shown that the antioxidant mixture found in an extract is more active than are individual constituents or mixtures of main constituents mixed in specific proportions.

The protective effect of procyanidins and catechins against AAPH-induced lipid oxidation showed a dependence on the types of molecules in the mixture and on different mechanisms of radical formation. The mechanism of antioxidant action may involve direct inhibition in generating reactive oxygen species, or in the scavenging of free radicals. It is probable, that procyanidin extracts contained not only monomers, dimers and trimers, which were isolated from them, but also tetramers, pentamers and hexamers which express higher antioxidant activity.

A greater efficiency of mixtures may explain the existence of an intermolecular synergism of antioxidant active structural elements present in the mixture or some regeneration mechanism of radicals to restore the parent molecule.

At the same time, it should be noted that, in the case of polyphenol mixtures in a solution, molecules may be rearranged and their structure and cross-orientation changed, leading to a decrease of such activity in the mixture, as was observed in the pine fractions and preparation.

5. Conclusions

The best effects were observed in the samples containing procyanidins of hawthorn at 6 and 12 ppm concentrations – they did not statistically differ from the antioxidative activity of trolox and BHT. The significantly worst results were observed in the samples containing 6 ppm of flavones of skullcap or procyanidins of pine.

Average antioxidative activities of two preparations were higher than the activity of individual constituents of those mixtures. To sum up, the separation of polyphenolic extracts into individual compounds does not seem to be beneficial in respect of their antioxidative activity.

Screening of antioxidant activity, using simple assays in order to predict positive or negative effects of extracts in food, has not yet been sufficiently examined.

Further studies of the activity of extracts, as well as on isolated compounds, are necessary, e.g. (1) evaluation of possible synergism, (2) definition of possible use of plant extracts as the antioxidant substances in food, (3) establishment of standard methods for active compounds in extracts.

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