

## EXPERIMENTAL PAPER

# Antiradical activity and amount of phenolic compounds in extracts obtained from some plant raw materials containing methylxanthine alkaloids

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### Summary

Raw materials containing methylxanthine alkaloids such as yerba mate, guaraná, white and green tea, coffee seeds, chocolate and cacao seed powder and extracts obtained from these raw materials were investigated for their antioxidant features and the amount of phenolic compounds. The level of phenolic compounds was measured with the colorimetric method using Folin-Ciocalteu's phenol reagent and antioxidant features was determined with the use of DPPH (2,2-diphenyl-1-picrylhydrazyl radical). Amounts of phenolic compounds were presented in percentages per mass of extracts and mass of raw materials. Antiradical potential was shown as the number of  $TAU_{DPPH}$  units per mg of extracts and g of raw materials. The highest number of antiradical units  $TAU_{DPPH}$  as well as the amount of phenolic compounds was calculated for white tea and its extracts and lowest for chocolate. The correlation coefficient between the content of phenolics and antiradical features of raw materials is equal to  $r=0.994$ .

**Key words:** antioxidants, free radical scavengers, plant extracts, plant phenolics, methylxanthine alkaloid raw materials, antiradical units

## INTRODUCTION

Plant raw materials containing methylxanthine alkaloids are rich in phenolic compounds [1-4]. Also extracts obtained from these sources contain large quantities of phenols with strong antioxidative activity. Tea leaves and extracts from tea are among the most effective. Among them white and green tea exhibit the strongest antioxidant or antiradical properties [5].

Guaraná, *Paullinia cupana* Kunth, is a member of the climbing plant family of *Sapindaceae*. The plant is native to the Amazon basin including Brazil. Guaraná seeds are rich in caffeine and other methylxanthine derivatives, also contains tannins and saponins. This raw material contains about 12% tannins, mainly proanthocyanidins, about 6% (+)-catechin and 3.8% (-)-epicatechin [6]. Guaraná was consumed for centuries by Indians, who added it to drinks and foods in order to decrease fatigue and to increase vigor [4, 7]. Nowadays, guaraná is used for the production of soft drinks. Guaraná powder is used as a nutritional supplement to enhance vigor, to inhibit the platelet aggregation, protect against gastric injury caused by ethanol, and for antimicrobial and antioxidant activity [4].

White tea (*Camellia sinensis* (L.) Kuntze) is a less processed kind of tea (steamed, then dried without withering) and exhibit slightly higher antiradical properties than green teas [5, 8]. It acts advantageously in the prevention of many diseases, such as heart and neurodegenerative diseases as well as cancers [8]. White tea is produced from buds and leaves of *Camellia sinensis*. The white color comes from the hair of unopened buds. White tea contains similar amount of catechin derivative as epigallocatechin gallate as green tea but the amount of epigallocatechin is higher than in green teas. Main catechins in white tea are catechin, epigallocatechin gallate, epigallocatechin, and epicatechin [5, 8].

Yerba mate beverage originates from the leaves of *Ilex paraguariensis* A. St.-Hil. It is consumed in South America countries such as Brazil (South part), Argentina, Uruguay and Paraguay. The raw material to be infused consists of dry leaves of *Ilex paraguariensis* belonging to the botanical family *Aquifoliaceae*. According to the literature, yerba mate has hypocholesterolemic and hepatoprotective activity, and also exhibits a stimulating effect on the central nervous system [1, 9]. There was also observed diuretic and antioxidant activity [1, 10, 11], an advantageous effect on the cardiovascular system [12, 13], and protection of DNA and LDL against oxidation [14]. Leaves of *Ilex paraguariensis* contain polyphenols such as phenolic acids (chlorogenic, caffeic, dicaffeoylquinic acids), flavonoids (quercetin, kaempferol, rutin), methylxanthines such as caffeine and smaller quantity of theobromine [9]. The leaves of yerba mate also contain amino acids, some vitamins such as C, B<sub>1</sub>, B<sub>2</sub>, and mineral components (Fe, P, Ca) [9]. In some cases during use of yerba mate serious adverse effects have been observed, such as oral, oropharyngeal and esophageal cancers [15].

Coffee is the most frequently consumed drink in the world, along with tea. Seeds of coffee contain numerous compounds with antiradical activity such as chlorogenic, 5-caffeoylquinic, feruloylquinic and caffeic acids, melanoidins and caffeine [16].

Cocoa beans originate from plants of *Theobroma cacao* L. The amount of total phenols in cocoa powder is about 6%. There are mainly flavan-3-ols, such as monomeric catechin or epicatechin, their oligomers (procyanidins) and flavonoids among other derivatives of quercetin [16, 17].

Chocolate is obtained from cacao. Over many years the use of cacao (*Theobroma cacao*) evolved into the product called chocolate. Chocolate is rich in lipids with high amounts of saturated fatty acids such as stearic (30–33%) and palmitic (28–30%) [18]. Besides, some amounts of sterols were identified as well as minerals, flavonoids, catechin, epicatechin, and procyanidins (in cocoa beans 12–48%). Chocolate has a concentration of flavan-3-ol higher than most plant-based foods [19, 20]. The polyphenols present in chocolate and cacao beans might exert advantageous vascular effects [21]. These compounds might reduce cardiovascular morbidity [22], and diseases with chronic character [23]. According to scientific opinion demonstrated in EFSA (European Food Safety Authority) monomeric catechins (mainly epicatechin) and oligomers (procyanidins) maintain "endothelium-dependent vasodilation which contributes to healthy blood flow" at a daily dose of 200 mg [24, 25].

It is necessary to mention that chocolate are high processed products and the amount of phenols and the antioxidant activity varies widely. The strongest antioxidant activity exhibit dark chocolate but weakest milk chocolate [26].

All above described raw materials are known to have high amounts of phenolic compounds such as tannins, mainly condensed [4, 27], catechins and gallic acid derivatives (epigallocatechin gallate, EGCG) [28], and other coupling of catechins such as procyanidins [29], and simple phenolic acids (gallic, caffeic) [30–32]. Other phenols belong to the very wide group of flavonoids such as, the most common, derivatives of quercetin, or kaempferol [31, 33].

The demonstration of methylxanthine raw materials' strong antiradical or antioxidant activity might expand their therapeutic use as a weapon to fight diseases caused by free radicals and reactive oxygen species.

Free radicals and reactive oxygen species (ROS) are formed in normal physiological processes and in some diseases, especially chronic [34]. The physiological level of free radicals and ROS is maintained with an enzymatic system (superoxide dismutase, catalase), and natural antioxidants such as glutathione, or NADPH, and vitamins E and C can decrease the amount of ROS [35–37]. During chronic inflammation, in diseases such as diabetes, free radicals are formed in excess and cannot be effectively eliminated from the organism. Then, providing strong antioxidants or free radical scavengers in diet or as a medicine could be advantageous.

As it was stated above, raw materials containing methylxanthines (caffeine, theobromine, theophylline) are rich in polyphenols with strong antioxidant properties. Thanks to the content of phenolic compounds, these raw materials could have many beneficial effects to health such as antiatherosclerotic effect.

Although methylxanthines exhibit some antioxidant properties, the main components responsible for antioxidant activity are polyphenols. There were described studies [26] that antioxidant activity of different types of chocolate positively correlated with the content of phenolic compounds.

The aim of this work was to measure the antioxidant activity of these raw materials and study the correlation between antioxidant activity and the amount of phenolic compounds in extracts.

## MATERIAL AND METHODS

### Raw materials

White tea leaf from *Camellia sinensis* (L.) Kuntze (wt) – 44.5 g  
Guaraná seeds from *Paullinia cupana* Kunth (gs) – 48.6 g  
Yerba mate leaf from *Ilex paraguariensis* A. St.-Hil. (ym) – 39.8 g  
Green tea leaf (gunpowder) from *Camellia sinensis* (L.) Kuntze (gt) – 50.0 g  
Costa Rica coffee seeds from *Coffea arabica* L. (cc) – 48.0 g  
Arabica coffee seeds from *Coffea arabica* L. (ca) – 46.0 g  
Chocolate (Ristora) (cr) – 48.1 g  
Chocolate (van Heuten) (ch) – 50.0 g  
Chocolate (Tazza) (ct) – 48.1 g  
Cacao (van Heuten) seed powder from *Theobroma cacao* L. (c) – 50.0 g  
All raw materials were of commercial origin, edible quality.

### Preparation of extracts

Raw materials were extracted according to the modified method described by Kasprzyk *et al.*, [38] with methanol (900 ml) at a temperature of 50°C for 48 h. Twenty percent of methanol extract (180 ml) was separated and concentrated to dryness under reduced pressure to obtain the WA residue. The remaining part of methanol extract (720 ml) was concentrated to dryness under reduced pressure and then dissolved in 600 ml of water at 45°C. Water solution of extract was stored at 4°C for 48 h. The precipitate was separated with filter paper (Filtrak, 388, 80 g/m<sup>2</sup>) and then dried under reduced pressure to obtain the WD residue.

After precipitate separation, aqueous solution was exhaustively extracted with ethyl acetate. Aqueous remaining and ethyl acetate solution were concentrated to dryness under reduced pressure to obtain WB and WC residues, respectively.

Extracts from white tea were marked additionally with “wt”, extracts from guaraná seeds with “gs”, extracts from yerba mate “ym”, extracts from green tea with “gt”, extracts from Costa Rica coffee “cc”, extracts from Arabica coffee “ca”, extracts from chocolate (Ristora) with “cr”, extracts from chocolate (van Heuten) with “ch”, extracts from chocolate “Tazza” with “ct”, and extracts from cacao (van Heuten) with “c”. For example, extracts obtained from white tea leaves are marked with WAwt, WBwt, WCwt and WDwt.

During extraction to the ethyl acetate, in most cases the emulsion was formed, which was individually separated and then condensed to dryness under reduced pressure to obtain WE extract.

## Measurement of total phenolic compounds

The amount of total phenolic compounds was measured with use the method of Singleton and Rossi [39]. Seven ml of water, then 0.5 ml of Folin and Ciocalteu's phenol reagent ( $3\text{H}_2\text{O} \times \text{P}_2\text{O}_5 \times 13\text{WO}_3 \times 5\text{MoO}_3 \times 10\text{H}_2\text{O}$ ) and 0.5 ml of methanol solution of extract was poured into the test tube. After 3 min, 2 ml of 20% aqueous solution of sodium carbonate was added. The mixture was heated at  $100^\circ\text{C}$  for 1 min. After cooling, absorbency was measured at 685 nm. The measurement was repeated for five times. Maximal error was calculated with total differential method. Phenolic compounds were expressed as gallic acid and calculated in percentage per weight of extract.

## Antiradical activity of extracts

Antiradical activity of extracts was measured by the method of Brand-Williams *et al.* [40]. The decrease of absorbency of DPPH (2,2-diphenyl-1-picrylhydrazyl radical) solution in methanol ( $94 \mu\text{mol/l}$ ) at 515 nm is measured in the presence of a substance with antiradical activity. The rate of the decrease of absorbance is proportional to antiradical activity of the substance.

DPPH was dissolved in methanol (gradient grade, Merck). The reagent was prepared a day before the experiment so that absorbency at 515 nm of solution was stable.

2 ml of DPPH solution ( $94 \mu\text{mol/l}$ ) was placed in glass cuvette with optical path of 1 cm.  $50 \mu\text{l}$  of methanol solution of extract was added. The absorbance of the solution was measured at 0 and 60 s.

The antiradical potential of the extract was demonstrated as a number of antiradical units  $TAU_{\text{DPPH}}$  per mg of extract ( $TAU_{\text{DPPH/mg}}$ ) and per g of raw material ( $TAU_{\text{DPPH/g}}$ ) calculated according to the equations (1) and (2), respectively.

One unit of antiradical activity is the quantity of antioxidant that scavenges  $1 \mu\text{mol}$  of DPPH radical in 1 ml of reaction mixture during 1 minute of reaction at  $25^\circ\text{C}$ .

$$TAU_{\text{DPPH/mg}} = 0.0799 \cdot \frac{A_{s0} - A_{s1}}{c} \quad (1)$$

The above equation was derived using absorption attenuation coefficient ( $\epsilon$ ) equal to:  $1.2509 \cdot 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  [40], where  $TAU_{\text{DPPH/mg}}$  is the number of antiradical units per mg of substance,  $A_{s0}$  is absorbency of DPPH solution at the beginning of the reaction,  $A_{s1}$  is absorbency of DPPH solution after 1 min of reaction,  $c$  is concentration of substance in reaction mixture [mg/ml].

The number of antiradical units per gram of raw material ( $TAU_{DPPH/g}$ ) was calculated by summing up all  $TAU_{DPPH/mg}$  units calculated for all extracts multiplied by the total mass of extracts obtained from given raw material according to the equation:

$$TAU_{DPPH/g} = \frac{(TAU_{DPPH/mgWA} \cdot m_{WA}) + (TAU_{DPPH/mgWB} \cdot m_{WB}) + (TAU_{DPPH/mgWC} \cdot m_{WC}) + (TAU_{DPPH/mgWD} \cdot m_{WD}) + (TAU_{DPPH/mgWE} \cdot m_{WE})}{W_R} \quad (2)$$

where  $TAU_{DPPH/g}$  is the number of antiradical units calculated per g of raw material;  $TAU_{DPPH/mgWA}$ ,  $TAU_{DPPH/mgWB}$ ,  $TAU_{DPPH/mgWC}$ ,  $TAU_{DPPH/mgWD}$ ,  $TAU_{DPPH/mgWE}$  is the number of antiradical units per mg of extract WA, WB, WC, WD and WE respectively;  $m_{WA}$ ,  $m_{WB}$ ,  $m_{WC}$ ,  $m_{WD}$ ,  $m_{WE}$  is whole mass of WA, WB, WC, WD, WE extracts [mg], respectively;  $W_R$  is mass of raw material [g] taken for extraction.

## RESULTS AND DISCUSSION

The number of antiradical units calculated per mg of extracts ( $TAU_{DPPH/mg}$ ) and amount of phenolic compounds in extracts and raw materials ( $Ph\%$ ) is demonstrated in table 1, figure 1, and figure 2. The general observation is that the highest values of  $TAU_{DPPH/mg}$  were obtained for extracts WC for all investigated raw materials; also the amount of phenolic compounds for this extract was the highest. The strongest antiradical properties exhibited extracts from white tea, among them WCwt was the strongest ( $6.86 \pm 0.55$ ) among all investigated extracts. Similar, in terms of antiradical activity, were extracts obtained from leaves of green tea. Among them the strongest was WCgt extract ( $6.73 \pm 0.21$ ).

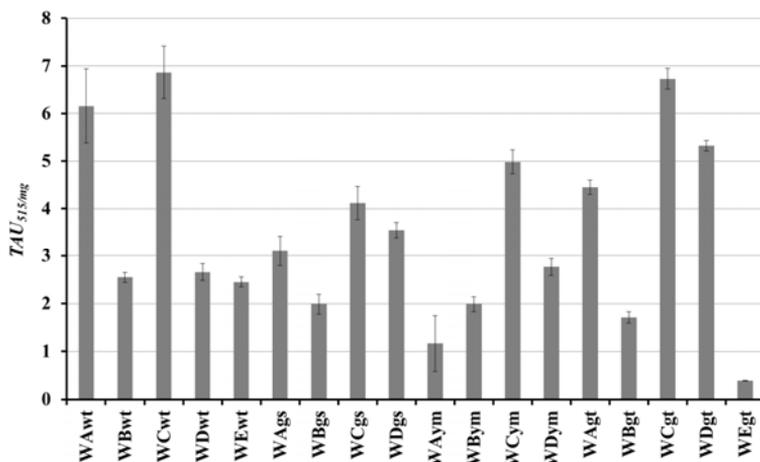


Figure 1.

Number of antiradical units ( $TAU_{DPPH/mg}$ ) per mg of extracts obtained from white tea (wt), extracts from guaraná seeds (gs), extracts from yerba mate (ym), extracts from green tea (gt).

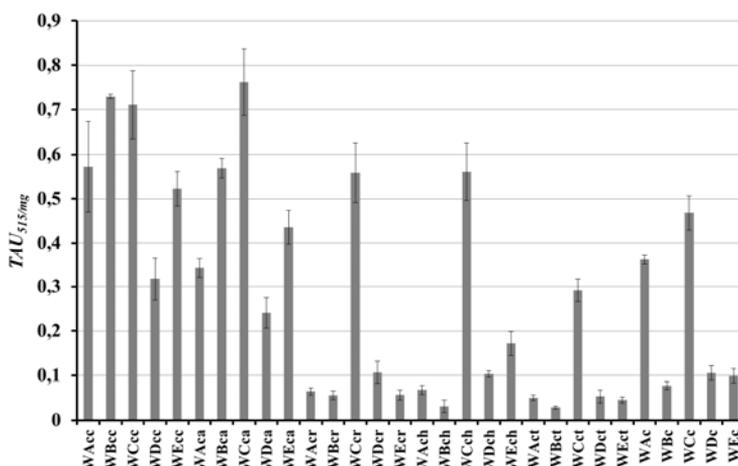


Figure 2.

Number of antiradical units ( $TAU_{DPPH/mg}$ ) per mg of extracts obtained from coffee Costa Rica (cc), coffee Arabica (ca), chocolate Ristora (cr), chocolate van Heuten (ch), chocolate Tazza (ct) and cacao (c).

Table 1.

The weight of extracts [mg], number of antiradical units per mg of extract ( $TAU_{DPPH/mg}$ ), amount of phenolic compounds in extracts expressed in percentage, number of antiradical units per g of raw material ( $TAU_{DPPH/g}$ ), amount of phenolic compounds in raw materials ( $Ph\%$ ) expressed in percentage.

Raw material	Extract	Weight of extract [mg]	$TAU_{DPPH/mg}$	The amount of phenolic compounds in extracts [w/w]	$TAU_{DPPH/g}$	The amount of phenolic compounds in raw materials $Ph\%$ [w/w]
wt	WAw	2523.9	$6.16 \pm 0.78$	$43.2 \pm 3.22$	1268.6 $\pm$ 133.5	12.26 $\pm$ 10.57
	WBw	4606.8	$2.55 \pm 0.10$	$21.1 \pm 2.25$		
	WCw	3160.6	$6.86 \pm 0.55$	$65.6 \pm 3.24$		
	WDw	2616.6	$2.66 \pm 0.17$	$49.2 \pm 2.23$		
	WEw	213.8	$2.45 \pm 0.11$	$15.8 \pm 0.89$		
gs	WAg	1654.5	$3.10 \pm 0.31$	$30.5 \pm 2.01$	474.8 $\pm$ 44.6	4.42 $\pm$ 3.93
	WBg	1899.6	$1.98 \pm 0.21$	$18.1 \pm 1.29$		
	WCg	1518.7	$4.12 \pm 0.35$	$37.1 \pm 2.55$		
	WDg	2226.3	$3.55 \pm 0.16$	$33.2 \pm 2.25$		
ym	WAm	1701.8	$1.17 \pm 0.45$	$23.5 \pm 1.46$	445.8 $\pm$ 62.4	4.21 $\pm$ 4.26
	WBy	4095.4	$1.98 \pm 0.16$	$17.6 \pm 1.37$		
	WCy	1220.8	$4.98 \pm 0.25$	$41.3 \pm 3.16$		
	WDy	555.7	$2.76 \pm 0.17$	$9.3 \pm 1.42$		
gt	WAg	2984.7	$4.45 \pm 0.15$	$30.4 \pm 5.07$	1184.4 $\pm$ 64.4	9.79 $\pm$ 0.56
	WBg	4808.4	$1.71 \pm 0.12$	$15.84 \pm 2.64$		
	WCg	2831.4	$6.73 \pm 0.21$	$59.2 \pm 9.87$		
	WDg	3474.2	$5.32 \pm 0.11$	$42.09 \pm 7.01$		
	WEg	420.5	$0.39 \pm 0.08$	$20.17 \pm 3.36$		

Tab. 1. cont.

Raw material	Extract	Weight of extract [mg]	$TAU_{DPPH/mg}$	The amount of phenolic compounds in extracts [w/w]	$TAU_{DPPH/g}$	The amount of phenolic compounds in raw materials Ph% [w/w]
cc	WAcc	1168.9	$0.57 \pm 0.10$	$10.98 \pm 3.85$	60.9 ± 6.5	1.36 ± 0.19
	WBcc	1168.3	$0.73 \pm 0.005$	$11.79 \pm 1.39$		
	WCcc	831.2	$0.71 \pm 0.075$	$24.06 \pm 0.83$		
	WDcc	1245.2	$0.32 \pm 0.05$	$9.39 \pm 1.25$		
	WEcc	76.7	$0.52 \pm 0.04$	$13.43 \pm 0.46$		
ca	WAca	998.0	$0.34 \pm 0.02$	$6.14 \pm 1.88$	41.8 ± 4.2	0.94 ± 0.15
	WBca	1237.9	$0.57 \pm 0.02$	$13.63 \pm 0.94$		
	WCca	644.8	$0.76 \pm 0.075$	$20.11 \pm 2.60$		
	WDca	1530.4	$0.24 \pm 0.034$	$4.63 \pm 1.56$		
	WEca	36.5	$0.44 \pm 0.038$	$12.53 \pm 1.0$		
cr	WAc	857.8	$0.065 \pm 0.008$	$1.51 \pm 0.13$	6.3 ± 1.1	0.088 ± 0.004
	WBcr	3238.5	$0.055 \pm 0.009$	$0.59 \pm 0.10$		
	WCcr	75.3	$0.56 \pm 0.067$	$9.85 \pm 1.72$		
	WDcr	212.3	$0.11 \pm 0.024$	$2.25 \pm 0.20$		
	WEcr	67.8	$0.06 \pm 0.01$	$1.54 \pm 0.19$		
ch	WAch	2033.4	$0.068 \pm 0.01$	$1.81 \pm 0.20$	10.4 ± 3.2	0.260 ± 0.052
	WBch	7757.0	$0.031 \pm 0.01$	$0.77 \pm 0.20$		
	WCch	131.6	$0.56 \pm 0.065$	$16.52 \pm 0.68$		
	WDch	348.6	$0.10 \pm 0.007$	$1.23 \pm 0.38$		
	WEch	194.4	$0.17 \pm 0.03$	$3.77 \pm 1.13$		
ct	WAct	671.4	$0.05 \pm 0.006$	$1.37 \pm 0.30$	3.0 ± 0.44	0.067 ± 0.02
	WBct	2405.9	$0.029 \pm 0.003$	$0.51 \pm 0.29$		
	WCct	86.2	$0.29 \pm 0.025$	$8.54 \pm 0.58$		
	WDct	207.1	$0.05 \pm 0.015$	$1.0 \pm 0.17$		
	WEct	92.4	$0.04 \pm 0.006$	$0.82 \pm 0.08$		
c	WAc	646.2	$0.28 \pm 0.008$	$5.73 \pm 0.55$	9.2 ± 0.87	0.282 ± 0.019
	WBc	1670.0	$0.061 \pm 0.007$	$3.72 \pm 0.17$		
	WCc	349.8	$0.37 \pm 0.03$	$9.88 \pm 0.52$		
	WDC	580.7	$0.082 \pm 0.012$	$1.26 \pm 0.18$		
	WEc	4.3	$0.078 \pm 0.012$	$2.56 \pm 0.19$		

WA - methanol extract, WB - water remaining extract, WC - ethyl acetate extract, WD - precipitate, WE - emulsion (layer formed between the water and ethyl acetate), "wt" white tea, "gs" guaraná seeds, "ym" yerba mate, "gt" green tea, "cc" coffee seeds (Costa Rica), "ca" coffee seeds (Arabica), "cr" chocolate Ristora, "ch" chocolate van Heuten, "ct" chocolate Tazza, "c" cacao. Value  $TAU_{DPPH/mg}$  for trolox is  $3.22 \pm 0.06$ .

White and green tea extracts appeared to be the strongest antiradicals, and white tea was even more active than green tea. Our previous research exhibited strong antiradical features of green tea leaves [41]. Green and white teas do not undergo a fermentation process. These two kinds of teas contain phenolic compounds such as gallic acid and gallic acid gallate [5], which appeared to be very effective antiradical scavengers.

The raw materials such as yerba mate (ym) and guaraná seeds (gs) appeared to have lower antiradical properties than those of white and green teas (tab. 1, fig. 3). Yerba mate contains many classes of caffeoyl derivatives (caffeic, chlorogenic and 5-caffeoylquinic acids and flavonoids, such as rutin [9]). Phenolic compounds present in yerba mate leaves exhibit the antioxidant activity *in vitro* and *in vivo*, and have the ability to scavenge free radicals and reactive oxygen species [10, 11].

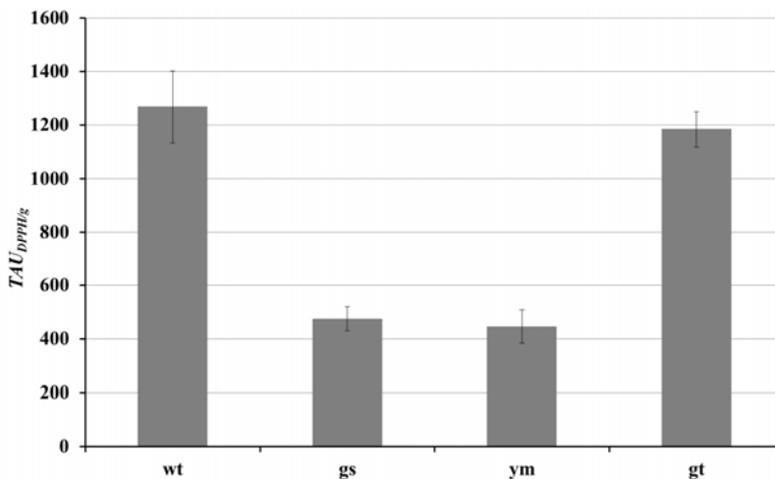


Figure 3.

Number of antiradical units per g of raw material ( $TAU_{DPPH/g}$ ) for white tea (wt), guaraná seeds (gs), yerba mate (ym), green tea (gt)

Guaraná seeds is a raw material rich in methylxanthine derivatives, and contain more caffeine than other raw materials [42]. This raw material contains polyphenols with strong antioxidant activity such as tannins, especially condensed tannins [4]. It is believed that condensed and hydrolysable tannins apart from antioxidant activity have other therapeutic properties [43, 44].

Our investigation showed that extracts obtained from coffee seeds (cc, ca) have average antioxidative activity (tab. 1, fig. 2). The antioxidant features are positively correlated with amount of phenolic compounds in these extracts (tab. 1). Correlation coefficient ( $r$ ) between amount of phenolic compounds in extracts and number of antiradical activity  $TAU_{DPPH/mg}$  was equal to 0.91 (fig. 4). Correlation coefficient ( $r$ ) between amount of phenols and number of activity units in raw materials ( $TAU_{DPPH/g}$ ) was equal to 0.99.

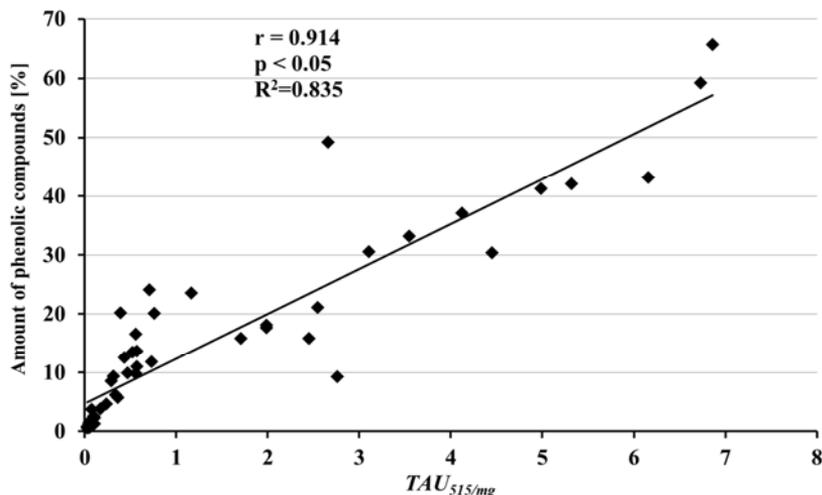


Figure 4.

Correlation coefficient ( $r$ ) between number of antiradical units per mg of extract ( $TAU_{515/mg}$ ) and amount of phenolic compounds in extracts (% w/w)

Seeds of coffee are known to contain effective antiradical phenolic compounds such as caffeic and chlorogenic acids [45]. The chlorogenic acid, apart from antioxidant activity, also exhibits some astringency and bitterness. Other phenolic compounds present in coffee seeds are ferulic, *p*-coumaric, vanillic, and syringic acids [46], which are rather weak antioxidants. Phenolic compounds play an important role in the quality and flavor of coffee seeds. Other compounds present in coffee seeds with antioxidant activity are melanoidins [47] and caffeine [48].

Our investigation showed that chocolates (cr, ch, ct) appeared to be the weakest antioxidants among all raw materials investigated in this work (tab. 1, fig. 5), which correlated positively with small amount of phenolic compounds. Chocolate has variable amounts of phenolic compounds. White chocolate contains low amounts of phenols, dark chocolate is rich in phenols [45]. The amount of phenolic compounds correlated positively with antioxidant activity in dark, milk and white chocolate [49]. According to Miller *et al.* [19] the antiradical activity varies from  $66.7 \pm 8.7$  ( $\mu\text{mol TE/g}$ ) for chocolate syrup to  $499 \pm 35.5$  ( $\mu\text{mol TE/g}$ ) for baking chocolate and even to  $816 \pm 37.6$  ( $\mu\text{mol TE/g}$ ) for cacao powder. The main phenolic compounds present in chocolate are epicatechin, catechin, procyanidin B2, procyanidin C1, and procyanidin B5 which are antioxidants, valuable for health [50].

In our research, cacao powder and extracts from this raw material were found to be weak antioxidants (tab. 1, fig. 5). The literature describes rather strong antiradical activity of cocoa [19, 51] due to presence of high amounts of phenolic compounds.

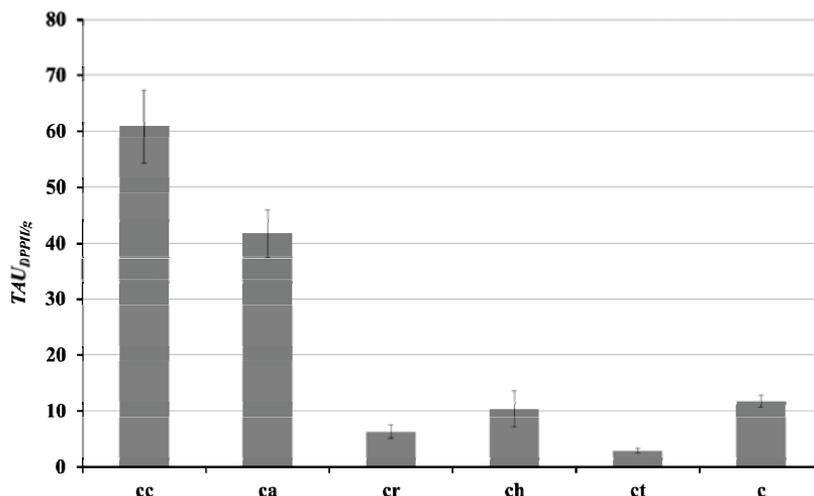


Figure 5.

Number of antiradical units per g of raw material ( $TAU_{DPPH/g}$ ) for Costa Rica coffee (cc), Arabica coffee (ca), Ristora chocolate (cr), van Heuten chocolate (ch), Tazza chocolate (ct), cacao (c)

## CONCLUSIONS

1. It can be concluded that the extracts of non-fermented, white and green teas exhibited very high antioxidant features. Much lower activity was observed for guaraná seeds and yerba mate leaves, and chocolates and cocoa powder appeared to be the weakest antioxidants.
2. The antioxidant properties highly positively correlated with the amount of phenolic compounds.

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## AKTYWNOŚĆ PRZECIWRODNIKOWA ORAZ ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH W WYCIĄGACH OTRZYMANYCH Z SUROWCÓW ROŚLINNYCH ZAWIERAJĄCYCH ALKALOIDY METYLOKSANTYNOWE

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### Streszczenie

Surowce takie jak yerba mate, guaraná, biała i zielona herbata, nasiona kawy, czekolada i kakao zawierające alkaloidy metyloksantynowe były badane na zawartość związków fenolowych i aktywność przeciwutleniającą. Poziom związków fenolowych określono metodą kolorymetryczną za pomocą odczynnika Folin-Ciocalteu, a właściwości przeciwutleniające mierzono stosując rodnik DPPH (difenyl-pikrylohydrozylowy). Ilość związków fenolowych wyrażono w procentach masy wyciągów i surowców. Potencjał przeciwrodnikowy określono jako liczbę jednostek  $TAU_{DPPH}$  na mg wyciągów i g surowców. Największą liczbę jednostek przeciwrodnikowych  $TAU_{DPPH}$  oraz najwyższą zawartość związków fenolowych wykazano dla białej herbaty i wyciągów otrzymanych z tego surowca, a najniższe wartości uzyskano dla czekolady. Współczynnik korelacji pomiędzy ilością związków fenolowych i aktywnością przeciwrodnikową surowców wynosił  $r=0,994$ .

**Słowa kluczowe:** przeciwutleniacze, zmiatacze wolnych rodników, wyciągi roślinne, fenole roślinne, surowce zawierające alkaloidy metyloksantynowe, jednostki przeciwrodnikowe