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Total phenol content and antioxidant activity of water solutions of plant extracts

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Summary

Water solutions of extracts were investigated for total phenol content, flavonoid content and antioxidant activity. Susceptibility to degradation of water solutions of plant extracts, under light and in the dark, during storage at room temperature was investigated in order to determine their stability prior to their application for fortification of food products. Large dispersion of total phenol (TP) content in the investigated model solutions of selected extracts (olive leaves, green tea, red grape, red wine, pine bark PE 5:1, pine bark PE 95 %, resveratrol), ranging from 11.10 mg GAE/100 mL to 92.19 mg GAE/100 mL was observed. Consequently, large dispersion of total flavonoids (TF) content (8.89 mg to 61.75 mg CTE/100 mL) was also observed. Since phenols have been mostly responsible for antioxidant activity of extracts, in most cases, antioxidant activity followed the TP content. That was proven by estimation of correlation coefficient between the total phenol content and antioxidant activity. Correlation coefficients between investigated parameters ranged from 0.5749 to 0.9604. During storage of 5 weeks at room temperature loss of phenols and flavonoids occurred. Antioxidant activity decreased with the decrease of TP and TF content. Degradations of phenols and flavonoids were more pronounced in samples stored at light.

Keywords: polyphenols, flavonoids, extracts solutions, antioxidant activity, storage

Introduction

Generally, today there is an upsurge of interest in phytochemicals as new sources of natural antioxidants in order to use them in foods and pharmaceutical preparations to replace synthetic antioxidants, which are being restricted due to their potential health risks and toxicity (Cai et al., 2004; Katalinić et al., 2006; Wong et al., 2006). A nutraceutical is any substance that is a food, or part of a food, and provides medical or health benefits, including the prevention or treatment of disease. Nutraceuticals may be isolated nutrients, dietary supplements, specific diets, designer foods, herbal products, processed foods, or processed beverages (Morris, 2003). Phenolic compounds, which are present naturally in vegetables, fruits and grains exhibit wide range of physiological properties and possess the ability to reduce oxidative damage associated with many diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, asthma, hepatitis, liver injury, arthritis, immune deficiency diseases and ageing (Pietta et al., 1998; Lee et al., 2000; Middleton et al., 2000). The oxidative stress, defined as ''the imbalance between oxidants and antioxidants in favour of the oxidants potentially leading to damage'', has been suggested to be the cause of aging and various disease in humans. In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept maintaining a healthy biological system (Davies, 2000; Tiwari, 2001, Katalinić et al., 2006). Phenolic compounds are ubiquitous in plants, and when plant foods are consumed, these phytochemicals contribute to the intake of natural antioxidants in the human diets (Balasundram, 2006). Next to high antioxidant activity, it was well documented (Asen et al. 1972; Wilska-Jeszka and Korzuchowska, 1996; Baranac et al., 1996; Baranac et al., 1997a; Baranac et al., 1997b; Baranac et al., 1997c; Dimitrić-Marković et al., 2000; Boulton, 2001; Bąkowska et al., 2003; Rein and Heinonen, 2004; Mollov et al., 2007; Awika, 2008) that phenolic compounds interact with anthocyanins and through co-pigmentation effect they can influence anthocyanins stability after addition and/or during storage. Thus, the addition of phenolic compounds can be a valuable tool for improvement of nutritive value of food products.

Since it is possible to fortify food products with addition of extracts rich in phenols, in this work stability of phenols, flavonoids and antioxidant activity of water solutions of selected extracts during storage at room temperature under light and in the dark were investigated. Also, correlation between total phenol content and antioxidant activity was evaluated.

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Materials and methods

Material

Crude extracts (olive leaves, green tea, red grape, red wine, pine bark PE 5:1, pine bark PE 95 %, resveratrol) were obtained from Naturex (France). 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2'azinobis-(3-ethylbenzthiazoline-6-sulphonicacid (ABTS) and 2,2-diphenyl-1-picrilhydrazyl (DPPH) were obtained from Fluka (Germany), gallic acid and catechin from Sigma (Germany), and all other reagents from Kemika (Croatia).

Sample preparation

0.1 % water solutions of selected crude extracts were prepared. Samples were stored at room temperature exposed to light, and in the dark, during 5 weeks. The total phenol content, flavonoid content and antioxidant activity were determined after preparation of solutions and during storage after 1, 2, 3, 4 and 5 weeks.

Estimation of total phenol content

The Folin-Ciocalteu method (Ough and Amerine, 1988) was used to determine the total phenol content. Gallic acid was used as a standard to produce the calibration curve. Total phenol content was expressed in mg of gallic acid equivalents (GAE)/100 mL of sample.

Estimation of total flavonoid content

The total flavonoid content was determined according to Makris et al. (2007). The total flavonoid content was calculated from a calibration curve using catechin as a standard, and expressed as mg catechin equivalents (CTE)/100 mL of sample.

The ferric reducing power (FRAP) assay

FRAP assay was done according to Benzie and Strain (1996). The results ware expressed in mg gallic acid equivalents (GAE)/100 mL of sample. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

The free radical scavenging ability by the use of a stable ABTS radical (ABTS) assay

The ABTS assay followed the method of Arnao et al. (2001) with some modifications. The results were expressed in mg gallic acid equivalents (GAE)/100 mL of sample. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

The free radical scavenging ability by the use of a stable DPPH radical (DPPH) assay

0.2 mL of the sample was diluted with methanol and 1 mL of DPPH solution (0.5mM) was added. After 15 minutes absorbance was read at 517 nm. The results were expressed in mg gallic acid equivalents (GAE)/100 mL of sample. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

Statistical analysis

Antioxidant activity, total phenol content and total flavonoid content, during storage, were analyzed by the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P < 0.05$. All statistical analyses were carried out using the software program STATISTICA 8 (StatSoft, Inc, USA). The results were expressed as means ± standard deviation.

Results and Discussion

All of the selected extracts are rich in polyphenols thus they would be appropriate for fortification of foods. For better understanding of their application it is necessary to determine their stability during storage as well as their stability in different conditions, like exposure to light and darkness.

Total phenol content

In most investigated cases, up to now, different plants (Hou et al., 2003; Wong et al., 2006; Balasundram et al., 2006; Katalinić et al., 2006; Al-Fatimi et al., 2007; Tawaha et al., 2007; Chang et al., 2007; Singh et al., 2009) were investigated for their phenolic content and antioxidant activity, thus extraction of phenolic compounds should be made before analyses. In those cases, practical aspects that need to be considered include extraction efficiency, availability of sufficient raw material, and toxicity or safety considerations (Balasundram, 2006). In our study we investigated crude extracts, avoiding laboratory extraction procedure which could also lead to extraction of other compounds that are contained in the plants thus we presume that extraction of other compounds were minimal. The total phenol (TP)

content was determined by Folin-Ciocalteu method. The Folin-Ciocalteu method gives a crude estimation of the total phenolic compounds present in a sample. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations (Prior et al., 2005). Since we studied purified extracts it can be assumed that the estimated TP content was real without influence of other compounds that can react with the Folin-Ciocalteu reagent.

As shown in Table 1. there was a large dispersion of the TP content in the investigated model solutions of selected extracts, ranging from 11.10 mg/100 g to 92.19 mg/100 g. The highest TP content, after preparation of 0.1 % water solution of extracts, showed the red grape extract and pine bark PE 95 % (92.19 and 72.33 mg/100 mL, respectively), while the lowest TP content was in olive leaves and pine bark PE 5:1 extracts (both 11.1 mg/100 mL).

Table 1. Total phenol (TP) content (mg GAE/100 mL) of 0.1 % water solution of extracts during 5 weeks of storage under light and in the dark at room temperature

Extract	0	1 week	2 weeks	3 weeks	4 weeks	5 weeks
	Light					
olive leaves	11.10^{a}	9.39^{b}	8.57^{c}	6.87^{d}	6.31^{d}	5.34^{e}
green tea	31.95^{a}	29.43^{b}	27.62^{c}	23.13^{d}	$22.69^{d,e}$	21.68^{e}
red grape	92.19^{a}	85.85^{b}	82.22^{c}	80.51^{d}	71.95^{e}	67.71^{f}
red wine	41.02^{a}	36.89^{b}	35.58^{b}	29.84^{c}	27.12^{d}	25.81^{d}
pine bark PE 5:1	11.10^{a}	8.78^{b}	$8.22^{b,c}$	8.09^{c}	7.97^{c}	7.16^{d}
pine bark PE 95%	72.33^{a}	68.22^{b}	66.40^{c}	64.39^{d}	62.88^{e}	56.33^{f}
resveratrol	21.98^{a}	21.28^{a}	18.76^{b}	18.35^{b}	18.05^{b}	14.32^{c}
	Dark					
olive leaves	11.10^{a}	10.60^{b}	8.77^{c}	7.16^{d}	6.71^{d}	5.90^{e}
green tea	31.95^{a}	30.34^{b}	28.13^{c}	26.81^{d}	25.00^{e}	23.69^{f}
red grape	92.19^{a}	85.95^{b}	82.92^{c}	80.00^{d}	72.05^{e}	68.11^{f}
red wine	41.02^{a}	38.80^{b}	37.70^{b}	35.68^{c}	34.77^{c}	31.15^{d}
pine bark PE 5:1	11.10^{a}	9.69^{b}	9.48^{b}	9.28^{b}	8.37^{c}	7.57^{d}
pine bark PE 95%	72.33^{a}	71.14^{b}	$70.43^{b,c}$	65.53^{c}	63.08^{d}	58.44^{e}
resveratrol	21.98^{a}	21.58^{a}	19.46^{b}	$18.90^{b,c}$	18.56^{c}	14.93^{d}

Values in the same row with different superscripts (a-f) are significantly different ($P < 0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

During storage the TP content decreased in all samples regardless of exposure to light or darkness. After 5 weeks of storage the highest TP content had red grape and pine bark PE 95 % and the lowest had olive leaves and pine bark PE 5:1. The samples stored in the dark had a higher TP content than samples stored under light. The difference in the TP content in the samples stored under light in comparison to the samples stored in the dark in some cases is not so high. Olive leaves, pine bark PE 5:1, red grape and resveratrol had insignificant difference in the TP content stored under light and in the dark, while all other samples had a higher difference. It can be concluded that those extracts (olive leaves, pine bark PE 5:1, red grape and resveratrol) were stable under light and in the dark, and that they were more stable than other extracts under light.

From the TP retention calculation (Table 6), after 5 weeks of storage (Table 2), it can be seen that the samples stored in the dark had higher retention of TP content. The highest retention of TP content had pine bark regardless of storage conditions (under light, 77.88 % and in the dark, 80.80 %). The lowest retention had olive leaves extract (48.11 % and 53.15 %).

Table 2. Total flavonoid (TF) content (mg CTE/100 mL) of 0.1 % water solution of extracts during 5 weeks of storage under light and in the dark at room temperature

Extract	0	1 week	2 weeks	3 weeks	4 weeks	5 weeks
	Light					
olive leaves	8.89^{a}	6.26^{b}	$6.02^{b,c}$	$5.52^{c,d}$	$4.95^{d,e}$	2.98^{f}
green tea	13.15^{a}	12.33^{b}	10.28^{c}	9.05^{d}	7.98^{e}	5.85^{f}
red grape	61.75^{a}	58.89^{b}	53.97^{c}	47.49^{d}	$46.34^{d,e}$	45.20^{e}
red wine	27.82^{a}	24.30^{b}	23.64^{b}	22.25^{c}	21.51^{c}	19.79^{d}
pine bark PE 5:1	9.30^{a}	7.57^{b}	6.43^{c}	6.26^{c}	6.02^{c}	4.95^{d}
pine bark PE 95%	48.72^{a}	$47.90^{a,b}$	46.43^{b}	42.41^{c}	$41.43^{c,d}$	39.87^{d}
resveratrol	20.20^{a}	19.95^{a}	18.64^{b}	18.31^{b}	17.49^{c}	13.88^{d}
	Dark					
olive leaves	8.89^{a}	7.90^{b}	7.32^{c}	6.26^{d}	5.85^{d}	4.62^{e}
green tea	13.15^{a}	13.07^{a}	12.49^{a}	9.38^{b}	8.72^{b}	6.10^{c}
red grape	61.75^{a}	60.28^{b}	57.08^{b}	51.59^{d}	51.18^{d}	46.84^{e}
red wine	27.82^{a}	27.16^{a}	25.61^{b}	24.62^{b}	21.75^{c}	19.95^{d}
pine bark PE 5:1	9.30^{a}	$8.64^{a,b}$	$8.15^{b,c}$	$7.82^{c,d}$	7.16^{d}	6.18^{e}
pine bark PE 95%	48.72^{a}	47.98^{a}	45.03^{b}c	43.72^{c}	43.15^{c}	40.52^{d}
resveratrol	20.20^{a}	19.87^{a}	18.15^{b}	17.57^{b}	17.16^{b}	13.72^{c}

Values in the same row with different superscripts (a-f) are significantly different ($P < 0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

Flavonoid content

Results of the TF content followed the TP content result, which means that when the TP content was higher the TF content was also higher (Table 2). The TF content of investigated water solutions of extracts was lower (in higher or lesser extent) than the TP content. The TF content of olive leaves, pine bark (5:1) and resveratrol was little lower than the TP content, while for all other samples these difference were more pronounced. The TF content ranged from 8.89 mg CTE/100 mL for olive leaves up to 61.75 mg CTE/100 mL for red grape extract solution. During storage, degradation of flavonoids occurred regardless of the storage conditions. As it was the case with the TP content, higher retention of the TF content after storage of 5 weeks had samples stored in the dark with an exception of resveratrol. Also, the highest retention of the TF content (Table 6) had pine bark PE 95 % regardless of the storage conditions (81.83 % and 83.17 %) and the lowest retention had olive leaves (33.52 % and 51.97 %) and green tea (44.49 % and 46.39 %).

Antioxidant activity

Polyphenols have been reported to be responsible for the antioxidant activities of botanical extracts (Wong et al., 2006). The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (Afanas'ev et al., 1989; Amarowicz et al., 2004). It is important to select and employ a stable and rapid method to assay antioxidant activity, because the determination of hundreds of samples is time-consuming. Several methods have been developed to assay free radical scavenging capacity and the total antioxidant activity of plant extracts (Cai et al., 2004). In our study, determination of antioxidant activity was conducted through the ferric reducing power (FRAP) assay, the free radical scavenging ability by the use of a stable ABTS radical (ABTS) assay, and the free radical scavenging ability by the use of a stable DPPH radical (DPPH) assay. The results of antioxidant activity are presented in Tables 3, 4 and 5.

Table 3. Antioxidant activity determined by FRAP assay (mg GAE/100 mL) of 0.1 % water solution of extracts during 5 weeks of storage under light and in the dark at room temperature

Extract	0	1 week	2 weeks	3 weeks	4 weeks	5 weeks
	Light					
olive leaves	2.94^{a}	1.97^{b}	1.90^{b}	1.46^{c}	1.42^{c}	1.05^{d}
green tea	14.54^{a}	13.03^{b}	12.4^{b}	9.88^{c}	9.81^{c}	8.99^{d}
red grape	28.55^{a}	28.21^{a}	25.45^{b}	19.78^{c}	19.18^{c}	16.00^{d}
red wine	13.47^{a}	11.74^{b}	11.67^{b}	8.86^{c}	8.11^{d}	6.77^{e}
pine bark PE 5:1	4.38^{a}	2.93^{b}	2.40^{b}	1.86^{d}	1.80^{d}	0.98^{e}
pine bark PE 95%	20.89^{a}	18.69^{b}	17.89^{c}	16.40^{d}	16.24^{d}	13.61^{e}
resveratrol	7.84^{a}	7.56^{a}	7.43^{a}	6.45^{b}	6.39^{b}	5.39^{c}
	Dark					
olive leaves	2.94^{a}	2.23^{b}	2.03^{b}	1.64^{c}	$1.52^{c,d}$	1.21^{d}
green tea	14.54^{a}	13.13^{b}	12.55^{b}	9.88^{c}	9.84^{c}	9.39^{c}
red grape	28.55^{a}	28.46^{a}	25.60^{b}	20.01^{c}	19.31^{c}	17.10^{d}
red wine	13.47^{a}	12.73^{b}	11.84^{c}	8.66^{d}	8.41^{d}	7.14^{e}
pine bark PE 5:1	4.38^{a}	3.35^{b}	2.33^{c}	1.87^{d}	1.82^{d}	1.02^{e}
pine bark PE 95%	20.89^{a}	19.43^{b}	17.48^{c}	16.29^{d}	16.25^{d}	13.86^{e}
resveratrol	7.84^{a}	$7.66^{a,b}$	$7.55^{a,b}$	$6.76^{b,c}$	$6.43^{c,d}$	5.57^{d}

Values in the same row with different superscripts (a-e) are significantly different ($P < 0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

The strongest antioxidant activity, which were evaluated by FRAP and ABTS assay, had red grape > pine bark PE 95 % > green tea > red wine > resveratrol > pine bark PE 5:1> olive leaves. DPPH assay showed different tendency, which means red grape > pine bark PE 95 % > red wine > green tea > resveratrol > pine bark PE 5:1> olive leaves. The results of DPPH assay were higher, except for red grape, in comparison to results obtained by the ABTS and FRAP method. While the ABTS and FRAP results showed that green tea and red wine (14.54 and 13.47 FRAP, 15.69 and 10.22 ABTS) had much lower values than red grape (28.55 FRAP and 37.26 ABTS), the DPPH results showed that the values were similar, 22.34, 25.76 and 22.56 for green tea, red grape and red wine, respectively. Those results suggest that different phenolic compounds react differently depending on the applied reagent for antioxidant determination. The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerised compounds (Bravo, 1998).

Table 4. Antioxidant activity determined by ABTS assay (mg GAE/100 mL) of 0.1 % water solution of extracts during 5 weeks of storage under light and in the dark at room temperature

Extract	0	1 week	2 weeks	3 weeks	4 weeks	5 weeks
	Light					
olive leaves	2.41^{a}	2.19^{a}	1.79^{b}	1.68^{b}	1.19^{c}	1.02^{d}
green tea	15.69^{a}	14.65^{b}	12.85^{c}	12.41^{c}	$12.03^{c,d}$	11.92^{d}
red grape	37.26^{a}	35.08^{b}	30.86^{c}	23.24^{d}	18.28^{e}	17.74^{e}
red wine	10.22^{a}	8.57^{b}	8.08^{b}	7.26^{c}	6.37^{d}	5.78^{e}
pine bark PE 5:1	2.45^{a}	2.26^{a}	2.05^{b}	$2.04^{b,c}$	$1.97^{b,c}$	1.78^{c}
pine bark PE 95%	21.93^{a}	19.95^{b}	19.58^{b}	19.25^{b}	16.78^{c}	14.75^{d}
resveratrol	8.17^{a}	6.96^{b}	6.33^{c}	5.96^{c}	5.38^{d}	4.77^{e}
	Dark					
olive leaves	2.41^{a}	2.33^{a}	1.80^{b}	1.70^{b}	1.32^{c}	1.09^{d}
green tea	15.69^{a}	14.81^{b}	13.95^{c}	13.51^{c}	12.94^{d}	11.97^{e}
red grape	37.26^{a}	35.71^{b}	32.42^{c}	24.87^{d}	18.33^{e}	18.02^{e}
red wine	10.22^{a}	10.08^{a}	9.97^{a}	8.30^{b}	6.62^{c}	5.98^{c}
pine bark PE 5:1	2.45^{a}	$2.34^{a,b}$	$2.15^{a,b}$	$2.13^{b,c}$	$1.82^{c,d}$	1.72^{d}
pine bark PE 95%	21.93^{a}	20.38^{b}	19.92^{b}	19.72^{b}	17.57^{c}	15.26^{d}
resveratrol	8.17^{a}	7.20^{b}	$6.70^{b,c}$	$6.13^{c,d}$	$5.53^{d,e}$	4.89^{e}

Values in the same row with different superscripts (a-e) are significantly different ($P < 0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

Table 5. Antioxidant activity determined by DPPH assay (mg GAE/100 mL) of 0.1 % water solution of extracts during 5 weeks of storage under light and in the dark at room temperature

Extract	0	1 week	2 weeks	3 weeks	4 weeks	5 weeks
	Light					
olive leaves	5.09^{a}	4.29^{b}	3.94^{b}	3.84^{b}	3.04^{c}	2.45^{d}
green tea	22.34^{a}	21.43^{b}	19.30^{c}	18.55^{d}	17.58^{e}	16.93^{e}
red grape	25.76^{a}	23.04^{b}	22.52^{b}	22.36^{b}	21.06^{c}	20.70^{c}
red wine	22.56^{a}	20.21^{b}	19.30^{b}	18.78^{d}	18.55^{d}	17.55^{e}
pine bark PE 5:1	5.81^{a}	5.05^{b}	4.92^{b}	4.13^{c}	3.81^{d}	2.77^{e}
pine bark PE 95%	24.65^{a}	$23.11^{a,b}$	$22.59^{b,c}$	$22.04^{b,c}$	$21.22^{c,d}$	19.60^{d}
resveratrol	15.34^{a}	14.71^{a}	13.51^{b}	13.28^{b}	$12.57^{b,c}$	11.82^{c}
	Dark					
olive leaves	5.09^{a}	4.78^{b}	4.21^{c}	4.11^{c}	3.29^{d}	2.99^{e}
green tea	22.34^{a}	21.89^{a}	20.09^{b}	19.34^{b}	18.13^{c}	17.61^{c}
red grape	25.76^{a}	24.53^{b}	23.40^{b}	22.43^{d}	$21.78^{d,e}$	21.58^{e}
red wine	22.56^{a}	21.86^{b}	20.51^{c}	19.27^{d}	18.85^{e}	17.68^{f}
pine bark PE 5:1	5.81^{a}	$5.61^{a,b}$	5.41^{b}	4.39^{c}	3.92^{d}	3.10^{e}
pine bark PE 95%	24.65^{a}	$23.97^{a,b}$	$23.18^{b,c}$	22.79^{c}	21.55^{d}	20.70^{d}
resveratrol	15.34^{a}	14.98^{b}	13.74^{b}	$13.51^{c,d}$	12.76^{d}	11.91^{e}

Values in the same row with different superscripts (a-f) are significantly different ($P < 0.05$) by analysis of variance (ANOVA) and Fisher's least significant difference (LSD).

Wong et al. (2006) reported that results obtained by the FRAP method (expressed as trolox equivalents) were higher than results obtained by the DPPH method of extract of selected plants, while Gil et al. (2002) obtained reverse results for stone fruit. They obtained higher antioxidant activity with the DPPH method (expressed as ascorbic acid equivalents) than with the FRAP method. According to Wong et al (2006), the probable reason for the lower DPPH values of the plants could be the presence of compounds that are not reactive towards DPPH. Antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently due to steric hindrance (Wong et al., 2006).

Table 6. Retention (%) of total phenol (TP) content and total flavonoid (TF) content after 5 weeks of storage

Extracts	TP content retention		TF content retention	
	light	dark	light	dark
olive leaves	48.11	53.15	33.52	51.97
green tea	67.86	74.15	44.49	46.39
red grape	73.45	73.88	73.20	75.85
red wine	62.92	75.94	71.14	71.71
pine bark PE 5:1	64.50	68.20	53.23	66.45
pine bark PE 95%	77.88	80.80	81.83	83.17
resveratrol	65.15	67.93	68.71	67.92

Regardless of the used method for antioxidant activity determination, decrease of antioxidant activity occurred during storage. As it was case with the TP content and TF content, decrease of antioxidant activity was also more pronounced in samples stored under light.

Correlation between the TP content with the TF content and antioxidant activity, as well as the TF content and antioxidant activity was also evaluated. Generally, it is very complicated to correlate the data on antioxidant activity of natural products in various works and measured by various methods (Roginsky and Lissi, 2004). The antioxidant capacities of phenols determined by a different method, only poorly correlate with each other (Roginsky and Lissi, 2004). From the results presented in Table 7. it can be seen that correlation coefficients ranged from 0.5749 to 0.9604 when all samples were considered for correlation evaluation, but when the results of green tea extract were eliminated, correlation coefficient was generally higher, 0.6756 to 0.9940. Roginsky and Lissi (2004) pointing out that the capacity of some tea catechins, epigallatocatechin and epigallatocatechin gallate, changed significantly when they reacted with DPPH and $ABTS^{\cdot+}$, on the one hand, and peroxyl radical, on the other hand. Since we assumed that in our study we evaluated only the TP content, lower correlation coefficients, when results of green tea extracts were taken into correlation evaluation, could be explained with the fact that various phenolic compounds respond differently in this assay, depending on the number of phenolic groups they have (Singleton and Rossi, 1965). Lower correlation coefficients were only between the TP content and DPPH, and the TF content and DPPH, thus our results suggested that the phenolic compounds mostly contributed to the antioxidant activity of the investigated extracts.

Table 7. Correlation between investigated parameters

all samples	TP content	TF content	FRAP	ABTS	DPPH
TP content	1	0.9604	0.9604	0.9216	0.6885
TF content		1	0.8646	0.8394	0.5749
FRAP			1	0.9605	0.7906
ABTS				1	0.6550
DPPH					1
without green tea	TP content	TF content	FRAP	ABTS	DPPH
TP content	1	0.9940	0.9924	0.9515	0.7980
TF content		1	0.9912	0.9608	0.8117
FRAP			1	0.9604	0.8169
ABTS				1	0.6756
DPPH					1

There have been lots of studies on correlation between antioxidant activity and the TP content. Zheng and Wang (2001) reported excellent correlation for medicinal plants and culinary herbs when antioxidant activity (determined using the oxygen radical absorbance assay, ORAC assay) was compared with the TP content. Kalt et al. (1999) found a strong correlation between antioxidant capacity and total phenols (0.83) in four species of berries. Tawaha et al. (2007) reported positive linear correlation between antioxidant activity determined by the ABTS assay and TP content for aqueous and methanolic extracts of Jordanian plants (0.892 and 0.851, respectively). Cai et al. (2004) also found highly significant linear correlation (methanolic/aqueous: 0.964/0.953) between the TP content and antioxidant activity of 112 Chinese medical plants. Katalinić et al. (2006) reported high linear correlation (0.9825) between the TP content and FRAP values of selected medical plant extracts. In contrast to these investigations, Wong et al. (2006) reported only satisfactory correlation between the TP content and antioxidant activity determined by the FRAP and DPPH method. In their opinion this low correlation can be ascribed to an error introduced in the assay used to determine the extracts ability to scavenge DPPH free radicals or reduce ferric ions. Their assays were based on the measurement of end point, so one could actually be measuring the antioxidant activity of the reaction by-products,

rather than the compounds present in the original mixture. Another source of error in their study could be the lack of specificity of the Folin-Ciocalteau phenol reagent (Wong et al., 2006). Alothman et al. (2009) found out that the correlations between TP content and TF content assays were 0.853 and 0.763 for guava and pisang mas, respectively, and 0.031 in the case of pineapple. Their results indicate that the flavonoids are an important phenolic group in representing the antioxidant capacity of guava and pisang mas but not of pineapple, where it could be related to other antioxidant compounds contained in pineapple fruit pulp. FRAP and DPPH assays showed the same trends. This is proved by the significant correlations between the FRAP values and DPPH values for all the fruits in the current study. Correlation values were 0.659 and 0.438 for guava and pineapple, respectively and 0.398 for pisang mas. Another significant correlation between the TP content and the antioxidant capacity of fruits extracts (FRAP and DPPH values) was obtained. These correlations confirm that the phenolic compounds are the main micro constituents contributing to the antioxidant activities of these fruits (Alothman et al., 2009). The lowest correlation coefficients were between DPPH values and other investigated parameters probably because reactions of phenols with ABTS radical cation are usually rapid, but their actions with DPPH radical differ from compound to compound (Sanchez-Moreno, 2002; Katalinić et al., 2006).

Conclusions

Stability of water solutions of selected crude extracts throughout evaluation of the antioxidant activity, total phenol content and total flavonoid content during 5 weeks of storage under light and in the dark at room temperature was conducted. The total phenol content, total flavonoid content and antioxidant activity differ among water solutions of selected crude extracts. Correlation between total phenol content and antioxidant activity was very high suggesting that phenolic compounds are the most responsible compounds contributing to antioxidant activity of investigated samples. It is well known that phenols have beneficial effect on health, so addition of crude extract to food products would be very interesting for functional foods preparation since addition of crude extracts into food products, like juices or jams, could be a valuable tool for fortification of foods with phenolic compounds, as well as for stabilisation of anthocyanins.

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