

ANTIOXIDANT POTENTIAL AND RADICAL SCAVENGING ACTIVITY OF DIFFERENT FERMENTATION DEGREE TEA LEAVES EXTRACTS

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ABSTRACT

Today's world needs a super antioxidant. Researches mainly aim to find new sources of plant world polyphenols, which accessible from everyday food could influence unfavorable aging processes in human body. There are many studies providing evidence that tea bioactive compounds (*Camellia sinensis* L.) possess antioxidant capacity, profitably influencing human health, by degenerative diseases morbidity decrease.

The scope of present research was to answer the question if there are any correlations between polyphenol content in processed tea leaves extracts and its antiradical and antioxidant activity. Evaluations were carried on leaves differentiated by the fermentation process degree - white, green, yellow, red and black tea, with use of DPPH, ABTS, FRAP, reducing power and chelating activity evaluation methods.

Keywords: Tea, *Camellia sinensis*, bioactive compounds, radical's scavenger, antioxidant.

INTRODUCTION

Tea leaves, *Camellia sinensis* is widely consumed beverage rich in strong antiradical and antioxidant constituents - polyphenols. Polyphenols, naturally occurring plant origin constituents beneficial for human body are subject matter of numerous researches (Niwa and Miyachi 1986, Worthy 1991, Yokozawa et al. 1998, Gramza and Regula 2007, Ho et al. 1997, Hollman 2001, Gramza et al. 2005a). It was proved that polyphenols rich diet could be helpful in scavenging free radicals, major agents in degenerative diseases development. Throughout the years the human consumption habits are significantly changing, as a result of popular "healthy lifestyle" (Khan and Mukhtar 2007, Gramza et al 2005a, Hollman and Arts 2000, Scalbert and Williamson 2000, Gramza-

Michalowska and Bajerska-Jarzêbowska 2007).

Tea leaves and its everyday beverage are one of common polyphenols sources, rich in catechins, and a decisive group for their antioxidative activity. Green tea leaves contain large amounts of flavonoids and phenolic acids which make up to 30 % of fresh green tea leaves dry weight and only 10 % dry weight of black tea (Wang et al. 2000). Basic flavonoids are catechins: catechins: (±)-catechin C, (-)-epicatechin EC, (+)-gallocatechin GC, (-)-epigallocatechin EGC, (-)-epicatechin gallate ECG, (-)-epigallocatechin gallate EGCG (Balentine et al 1997, Graham 1992, Ho et al. 1997, Ninomiya et al. 1997, Gramza et al. 2006), which undergo oxidation to form theaflavins and thearubigenes in the manufacturing of black tea (Ho et al. 1992, Xie et al. 1993, Yanishlieva-Maslarowa and Heinonen 2001, Hagerman and Carlson 1998).

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Research suggested that presence of ortho-hydroxylation on B-ring of flavonoids molecule, number of free hydroxyl groups, C2-C3 double bond in C-ring and the presence of 3-hydroxyl group are listed as major conditions for strong antiradical and antioxidant properties of tea polyphenols (Bors et al. 1990, 1997, Cao et al. 1977).

Green tea contains higher amounts of simple polyphenols like catechins, however black tea contains larger amounts of gallic acid, released from catechins gallates, and complexed compounds from catechins oxidation - theaflavins and thearubigenes formed during fermentation (Thanaraj and Seshardi 1990, Xie et al. 1998, Gramza et al. 2006). Differences in tea leaves antioxidant and antiradical activity could be the result of many factors influencing plant and extracts itself. Among those factors it could be specified that most important are tea species differences (kind and leaf structure), influencing the leaching kinetics of active substances, harvesting methods, collecting time, and what could be the most important, tea production traditions and fermentation process (Chu and Juneja 1997, Wang et al. 2000, Gramza and Korczak 2005, Fernandez et al. 2002, Gramza-Michalowska et al. 2007b). Literature also showed that conditions of plant material preparation and extraction processes are very important factors influencing extract's chemical composition (Khokhar and Magnusdottir 2002).

There is growing research interest on radicals generated by different environment factors influence on human health and food stability. However many researches need to be provided, since no "super antioxidant" had been found yet. Since it was found that polyphenols possess antioxidant activity, many possible mechanisms had been proposed: radicals and oxygen scavenging, chelating metal ions, absorbing the UV radiation, decomposing peroxides and nonradical products or partial

regenerating of primarily antioxidants (Gramza and Korczak 2005).

The aim of the research was to qualify the effectiveness of different fermentation degree tea extracts as the radical scavengers and antioxidants. There is no data on polyphenol content and antioxidative activity of white and yellow tea extracts, compared to other popular teas. That is why the study involved aqueous and ethanol extracts of white, green, yellow, oolong and black teas.

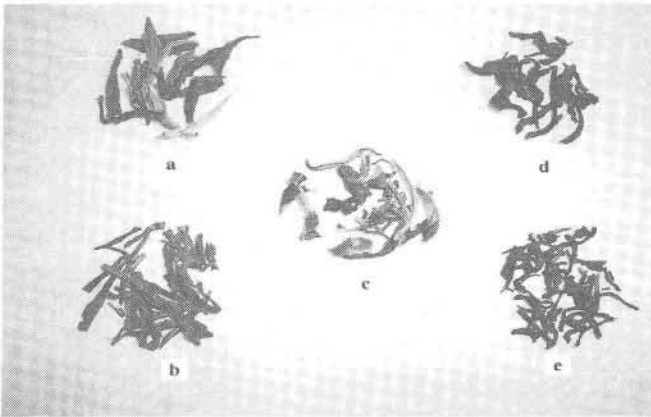
MATERIALS AND METHODS

Reagents: 2, 2'-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-Tipryridyl-s-triazine (TPTZ), Folin-Ciocalteu Reagent (Fluka); phosphate and acetic buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, iron chloride(III) hexahydrate, ferrozine, EDTA (ethylenediamine tetraacetic acid) (Sigma-Aldrich); potassium ferricyanide, trichloroacetic acid, ethanol, iron chloride, anhydrous sodium carbonate (POCH). All of purchased reagents used were of analytical grade.

Plant material: Base plant material used for the research was tea leaves (*Camellia sinensis* L.) differentiated by the fermentation degree, and was as follows: white (Pai Mu Tan), green (China Lung Ching), yellow (China Kekecha), oolong or red (Formosa Oolong) and black (Yunan Golden Leaf) (Photo 1). Two kinds of extracts had been prepared: aqueous and ethanol (Gramza et al. 2006). Briefly, aqueous extracts were prepared by triple active boiling of grinded tea leaves (15 min at 80°C), afterwards collected extracts were filtered (Whataman) and lyophilized under vacuum (HETO). Ethanol extracts were prepared by triple 24 hours maceration of tea leaves in 80% ethanol at ambient temperature conditions. Ethanol extracts were collected, filtered and

ethanol was evaporated on rotary evaporator (RVO 200A, INGOS). Rate of production yield presents table 1. The powdered extracts were kept frozen (-18°C) until further use. Range of tea extracts concentration was experimentally determined as follows: 100, 200, 500, 1000 ppm. To compare activity of tea extracts, rosemary (*Rosmarinus officinalis*) ethanol extract and Phytrox® commercial rosemary extract (Jan Dekker) in concentration of 200 ppm were chosen for the research.

Photo 1. Tea leaves (*Camellia sinensis*): a) white - Pai Mu Tan, b) green - China Lung Ching, c) yellow - China Kekecha, d) oolong or red - Formosa Oolong, and e) black - Yunan Golden Leaf



Total polyphenol content: The content of total polyphenols in the extracts was determined according to method presented by Horwitz (1970), and results were expressed as catechin equivalents in mg/g dry weight of tea extracts. Standard concentrations of catechin between 0–600 mg/mL were used to prepare a calibration curve.

ANTIRADICAL ACTIVITY

DPPH• Free radical scavenging method: The effect of tea aqueous and ethanol extracts was estimated according to the procedure described by Sanchez – Moreno et al. with slight modifications (1998). An aliquot of ethanol (0.1 ml), solution containing different established experimentally extracts concentrations (100, 200, 500, 1000 ppm), was added to 3.9 ml of

DPPH• 0.025 g litre⁻¹ in ethanol prepared daily. Absorbance decrease of samples was measured at 515 nm on Carl Zeiss Spectrophotometer (Jena Optik). DPPH• stock solution, was stored at 4°C until it was used. Violet color of DPPH• solution disappears in presence of antioxidant, which scavenges free radicals in measured medium. Faster the absorbance decreases stronger the antioxidant. The antiradical value represents percent of radicals scavenging ability according to: % scavenging effect = $[(Abk - Abb) / Abk] * 100$
Abb – sample absorbance
Abk - control sample absorbance
Free radical scavenging percentage was evaluated on the basis of standard curve for Trolox.

ABTS+• FREE RADICAL SCAVENGING METHOD:

Radical scavenging activity of tea extracts was measured according to assay described by Re et al. (1999). TEAC (Trolox Equivalent Antioxidant Capacity) evaluation is based on stability of the antioxidant to scavenge the blue-green colored ABTS+• radical cation in comparison to scavenging ability of water soluble vitamin E analogue – Trolox. To generate ABTS+• potassium persulfate ($K_2S_2O_8$) was used. The ABTS+• solutions, with stable absorbance at 734 nm for at least 2 h, were used for the determination of assay with tea extracts in ethanol to give the final concentration required. Antiradical activity of examined samples was presented as percentage of radicals scavenging and calculated using the same equation as in DPPH• method.

ANTIOXIDANT ACTIVITY

RP: Reducing power evaluation is based on spectrophotometric measurement of color change in sample after potassium ferricyanide addition (Jin-Wei et al. 2005). Absorbance is measured at wavelength $\lambda=700\text{nm}$. Color intensity is proportional to reducing power of sample; higher sample absorbance, higher

reducing power. Results of reducing power are presented as absorbance of samples.

FRAP: Ferric Reducing Ability of Plasma assay is based on ability to reduce complex of Fe(III)-2,4,6-tri(2-pirydy)-s-triazine (TPTZ) into Fe(II)-TPTZ by examined sample with reducing activity (Benzie and Strain 1996). Intensity of produced blue color is measured spectrophotometrically at $\lambda=583\text{nm}$. Results are presented as $\mu\text{M Fe}$ per 1 g of tea dry weight. CA: Chelating activity measurement is based on Fe²⁺ ions binding (chelating) ability of polyphenols (Tang et al 2002). Results are obtained after spectrophotometric analysis of absorbance changes with $\lambda=562\text{nm}$ of wavelength according to the control sample, and presented as % of chelating activity.

STATISTICAL ANALYSIS

The results were obtained from a minimum of three independent experiments and averaged. Data were analyzed by the analysis of variance ($p < 0,05$) to estimate the differences between values of tested extracts. Results were processed by the computer program Statistica 7.0.

RESULTS AND DISCUSSION

Evaluation of antiradical and antioxidant activity of tea extracts involved five kinds of tea leaves, differing with the fermentation process degree. Research included following tea leaves: white - Pai Mu Tan, green - China Lung Ching, yellow - China Kekecha, oolong or red - Formosa Oolong and black - Yunan Golden Leaf. Tea leaves extraction procedures involved two methods: aqueous extraction (to simulate European

traditional brewing method); and ethanol extraction (to simulate the infusion of the herbs - tincture). Highest rate of production yield was

Table 2. Total polyphenol content in plant extracts [mg catechin/g dw]

TEA	Total polyphenols [mg catechin/g dw]	
	Aqueous extract	Ethanol extract
WHITE	245,55 ^c ± 1,50	522,78 ^c ± 0,75
GREEN	236,78 ^b ± 0,25	574,63 ^d ± 7,51
YELLOW	319,68 ^d ± 0,50	698,84 ^d ± 2,50
OLOONG	248,31 ^c ± 1,25	361,25 ^b ± 2,75
BLACK	195,96 ^a ± 0,50	361,25 ^b ± 2,50
ROSEMARY	-	342,97 ^a ± 0,75

a, b – statistically significant differences in a column

evaluated in aqueous extract of white tea, lowest in ethanol extract of black tea (Table 1) and was lower with higher tea leaves fermentation process degree. It was found that tea ethanol extracts had been characterized by significantly lower extraction efficiency than aqueous extracts.

Total polyphenol content was measured with use of Folin-Ciocalteu phenol reagent. Results expressed as mg catechin equivalents per 1g of dry extract are presented in Table 2. The level of total phenolics in aqueous extracts varied between 195,96 and 319,68 mg/g, and was significantly lower in comparison to ethanol extracts which ranged from 361,25 to 698,84 mg/g of extract's dry weight. Rosemary ethanol extract was evaluated to contain lowest amount of polyphenols among ethanol extracts. Statistical analysis showed differences in total polyphenol contents, and dependency from solvent kind used for extraction ($p < 0,05$). In ethanol extracts it was found, that with increasing fermentation degree of tea leaves, polyphenol content decreases. Similar dependency was not found in aqueous extracts.

Atoui et al. (2005) and Yokozawa et al. (1998) researches showed that green tea contains more polyphenols than black tea. Satoh et al (2005) evaluated polyphenol content in different teas aqueous extracts and found that roasted tea

Table 1. Rate of tea extracts production yield [%]

TEA	Extraction yield [%]	
	Aqueous extract	Ethanol extract
WHITE	26,06 ^c ± 0,41	12,23 ^d ± 0,17
GREEN	24,49 ^b ± 0,53	12,61 ^d ± 0,37
YELLOW	24,17 ^a ± 0,27	10,91 ^c ± 0,21
OLOONG	19,82 ^a ± 0,27	7,72 ^b ± 0,08
BLACK	19,17 ^a ± 0,22	6,67 ^a ± 0,08

a, b – statistically significant differences in a column

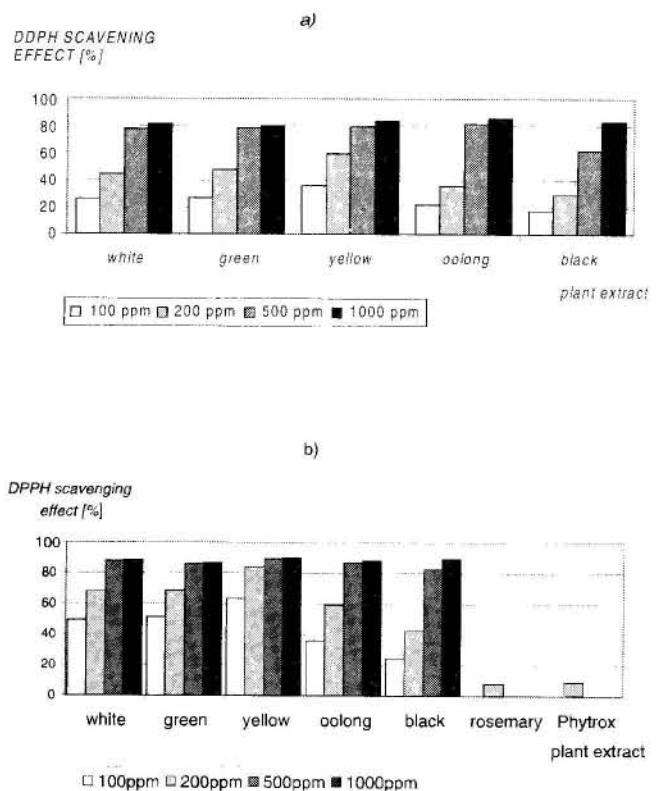
contained lower levels of total phenolics than green, oolong, or black tea (green tea > oolong tea > black tea > roasted tea). Results of present research seem to confirm that thesis, showing that with increase of fermentation degree total polyphenol content decreases. Generally higher polyphenols content was evaluated in yellow tea extracts, what could be the result of slight fermentation process, allowing producing lower amounts of complexed compounds than in fully fermented teas. Although green tea contains larger amounts of simple flavonoids - catechins, black tea contains larger quantity of gallic acid, released from catechins gallates, and complexed compounds from catechin oxidation - theaflavins and thearubigenes formed during fermentation (Thanaraj and Seshardi 1990, Xie et al. 1998, Gramza et al. 2006). Differences in tea leaves polyphenols levels could be also a result of tea kind and leaf structure, influencing the leaching kinetics of these substances (Chu and Juneja 1997, Wang et al. 2000, Khokhar and Magnusdottir 2002).

Research of Turkmen et al. (2006) showed that tea extracts total polyphenol content and antioxidant activity could be different according to solvent polarity. Khokhar and Magnusdottir (2002) suggested that both aqueous and ethanol extracts black tea contains more polyphenols than green tea. However results Manzocco et al. (1998), evaluated higher polyphenol content in green than in black tea extracts. In present results tea ethanol extracts are similarly characterized by higher content of total polyphenols, what was also presented in other research (Gramza et al. 2006). Results of Chen et al. (2007) showed adverse results were rosemary extract contained 185,04 [mg gallic acid/g], and green tea extract only 64,95 [mg gallic acid/g].

Polyphenols are characterized by high antiradical potential. Activity of tea extracts in radical scavenging analysis was measured with the most popular DPPH• and ABTS+• methods

(Gramza-Michalowska 2007). Results of DPPH• radicals scavenging activity showed that highest radical scavenging activity exhibited yellow tea extracts (Figure 1), and lowest black tea extracts. Statistical analysis of data received showed that tea extracts possessed radical scavenging ability, depending on their concentration. It was evaluated that with increase of extracts concentration; antiradical activity also does increase, reaching highest activity in

Figure 1. DPPH antiradical activity of aqueous (a) and ethanol (b) plants extracts expressed as percent of DPPH radical scavenging.

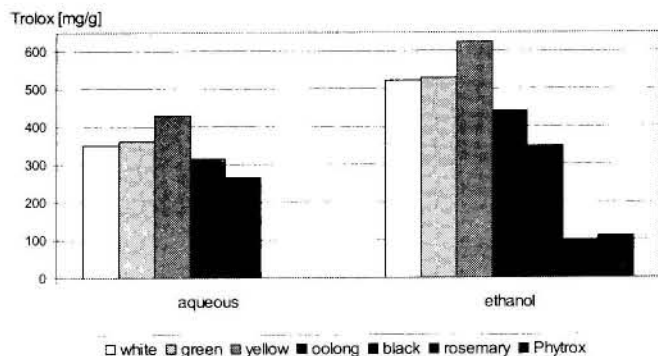


concentration of 1000ppm. Comparing to activity of well known natural antioxidant like rosemary extracts (ethanol extract and Phytrox) it was stated that tea extracts had been significantly stronger DPPH• radicals scavengers.

Analysis of DPPH• antiradical activity of tea extracts calculated on equal Trolox concentration showed that all tea extracts represented high activity, significantly higher than

of rosemary extracts (Fig. 2). Among tea extracts yellow tea aqueous and ethanol extracts activity was equal to higher Trolox amount, and it was 431,1 and 625,2 [mgT/g] respectively. Also green and white tea was characterized by high activity. Lowest impact on radical scavenging effect possessed black tea extract.

Figure 2. DPPH Antiradical activity of tea and rosemary extracts calculated on Trolox concentration [mg Tx/g dw]



Taking in to account both aqueous and ethanol extracts it was found that again ethanol extracts exhibited significantly higher activity than aqueous. Analysis of DPPH• scavenging activity showed that aqueous green tea extract possessed higher radical scavenging effectivity than black one (Yokozawa et al. 1998). Yen and Chen have also examined DPPH• radical scavenging activity of tea, and suggested that higher antiradical potential represented aqueous extract of green tea, than oolong and black tea (1995). Results of present research confirm this thesis in examined tea extracts. Aoshima and Ayabe (2007) examined DPPH• radical scavenging activity of tea, and stated that highest activity exhibited green tea extract, than black tea and lowest activity was evaluated in oolong tea. Satoh et al. (2005) graduated tea extracts as DPPH• radical scavengers in the following order: green tea>roasted tea>oolong tea>black tea. Statistical analysis allowed confirming that antiradical activity of tea extracts compounds is a result of concentration (Table 3).

Significantly higher antiradical activity was evaluated in ethanol extracts than the aqueous. Research of Gramza and coworkers confirm present investigations (2005b). Analysis of

rosemary extract showed minor antiradical activity, significantly lower than other plant extracts. Statistical analysis evaluations confirmed the correlations between antiradical activity and total polyphenols in both aqueous and ethanol tea extracts ($r=0,93$ $p<0,05$ and $r = 0,97$ $p<0,05$ respectively).

Table 3. Correlations between extracts concentrations and DPPH antiradical activity

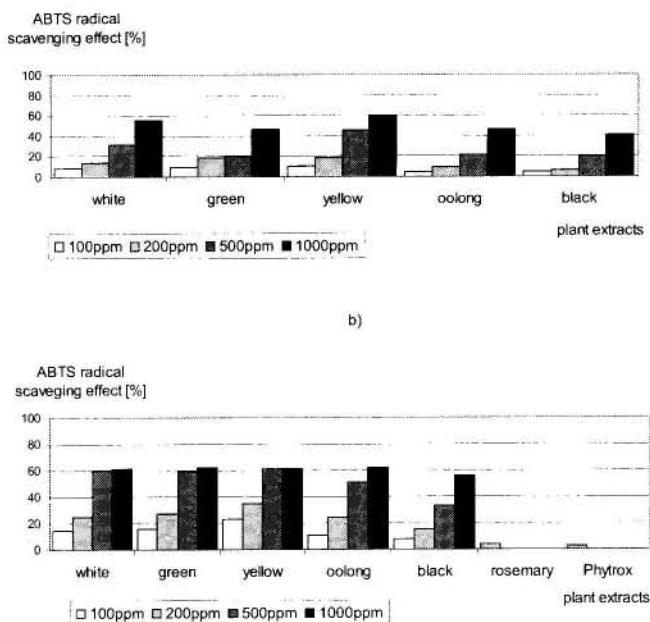
TEA	Aqueous extract	Ethanol extract
WHITE	$r = 0,97^{**}$	$r = 0,92^{**}$
GREEN	$r = 0,98^{**}$	$r = 0,91^{**}$
YELLOW	$r = 0,95^{**}$	$r = 0,90^{**}$
OOLONG	$r = 0,97^{**}$	$r = 0,97^{**}$
BLACK	$r = 0,95^{**}$	$r = 0,98^{**}$

*-no significant correlation ($p<0,05$)

** -significant correlation ($p<0,05$)

Antiradical activity of tea extracts was also examined with use of ABTS+• radical. On the basis of present research it was found that highest radical scavenging activity possessed yellow and white tea extract, lower however extract of black tea (Figure 3). Conducted analysis showed that

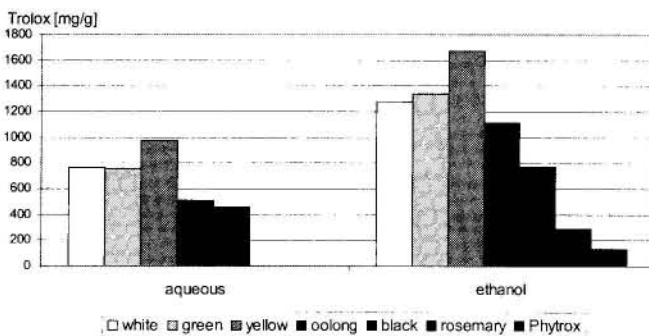
Figure 3. Antiradical activity of aqueous (a) and ethanol (b) plants extracts expressed as percent of ABTS+• radical scavenging.



with the increase of concentration also the ABTS+• radical scavenging activity increases. Rosemary extracts did not scavenge ABTS+• radicals as well as tea extracts and was significantly stronger than commercial rosemary extract – Phytrox ($p < 0,05$). According to the concentration used it was found that best antiradical activity was evaluated in concentration of 1000 ppm in each examined tea extract.

ABTS+• radical scavenging counted over the equal Trolox activity showed that yellow tea extracts possessed highest radical scavenging ability in each of extract's groups (Figure 4). Trolox equivalent amount was 973,1 for aqueous and 1671,9 [mgTx/g dw] for ethanol extract. Green and white tea extracts also exhibited high scavenging ability, lower than yellow tea, but higher than fermented tea, rosemary extract (291,2) and Phytrox (131,7).

Figure 4. ABTS Antiradical activity of tea and rosemary extracts calculated on Trolox concentration [mg Tx/g dw]



research, which showed that higher antiradical activity in ABTS+• method characterized black tea extract (Gramza et al. 2005b). Green tea extract possessed significantly lower radical scavenging activity. However results of Arts et al. (2002) confirm present results, showing higher ABTS+• radical scavenging activity of green than black tea extract. On the basis of statistical analysis it was stated that activity in scavenging of ABTS+• radical is strongly correlated with total polyphenol content in ethanol extracts ($r = 0,99$, $p < 0,05$), however weak correlation was found in

aqueous extracts ($r = 0,78$, $p < 0,05$). Analysis of correlation between ABTS+• radical scavenging ability and its concentration had been found to be significant in all samples except aqueous extracts of oolong and black tea (Table 4).

Table 4. Correlations between extracts concentrations and ABTS+• radical scavenging activity.

TEA	Aqueous extract	Ethanol extract
WHITE	$r = 0,88^{**}$	$r = 0,98^{**}$
GREEN	$r = 0,86^{**}$	$r = 0,98^{**}$
YELLOW	$r = 0,93^{**}$	$r = 0,95^{**}$
OOLONG	$r = 0,28^*$	$r = 0,90^{**}$
BLACK	$r = 0,10^*$	$r = 0,98^{**}$

*-no significant correlation ($p < 0,05$)

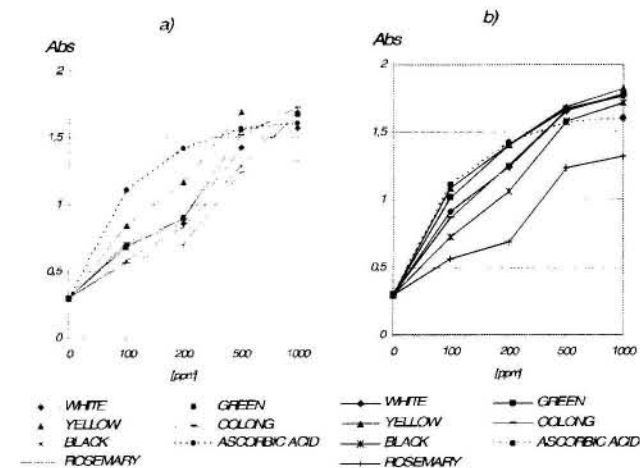
** -significant correlation ($p < 0,05$)

It was also evaluated that, DPPH• and ABTS+• radicals scavenging activity was correlated with degree of tea leaves fermentation. Highest activity was stated in tea extracts slightly fermented and non fermented leaves; lower activity was found in fermented teas. Results suggested that antiradical activity of tea extracts depends on catechins content; lower activity could be a result of higher content of tannins, high molecular weight theaflavins and thearubigenes. Present research is in agreement with results of Miller *et al.* (1996), who conducted analysis on antioxidant activity of theaflavins and its gallic esters. It was found that the radicals scavenging activity increased together with the degree of esterification. Other research of antiradical activity of tea extracts measured with two methods of scavenging the stable free radicals ABTS+• and DPPH• showed different antiradical activity (Gramza et al. 2005b). Highest activity in scavenging ABTS+• showed black tea aqueous and ethanol extracts, however in DPPH• radical best activity exhibited green and black tea ethanol extracts. It was also stated that aqueous extracts possessed 50% lower activity than equivalent ethanol extracts.

Evaluation of plant extracts reducing power was conducted on the basis of color changes after addition of potassium ferricyanide.

Transformation of Fe³⁺ - Fe²⁺ was determined in presence of plant extracts and ascorbic acid. Higher absorbance at λ=700nm represents higher reducing power of plant sample. The effects of various tea extracts on the reducing power are shown in Figure 5. In general reducing power of tea extracts was increasing with concentration, and was highest at 1000ppm. Yellow tea, both ethanol and aqueous extracts exhibited highest reducing power among examined extracts (Fig 5). Reducing power of white, oolong and black tea aqueous extracts was lower than of ascorbic acid, however in concentration of 1000ppm only black tea extract showed lower activity. According to ethanol extracts it was evaluated among all extracts only yellow tea extract presented highest activity, but significantly lower than ascorbic acid. Similarly to aqueous extracts concentration of 1000ppm characterized activity higher than ascorbic acid, except for white tea and rosemary extract. Statistical analysis suggested correlation between reducing power of tea extracts and its antioxidative activity. The results reported in present study are in agreement of Yen and Duh results (1993), indicating that reducing power is associated with the antioxidant activity.

Figure 5. Reducing power of various plant extracts; a) aqueous, b) ethanol



Farhoosh et al (2007) investigated reducing power of aqueous tea extracts and found that green tea extract was characterized by higher

reducing power than black tea. However the antioxidant activity of tea extracts was not concomitant with their reducing power. Authors suggested that the antioxidant activity of tea extracts likely involved also other mechanisms to those of reductones. Reported results seem to confirm this thesis. It was found that highest reducing power was evaluated in extracts concentration of 1000ppm. Ethanol tea extracts possessed highest reducing power, than it was estimated in aqueous extracts samples. All extracts did not differ significantly in the same concentrations; significant difference had been noticed according to extract concentration. It was found that all tea aqueous extracts were significantly weaker reducing agents in concentrations of 100 and 200ppm, higher concentrations gave the same reducing power like ascorbic acid. In ethanol extracts however reducing power was similar to ascorbic acid just from concentration of 200ppm. Rosemary ethanol extract was significantly weaker reducing agent than other plant extracts ($p < 0,05$).

Yen and Chen (1995) showed that highest reducing power than green or black tea exhibited red tea extract. According to results of this paper it was suggested, that green and oolong tea extract exhibited the same activity, no significant differences had been found ($p < 0,05$). Statistical analysis of relationship between both aqueous and ethanol tea extracts reducing power and total polyphenol content showed significant dependency ($r = 0,95$ and $r = 0,98$). Reducing power was also highly correlated with extracts concentrations ($p < 0,05$), as shown on table 5.

Table 5. Correlations between tea extracts concentrations and reducing power.

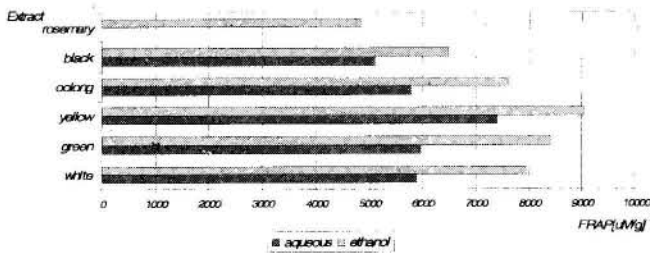
TEA	Aqueous extract	Ethanol extract
WHITE	$r = 0,91^{**}$	$r = 0,89^{**}$
GREEN	$r = 0,90^{**}$	$r = 0,85^{**}$
YELLOW	$r = 0,84^{**}$	$r = 0,89^{**}$
OOLONG	$r = 0,93^{**}$	$r = 0,87^{**}$
BLACK	$r = 0,97^{**}$	$r = 0,90^{**}$

*-no significant correlation ($p < 0,05$)

**-significant correlation ($p < 0,05$)

Antioxidative activity of examined plant extracts was measured with FRAP method (Ferric Reducing Antioxidant Power). Results of the determinations presented as $\mu\text{M Fe/g dw}$, showed that equally aqueous and ethanol extract of yellow tea exhibited higher ferric reducing activity, lowest black tea extracts ($p < 0,05$). Comparison showed significantly lowest reducing power of rosemary extract in FRAP analysis (Figure 6).

Figure 6. Ferric reducing antioxidant power of plant extracts [$\mu\text{M Fe/g dw}$]



As the result of the above mentioned analysis in reducing ability of aqueous and ethanol plants extracts were ranked as follows: rosemary < black tea < oolong tea < white tea < green tea < yellow tea. Benzie and Sheto (1999) investigated tea FRAP, and showed that highest antioxidative activity, was determined in green, than red and black tea, what confirmed present research. Similar results presented Bravo et al. (2007), were black tea needed lower amount of Trolox to cover the activity of green tea extract in FRAP analysis.

Ethanol extracts showed significantly higher ferric reducing antioxidant power, than aqueous extracts ($p < 0,05$). Comparison of examined tea extracts with rosemary extract activity, resulted in significantly lower activity found in rosemary extract ($p < 0,05$). Statistical analysis of relationships between ferric reducing antioxidant power of aqueous and ethanol tea extracts and total polyphenol content showed high correlations (respectively $r = 0,98$ and $r = 0,96$, $p < 0,05$).

Results of chelating ability of examined plant extracts presents table 6 and 7. It was stated that Fe^{2+} chelating ability of tea extracts, both aqueous and ethanol, increased with increase of concentration. Among aqueous extracts highest ability possessed black tea, lowest white tea extract. Similar results had been shown in ethanol extracts. Rosemary extract in comparison with tea extracts showed weak Fe^{2+} chelating ability. However all examined plant extracts exhibited significantly lower activity than EDTA, a strong chelating agent.

Table 6. Fe^{2+} chelating ability of aqueous tea extracts and EDTA [%]

TEA	100 ppm	200 ppm	500 ppm	1000 ppm
WHITE	14,39 ^{a,AB}	20,70 ^{b,AB}	27,64 ^{c,BC}	39,25 ^{d,A}
GREEN	15,38 ^{a,AB}	16,89 ^{a,A}	14,07 ^{a,A}	43,36 ^{b,BC}
YELLOW	11,02 ^{a,A}	19,57 ^{b,AB}	25,88 ^{c,B}	42,11 ^{d,B}
OOLONG	13,07 ^{a,AB}	21,70 ^{b,B}	30,46 ^{c,C}	42,35 ^{d,B}
BLACK	18,70 ^{a,B}	19,84 ^{a,AB}	30,83 ^{b,C}	44,47 ^{c,C}
EDTA	96,27 ^{a,C}	97,87 ^{ab,C}	97,88 ^{ab,D}	98,67 ^{b,D}

a, b – statistically significant differences in a verse
A, B – statistically significant differences in a column

Table 7. Fe^{2+} chelating ability of ethanol tea extracts and EDTA [%]

TEA	100 ppm	200 ppm	500 ppm	1000 ppm
WHITE	23,52 ^{a,B}	29,06 ^{b,B}	39,86 ^{c,C}	51,74 ^{d,D}
GREEN	23,52 ^{a,B}	26,84 ^{a,B}	36,40 ^{b,B}	46,28 ^{c,B}
YELLOW	26,19 ^{a,B}	28,75 ^{a,B}	35,99 ^{b,B}	46,18 ^{c,B}
OOLONG	30,54 ^{a,B}	32,24 ^{b,C}	41,55 ^{c,C}	49,10 ^{d,C}
BLACK	30,51 ^{a,B}	34,89 ^{a,D}	45,31 ^{b,D}	56,45 ^{c,E}
ROSEMARY	9,15 ^{a,A}	9,70 ^{a,A}	29,32 ^{b,A}	39,19 ^{c,A}
EDTA	96,27 ^{a,C}	97,87 ^{ab,E}	97,88 ^{ab,E}	98,67 ^{b,F}

a, b – statistically significant differences in a verse
A, B – statistically significant differences in a column

Fe^{2+} chelating ability of tea extracts was found to be dependent from leaves fermentation degree. Higher chelating activity was evaluated in fermented tea extracts. Significantly higher chelating ability of black tea could be a result of oxidated polyphenols presence – theaflavins, thearubigenes; and gallic acid, produced in large amounts during the fermentation process

(Gramza et al. 2004, 2005c). Results of Druzynska et al. (2007) indicated that aqueous green tea extract possessed higher chelating activity than ethanol extract. Those findings are in agreement with present research, showing higher activity of ethanol extracts. Significantly lower activity of all examined extracts was found according to EDTA chelating activity ($p < 0,05$). Tang et al. (2002) results showed that green tea extract's catechins exhibited lower chelating ability than EDTA, what also found a confirmation in presented results.

Result of statistical analysis showed no correlations between chelating ability and total polyphenol content in aqueous extracts ($r = 0,43$ $p < 0,05$). However the relationship between total polyphenol content and chelating ability was found in ethanol extracts ($r = 0,88$ $p < 0,05$). Also tea extracts concentration was influencing significantly its Fe²⁺ chelating ability (Table 8). Strong Fe²⁺ chelating properties of Yunnan tea

Table 8. Correlations between tea extracts concentration and Fe²⁺ chelating ability.

TEA	Aqueous extract	Ethanol extract
WHITE	$r = 0,98^{**}$	$r = 0,98^{**}$
GREEN	$r = 0,88^{**}$	$r = 0,98^{**}$
YELLOW	$r = 0,98^{**}$	$r = 0,99^{**}$
OOLONG	$r = 0,97^{**}$	$r = 0,98^{**}$
BLACK	$r = 0,99^{**}$	$r = 0,98^{**}$

*-no significant correlation ($p < 0,05$)

**-significant correlation ($p < 0,05$)

extracts were evaluated in other research, highest for green tea ethanol extract (Gramza et al. 2004). It was also found that there was a correlation between Fe²⁺ chelating ability and tea extracts properties in protection of lipids oxidation process.

CONCLUSIONS

Results of the present work indicate that aqueous and ethanol extracts of *Camellia sinensis* exhibited significant radicals scavenging activity, reducing power and chelating ability. Highest activity in all examined methods was found in

sample of yellow tea extracts aqueous as well as in ethanol. Moreover the correlations between total polyphenol contents of tea extracts tested and its activity had been found. Results of present work confirmed previous results of Yen and Chen (1995), showing that antioxidative and antiradical activity of tea extracts varied with the extent of fermentation time during tea manufacture. All activities of semifermented teas were greater than those of nonfermented and fully fermented teas. On the basis of the results of this study it was also indicated that activity of tea leaves extracts dependent of different fermentation degree, and was higher in slightly fermented yellow tea. Future research area would involve extracts purification, as it was found in previous work that green tea ethanol extract, subjected to different purification processes showed decrease of total polyphenols content; however without its antiradical and antioxidant activity decrease (Gramza-Michalowska et al. 2007a). Also the possible further usage directions as polyphenols carrier in medications, supplements and food products could be proposed.

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