Catechin-like antioxidative potential of selected tea products

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S u m m a r y

The presented study was aimed to establish the levels of antioxidative potential of five types of tea (white, red, green, black and blue/turquoise) originating from different geographical regions. Furthermore, the content and qualitative composition of phenolics in aqueous infusions of the tested tea products was compared. The highest antioxidant activity against DPPH• free radical was found in extracts obtained from white teas, whereas the lowest in extracts from red teas. Moreover, the highest total phenolics content was shown in the extracts prepared from the white teas. In addition, the HPLC analysis allowed to identify the following catechin derivatives in the tea extracts: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin gallate. Simultaneously, it was shown that the dominant compound in extracts from the all tested teas was (+)-catechin.

Key words: *tea, total phenolics, catechins, antioxidative potential*

INTRODUCTION

The intracellular redox potential is disturbed by reactive oxygen species (ROS), and the free oxygen radicals are produced in different metabolic reactions and processes in living organisms [1, 2]. Physiological level of ROS provides normal functioning of cells, their growth, differentiation and proliferation. On the other hand, higher amount of free oxygen radicals is released when the cells are subjected to various stressful factors connected with environmental pollution, infections or inappropriate diet [3]. Excessive ROS concentration may cause oxidative stress, leading to damages of cell membranes, lipids, proteins or nucleic acids, and subsequently may lead to circulatory and nervous system disorders, generalized arterio-atherosclerosis, diabetes and cancer [4].

In tissues of living organisms various biomolecules occur that inhibit free radicals' generation or participate in their turnovers into inactive derivatives. Among them enzymatic and non-enzymatic antioxidants may be distinguished [5]. Plant-derived food is also abundant source of antiradical constituents, since it contains polyphenolic compounds acting as antioxidants. Particularly high amounts of these substances were found in fruits, vegetables, cereal products, *Leguminosae* plants, herbs, spices and teas [5-9].

The plant polyphenolics are large and important group of biomolecules in tea leaves, and their content may reach even up to 36% of dry mass [10-12, 27]. Main representants of the polyphenols in tea are catechin derivatives that possess the highest antioxidative potential. They prevent ROS formation by inhibitory effect on activity of the enzymes participating in their generation. Furthermore, these constituents are involved in ROS scavenging or complexation of metal ions catalyzing free radical reactions [21]. Nowadays, the tea market has been improved and embraced a new tea products referring to white, red, green, black and blue/turquoise teas. There are lots of contradictory data referring to their antioxidative potential and role of the polyphenolics in scavenging of the free radicals.

Therefore, the purpose of conducted study was to compare the antioxidative potential of different types of tea and determine the content and qualitative composition of polyphenolic compounds in infusions of the tea leaves and tips.

MATERIALS AND METHODS

Material for chemical analysis were commercial tea products that were prepared from leaves and buds of tea bush, *Camellia sinensis* L. (Kuntze), in form of dried usable parts of the plants. Five types of tea (white, red, green, black and blue or turquoise) from different regions were selected for the analyses (one African tea from Malawi and fourteen Asian teas cultivated in India, Vietnam, Japan, Taiwan, Sri Lanka and the Chinese provinces of Yunnan and Fujian).

Aqueous infusions of tea were prepared by adding 100 ml of boiling distilled water to 5 g of powdered tea sample and extracted for 30 min on a laboratory shaker. The obtained infusions were cooled to room temperature and then centrifuged at 10 000·g for 20 min. Antiradical activity of the tea extracts against $DPPH^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) was measured according to Brand-Williams et al. [16]. Total phenolics content was determined with the Folin-Ciocalteu reagent [15]. The concentration of catechins was measured using colorimetric method described by Czapski and Szwejda [14]. Quantitative analysis of catechins was performed using high performance liquid chromatography (HPLC) based on the method of de Rijke et al. [17]. Tea extracts were partitioned with ethyl acetate and the organic phase after desiccation was evaporated to dryness and dissolved in 80% methanol. The MetOH solutions were injected on to Microsorb - MV 100-5 C18 column with Chrom Sep Guard Cartridge (HPLC Varian ProStar 210 system). The catechin partition was carried out using linear gradient of mobile phase from 20% Solvent B to 100% Solvent B at a flow rate of 1 ml·min^{-1} (solvent A-1% phosphoric acid; solvent B-40% acetonitrile with 1% phosphoric acid). Identification of the catechins was performed with Photodiode Array Detector Prostar 335. Level of the identified compounds in the studied teas was determined using the Sigma Chemical standards.

The obtained results were subjected to the analysis of variance (ANOVA), and the significance of differences between means was evaluated by Duncan's test at $p \leq 0.01$.

RESULTS AND DISCUSSION

The obtained results showed that the highest antioxidant activity against DPPH$^{\bullet}$ was found in water extracts from white teas and the lowest in extracts from red teas. Quite high antioxidant potential also exhibited the studied green, blue and widely used black teas. The observed trends were quite stable and were not dependent on the origin of tea plants (tab. 1, fig. 1).

Table 1.

Concentration of the tea extracts (g/100 ml) causing 50% inhibition of the DPPH$^{\bullet}$ radical (IC_{50})

Tea product		Location of shrubs	Antioxidative potential (IC_{50})
White tea	Long Zhu White	China	0.040 k
	Zomba Pearls	Africa	0.050 j
	White Monkey	China	0.075 i
Green tea	Biluochun	China	0.050 j
	Sencha	Japan	0.075 i
	Gyokuro	Japan	0.175 f
Blue tea	Dongding Oolong	Taiwan	0.260 e
	Milk Oolong	China	0.075 i
	Tie Guanyin	China	0.075 i
Black tea	Yunnan Special Gold	China	0.275 d
	Assam Dagapur SFTGFOP1	India	0.120 h
	Darjeeling Hypnotic FTGFOP 1	India	0.125 g
Red tea	Pu'er	China	0.475 c
	Pu'er Organic	China	0.675 b
	Pu'er Royal	China	0.730 a

Values not followed by the same letters are significantly different at $p \leq 0.01$ (Duncan's test).

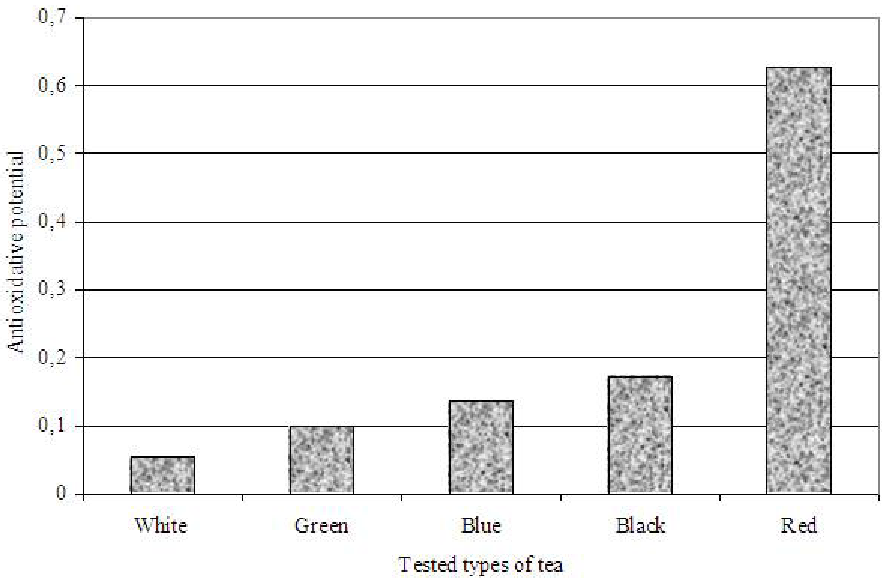

Figure 1.
An average concentration of the tea extracts (g/100 ml) caused 50% inhibition of the DPPH• radical (IC_{50})

The highest total phenolics content was shown in the water extracts prepared from the white teas. Relatively high amount of these substances was also found in blue tea products. Water infusions of the green and black teas possessed intermediate level of the phenolic compounds unlike infusions from red teas in which the level of the polyphenolics was the lowest (tab. 2, fig. 2). The obtained results are similar to previously reported which indicated much higher content of polyphenols within white and green teas than black teas [28-30]. However, some authors point out that meaningful comparison of the phenolic compounds content in different kind of teas is possible only when the methods of its cultivation and processing are similar [25].

However, the HPLC analysis allowed to identify the following catechin derivatives in the tea extracts: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin gallate. The dominant compound in water extracts from all studied teas was (+)-catechin. White tea extracts contained much higher content of (+)-catechin than the other tested tea infusions. The extracts with high level of (+)-catechin usually contained also more (-)-epicatechin, and this tendency was quite stable. The (-)-epigallocatechin, and (-)-epicatechin gallate occurred only in some water extracts from the studied teas in pretty low content. Sum of the identified catechins showed that blue, green and white teas contain much more of these substances than black teas, with exception of Darjeeling Hypnotic one. What is important, the identified catechins were present at very low amounts in the red tea water infusions.

Table 2.

Content of total phenols and total catechins (mg/g dry weight) in studied tea products

Tea products		Location of shrubs	Total phenols	Total catechins
White tea	Long Zhu White	China	125.90 a	14.65 d
	Zomba Pearls	Africa	136.10 a	15.68 d
	White Monkey	China	95.60 b	15.47 d
Green tea	Biluochun	China	85.90 bc	16.50 d
	Sencha	Japan	89.80 bc	18.04 cd
	Gyokuro	Japan	63.33 ef	23.98 b
Blue tea	Dongding Oolong	Taiwan	93.50 b	15.23 d
	Milk Oolong	China	94.80 b	33.02 a
	Tie Guanyin	China	85.70 bc	18.71 cd
Black tea	Yunnan Special Gold	China	49.20 g	0.15 e
	Assam Dagapur SFTGFOP1	India	78.70 cd	1.91 e
	Darjeeling Hypnotic FTGFOP 1	India	93.30 b	19.85 c
Red tea	Pu'er	China	31.20 h	T
	Pu'er Organic	China	70.08 de	T
	Pu'er Royal	China	51.36 fg	T

T – trace amount; values not followed by the same letters are significantly different at $p \leq 0.01$ (Duncan's test).

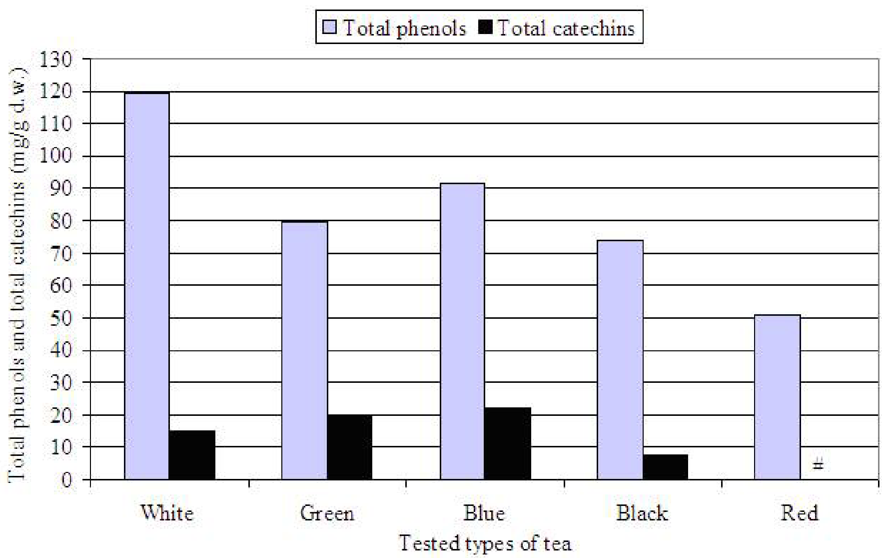

Figure 2.
An average content of total phenols and total catechins (mg/g dry weight) in the studied tea products
– trace amount

The literature data demonstrate clearly that degree of fermentation during the manufacturing process of tea had an influence on the catechin concentration in final product. Green teas contained higher amount of (-)-epigallocatechin gallate and (-)-epicatechin gallate in comparison to black teas [10-13, 20], whereas Oolong teas were rich in (+)-catechin and (-)-epigallocatechin [19, 24, 28]. Moreover, the white teas contained high level of (-)-epigallocatechin and (-)-gallocatechin gallate [25, 27], while Pu'er teas possessed the lowest amount of flavonoids [24].

The analyses performed revealed that the highest content of total catechins was found in water extracts of partly fermented blue teas, unfermented green teas and in slightly fermented white teas. The lower level of the catechins was determined in extracts of black teas that were subjected to full fermentation process, whereas trace amounts of catechins were found in extracts of the red teas (Pu'er type) that were non-enzymatically fermented (tab. 2, fig. 2). Thus, the fermentation process significantly affected the catechin amount in tested tea products. Peterson et al. [24] established that the total content of catechins declined with increasing degree of fermentation process of tea leaves. Average level of (+)-catechin in green tea was 135.75 mg/g d.w., instead of Oolong tea (54.47 mg/g d.w.) and black tea (41.82 mg/g d.w.).

It is also suggested that catechins occur in higher amounts in green and white teas, whereas teaflavin and tearubigin originated in fermentation process are present mostly in Oolong and black teas. In general, the level of the catechin constituents in about 90% is responsible for the total antioxidant activity of green tea extracts. Conversely, black teas possess significantly lower antioxidant properties that are connected with teaflavin presence, and in their extracts, content of teaflavin is significantly differentiated and depends on specific conditions of the technological process. The extracts of the black teas were characterized by lower capacity in binding of free radicals of $DPPH^{\bullet}$ in comparison with green teas [5]. Unachukwu et al. [25] showed that water extracts of white teas may vary widely in level of total catechins (14.40-369.60 mg/g d.w.), similarly to tea extracts (21.38-228.20 mg/g d.w.). The obtained results are similar to previously published data revealing that slightly fermented tea contains approx. 10% higher ability to eliminate free radical of $DPPH^{\bullet}$ in comparison with non-fermented green teas. Antioxidative potential of tested types of tea may be classified in the following order: partially fermented tea > non-fermented tea > fermented tea [18].

To summarize, it should be underlined that performed chemical analyses and previously published data [21-23, 30] indicated that catechins possess the high impact on antioxidative activity of the water infusion of teas. The catechins content in blue, green and white teas varied from 15 to 42% dry mass of leaves, whereas in the most often used black teas, only in a range of 3-10% d.m. Other analyzed parameters, like total level of phenolic compounds and/or total flavonoids content might also be sensitive indicators of the antioxidative potential of the water tea extracts.

CONCLUSIONS

1. The studied products showed different antioxidant potential towards the DPPH• radical.
2. The partially fermented white teas showed the highest DPPH• antioxidant potential, except the red teas and the most popular black teas with the lowest antioxidative potential among the studied tea products.
3. Thus, higher usage of white, green and blue teas, instead of less vulnerable black teas with lower antioxidative potential is recommended.

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MOŻLIWOŚCI ANTYOKSYDACYJNE POCHODNYCH KATECHINY W WYBRANYCH GATUNKACH HERBATY

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S t r e s z c z e n i e

Celem niniejszej pracy było określenie poziomu potencjału antyoksydacyjnego pięciu rodzajów herbat (białe, czerwone, zielone, czarne i niebieskie/turkusowe) pochodzących z różnych regionów geograficznych. Analizie porównawczej zostały także poddane zawartość i skład jakościowy związków fenolowych w wodnych ekstraktach badanych herbat. Najwyższą aktywność antyoksydacyjną wobec wolnego rodnika $DPPH^{\bullet}$ stwierdzono w ekstraktach otrzymanych z herbat białych, zaś najniższą w ekstraktach uzyskanych z herbat czerwonych. Wykazano ponadto, że najwyższą zawartością związków fenolowych ogółem charakteryzowały się wyciągi przygotowane z herbat białych. Dodatkowo, przeprowadzona analiza HPLC umożliwiła zidentyfikowanie w ekstraktach badanych herbat następujących pochodnych katechiny: (+)-katechiny, (-)-epikatechiny, (-)-epigalokatechiny i galusanu (-)-epikatechiny. Jednocześnie wykazano, że dominującą pochodną w ekstraktach z wszystkich testowanych herbat była (+)-katechina.

Słowa kluczowe: *herbata, związki fenolowe, katechiny, potencjał antyoksydacyjny*