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Investigation of antiradical potential of different kinds of teas and extracts from these teas using antiradical activity units (TAU)*

Badanie potencjału przeciwwolnorodnikowego różnych rodzajów herbat oraz wyciągów uzyskanych z tych herbat za pomocą jednostki aktywności przeciwwolnorodnikowej (TAU)

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Summary

Introduction: Green, black and pu-erh teas are known to have strong antiradical properties in comparison to other plant raw materials.

In this study fifteen different teas belonging to three types, green, black, and pu-erh tea (five of each kind), were investigated for their antiradical properties. Antiradical activity of extracts and raw materials was measured using DPPH and $ABTS^{\bullet+}$ radicals.

Material/Methods: The antiradical potential of teas was measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) and $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt). The TAU_{515} and TAU_{734} (antiradical activity units) were defined with the tests of DPPH and $ABTS^{\bullet+}$, respectively, and the number of units was calculated per mg of extracts ($TAU_{515/mg}$ and $TAU_{734/mg}$) and g of raw materials ($TAU_{515/g}$ and $TAU_{734/g}$).

Results/Discussion: When the extracts were investigated, the highest number of antiradical units ($TAU_{515/mg}$; $TAU_{734/mg}$) was found per mg of ethyl acetate extract obtained from green tea leaves assayed with DPPH and $ABTS^{\bullet+}$: 57.7±0.8; 106±2.0, units respectively. When the number of antiradical units $TAU_{515/g}$ was calculated per g of raw material, the highest antiradical potential (7601±92) was observed for green tea leaves. The greatest $TAU_{734/g}$ value (14 303±354) was also calculated for green tea leaves. The lowest value of antiradical activity units $TAU_{515/g}$ (684±30) and $TAU_{734/g}$ (1870±180) was calculated for pu-erh tea leaves.

A high positive correlation was found between the antiradical activity of the raw materials and the content of phenolic compounds in these raw materials.

Key words: **tea leaves • phenolic compounds • antiradical properties**

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Streszczenie

Wprowadzenie: Herbata zielona, czarna i pu-erh mają, w porównaniu do innych surowców roślinnych, silne właściwości przeciwwolnorodnikowe.

W pracy zbadano15 różnych herbat należących do trzech typów, zielonych, czarnych i pu-erh (po pięć rodzajów z każdego typu) w kierunku ich właściwości przeciwwolnorodnikowych. Aktywność przeciwwolnorodnikową wyciągów i surowców zmierzono za pomocą rodników DPPH i $ABTS^{\bullet+}$.

Materiały/Metody: Aktywność przeciwwolnorodnikową herbat zmierzono za pomocą DPPH (rodnika 1,1-difenylo--2-pikrylohydrazylowego) i $ABTS^{\bullet+}$ (soli diamonowej kwasu 2,2'-azyno-bis(3-ethylobenzotiazolino-6-sulfonowego)). Zdefiniowano jednostki TAU_{515} i TAU_{734} (jednostki aktywności przeciwwolnorodnikowej) odpowiednio dla testów z DPPH i $ABTS^{\bullet+}$ i określono liczbę jednostek na mg wyciągów ($TAU_{515/mg}$ i $TAU_{734/mg}$) i g surowców ($TAU_{515/g}$ i $TAU_{734/g}$).

Wyniki/Dyskusja: Gdy badano wyciągi, największą liczbę jednostek aktywności przeciwwolnorodnikowej $TAU_{515/mg}$ i $TAU_{734/mg}$ stwierdzono w mg wyciągu octanowego otrzymanego z liści herbaty zielonej: odpowiednio 57,7±0,8; 106±2,0 jednostek.

Gdy liczbę jednostek aktywności przeciwwolnorodnikowej ($TAU_{515/g}$) policzono na g surowca, największy potencjał wolnorodnikowy (7601±92) zaobserwowano dla liści herbaty zielonej, największą wartość $TAU_{734/g}$ (14303±354) obliczono również dla liści herbaty zielonej. Najniższą aktywność przeciwwolnorodnikową $TAU_{515/g}$ (684±30) i $TAU_{734/g}$ (1870±180) obliczono dla liści herbaty pu-erh.

Zaobserwowano wysoką, dodatnią korelację pomiędzy aktywnością przeciwwolnorodnikową surowców i zawartością związków fenolowych w tych surowcach.

Słowa kluczowe: liście herbaty • związki fenolowe • właściwości przeciwwolnorodnikowe

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Abbreviations: $TAU_{515/mg}$ **and** $TAU_{734/mg}$ – the number of total antiradical units calculated per mg of extract using DPPH and $ABTS^{\bullet+}$ respectively; $TAU_{515/g}$ **and** $TAU_{734/g}$ – the number of total antiradical units calculated per g of raw material using DPPH and $ABTS^{\bullet+}$ respectively.

Introduction

The leaves of tea (*Camellia sinensis* (L.) Kuntze) are known to have strong antiradical and antioxidant properties [26]. Brewed tea has been consumed for many centuries and health benefits have been attributed to tea since the very beginning of tea history. Nowadays tea of different kinds (especially green and black) is the beverage most widely consumed, next to water, in the whole world [18]. Some research made *in vitro* and using animals provides evidence that the polyphenolic compounds present in teas act beneficially in several chronic diseases [4,7,11]. For example, epigallocatechin gallate (EGCG) is attributed to be responsible for the ability of green tea to promote health [19]. Both green tea and black tea are considered to have anti-carcinogenic properties, which has been proved in many animal models [15,25].

Black tea is the most consumed tea in the world. More than 70% of all tea produced is black tea [6]. Many studies have demonstrated the health benefits of black tea consumption [14].

It was demonstrated that risk of myocardial infarction was lower by 11% when consumption of the beverage was three cups a day (about 700 ml) [12]. Other authors reported a considerable decrease of coronary heart disease (CHD) when more than a cup of tea is drunk daily [17]. Some authors did not observe any beneficial effect [9], while others observed a positive correlation between tea consumption and a lower risk of CHD mortality [10] as well as hypertension, plaques of the carotid artery, and increased homocysteine concentration in plasma [14].

Brewed tea is rich in various types of polyphenolic compounds [6]. Ruxton [15] investigated the content of phenols

in brewed black and green tea. Black tea in decreasing order is rich in epigallocatechin (10.43 mg/100 ml), epicatechin (2.33), quercetin (2.07), theaflavins (1.58), catechin (1.52), kaempferol (1.34), gallocatechin (1.26) and myricetin (0.45). Green tea is rich in epigallocatechin (17.08 mg/100 ml), epicatechin (8.47), catechin (2.73), quercetin (2.69), kaempferol (1.42), myricetin (1.10) and theaflavins (0.05) [14].

Tea polyphenols possess strong antiradical and antioxidant properties, and health benefits of consumption of tea are attributed partially to the antiradical and antioxidant properties of tea [6]. Tea extracts have been shown to scavenge singlet oxygen [8], hydroxyl radical [23], peroxyl radical [16], superoxide radical [24] and many other dangerous compounds which lead, among other things, to the destruction of lipids, proteins and nucleic acids [21].

The antioxidant activity of green tea extracts is always higher than that of black tea [1]. Especially effective as an antioxidant is epigallocatechin gallate (EGCG), which was detected in high concentrations in green tea extracts [5]. Green and black tea, *in vitro*, can inhibit lipoprotein oxidation initiated in the presence of Cu^{2+}.

The antioxidant and antiradical activity of teas has been measured by several methods. The methods can be divided into so-called hydrogen atom transfer (HAT) and electron transfer methods (ET). The methods called FRAP (ferric reducing ability of plasma, also ferric ion reducing antioxidant power) belong to ET methods and use Trolox (water soluble vitamin E analogue) as a standard. The methods using DPPA and $ABTS^{\bullet+}$ radicals belong to the HAT methods [3]. Another problem is the way of demonstration of antiradical features of substances. Some authors use standard substances known for their antiradical activity such as vitamin C or Trolox [14].

However, there is a lack of papers demonstrating antiradical activity of raw materials, extracts or substances as a general pool of hypothetical, defined units.

In this work the antiradical activity of different types of teas was measured and presented as the number of antiradical units TAU_{515} and TAU_{734} for the tests with DPPH and $ABTS^{\bullet+}$ radicals per mg of extracts and g of raw materials.

Material and Methods

Raw material

Green teas

The leaf of green tea from China bought from PPH Biofluid sp.j. was marked with $\mathbf{G_1}$, the green classic English tea leaf from India was marked with $\mathbf{G_2}$, natural green tea Dilmah Sri Lanka (Sri Lanka) with $\mathbf{G_3}$, the leaf of green tea Sir Roger (China) with $\mathbf{G_4}$, and the green tea Teekanne (China) with $\mathbf{G_5}$.

Black teas

The leaf of classic English tea, Tetley from India was marked with $\mathbf{B_1}$, Premium tea Ceylon, Dilmah (Sri Lanka) was marked with $\mathbf{B_2}$, Yellow Label tea, Lipton, a mixture of about 20 kinds of black teas, with $\mathbf{B_3}$, Yunnan Black Tea, Oskar International Trading sp. z o.o., from China, with $\mathbf{B_4}$, and Assam, Teekanne from India with $\mathbf{B_5}$.

Pu-erh teas

Pu-erh Bio-Active sp. z o.o. (China) – $\mathbf{P_1}$, pu-erh PPH Biofluid sp.j. (China) – $\mathbf{P_2}$, pu-erh Vitax Preminum Foods SA (China) – $\mathbf{P_3}$, pu-erh Bastek Coffee & Tea (China) – $\mathbf{P_4}$, pu-erh Herbapol Lublin SA (China) – $\mathbf{P_5}$.

Preparation of extracts

50 g of raw material was mixed with 900 ml of methanol and water (1:3). The raw material was heated at 70°C for 30 min, then was extracted at room temperature for 24 hours. The extract was filtered (filter discs, grade 388, Filtrak). Methanol was evaporated under reduced pressure and the remaining water was extracted three times with ethyl acetate (3×200 ml). The ethyl acetate extract and remaining water was evaporated to dryness under reduced pressure to obtain the dry residues **E** (ethyl acetate) and A remaining water.

The obtained extracts were additionally marked with the letter of the raw material. For example, ethyl acetate extract obtained from natural green tea Dilmah Sri Lanka (Sri Lanka) $\mathbf{G_3}$ was marked as $\mathbf{G_3E}$.

Colorimetric measurement of phenolic compounds

The amount of phenolic compound was measured with the method of Singleton and Rossi [20].

Reagents

Reagent 1

5.77 g of sodium tungstate ($Na_2WO_4 \times 2\ H_2O$) was dissolved in 75 ml of water. Then 8 ml of 85% solution of H_3PO_4 was added. Then the reagent was heated at 100°C for 3 hours. After cooling the volume was adjusted to 100 ml with the water.

Reagent 2

An 18% (w/o) aqueous solution of Na_2CO_3 was prepared.

Measurement of phenolic compounds

0.5 ml of reagent 1 was added to 0.5 ml of the sample (extract in 50% methanol solution at the concentration 0.17 mg/ml). Then 8.5 ml of 2 was added. The absorbance at 750 nm was measured 2 min after 2 reagent addition. The absorbance was measured in a 1-cm glass cuvette against a blind sample (0.5 ml of 50% methanol was added instead of a sample with the extract). The measurement was repeated three times and the standard deviation was calculated.

Measurement of antiradical activity of extracts with the DPPH radical

The antiradical or antioxidant activity could be demonstrated in many different ways. Among others it is compared

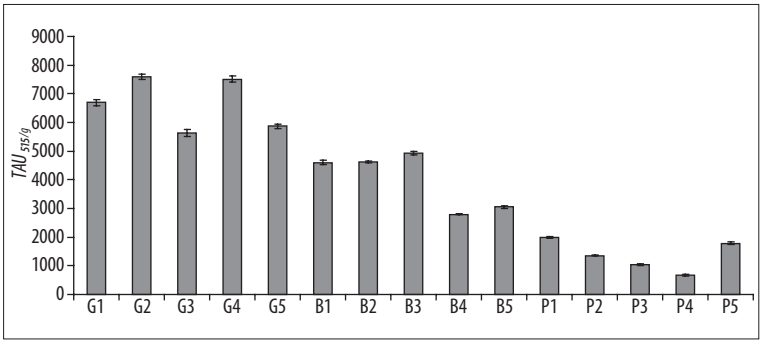

Fig. 1. The number of antiradical units $TAU_{515/g}$ calculated per g of raw material. G1-G5 – green tea, B1-B5 – black tea, P1-P5 – pu-erh

to the known antiradical standard substances such as vitamin C or Trolox. However, no authors try to demonstrate antiradical activity as a general pool of units in a determined mass of raw material and/or extracts.

For that reason we define the antiradical units in a similar way as it is done for the demonstration of enzyme activity [22]. We calculated these hypothetical units per mg of extracts and g of raw materials. We calculated the number of antiradical units per g of raw material as the sum of units calculated for extracts obtained from that raw material. The antiradical activity of extracts presented as the number of units per mg is compared to that of Trolox.

The obtained tea extracts contained compounds soluble in methanol as well as in water. Therefore two different tests were chosen to study the antiradical activity of these extracts: the method using the DPPH radical, which is suitable for fractions soluble in methanol, and the method with the $ABTS^{\bullet+}$ radical, more suitable for fractions soluble in water.

The method with DPPH was described by Brand-Williams et al. [2]. Antiradical properties were presented as the number of antiradical units TAU_{515} per mg of extract ($TAU_{515/mg}$) and per gram of raw material ($TAU_{515/g}$).

One unit is defined as the amount of substance in 1 ml of reaction mixture which causes a decrease in absorbance of 1 after 1 minute at 515 nm in a glass cuvette with a 1-cm optical path.

Preparation of reagent

DPPH radical (Sigma-Aldrich) was dissolved in methanol (Merck) at the concentration of 0.094 mmol/l. The reagent was stored at 4°C for 24 h before use. Before the test the absorbance of DPPH solution was adjusted to the value equal to 1 with methanol addition.

Measurement of DPPH radical scavenging

To 1.46 ml of DPPH methanol solution, 0.04 ml of extract solution in methanol was added. Absorbance at 515 nm was measured in glass cuvettes with an optical path of 1 cm at 1 minute after extract addition to the sample. All measurements were repeated three times and standard deviation was calculated. The control sample was prepared by the addition of 0.04 ml of methanol to 1.46 ml of DPPH radical.

The number of antiradical units $TAU_{515/mg}$ was calculated per mg of extract according to the equation:

$$TAU_{515/mg} = \frac{A_0 - A_1}{m}$$

where $TAU_{515/mg}$ = number of antiradical units calculated per mg of extract; A_0 = absorbance of sample at the beginning of the reaction; A_1 = absorbance of sample after 1 minute of reaction; m = weight of extract [mg] in 1 ml of measured sample (in cuvette).

The number of antiradical units $TAU_{515/g}$ was calculated per 1 g of raw material according to the equation:

$$TAU_{515/g} = \frac{(TAU_{515A/mg} \cdot m_a) + (TAU_{515/mg} \cdot m_e)}{50}$$

where $TAU_{515/g}$ is the number of antiradical units calculated per g of raw material; $TAU_{515A/mg}$ is the number of antiradical units calculated per 1 mg of aqueous extract; m_a is the whole mass of aqueous extract [mg]; $TAU_{515El/mg}$ is the number of antiradical units calculated per mg of ethyl acetate extract; m_e is the whole mass of ethyl acetate extract [mg]; and *50* is the weight of raw material taken for extraction [g].

The maximal error ($\Delta TAU_{515/mg}$ and $\Delta TAU_{515/g}$) was calculated according to the total differential method.

Measurement of antiradical activity of extracts with the $ABTS^{\bullet+}$ radical

Antiradical activity of extracts was measured with the method of Re et al. [13].

Reagents

ABTS solution at 7 mmol/l in water and aqueous solution of $K_2S_2O_8$ at 2.45 mmol/l were prepared. The two solutions were mixed in the volume ratio 1:1 and stored for 16 hours in a dark place at room temperature. During that time $ABTS^{\bullet+}$ radical was formed. The final solution was diluted with water to the value of absorbance at 734 nm equal to 1.

Measurement of $ABTS^{\bullet+}$ scavenging

0.015 ml of aqueous solution of extract was added to 1.5 ml of aqueous solution of $ABTS^{\bullet+}$. The absorbance at 734 nm was measured at the beginning and after 1 minute of reaction. The measurement was performed three times and standard deviation was calculated.

The number of antiradical units TAU_{734} per mg of extract ($TAU_{734/mg}$) and g of raw material ($TAU_{734/g}$) was calculated

Table 1. Weight of extracts, number of antiradical units ($TAU_{515/mg}$) per mg of extract, number of antiradical units ($TAU_{515/g}$) per g of raw material, amount of phenolic compounds expressed in percentage per dry weight of raw material, the antiradical unit distribution between ethyl acetate and aqueous extracts. Value of $TAU_{515/mg}$ for Trolox was 51.5±0.9

Raw material	Extract	Weight of extract [mg]	$TAU_{515/mg}$	Antiradical unit distribution [%]	Amount of phenolic compounds [%] (w/w)	$TAU_{515/g}$
G_1	G_1A	9817.3	8.6±0.2	25	5.26±0.23	6701±106
	G_1E	4588.2	54.6±0.8	75		
G_2	G_2A	13492	6.1±0.1	22	5.84±0.36	7601±92
	G_2E	5963.9	49.9±0.5	78		
G_3	G_3A	10726.4	10.2±0.3	29	4.22±0.31	5637±125
	G_3E	3332.2	51.6±1	71		
G_4	G_4A	10678.6	9.5±0.2	27	5.74±0.25	7515±117
	G_4E	4748.5	57.7±0.8	73		
G_5	G_5A	11849	10±0.3	40	4.67±0.24	5860±80
	G_5E	3423.3	51±0.6	60		
B_1	B_1A	13182.2	11.9±0.3	68	5.02±0.27	4599±82
	B_1E	1981.9	36.9±0.3	32		
B_2	B_2A	12193.1	11.3±0.1	60	3.89±0.46	4610±33
	B_2E	2211.6	41.7±0.3	40		
B_3	B_3A	12193.1	11±0.1	55	4.26±0.27	4931±55
	B_3E	2683.8	41.7±0.3	45		
B_4	B_4A	10856.4	7.8±0.1	61	3.49±0.33	2779±28
	B_4E	1655.5	32.8±0.3	39		
B_5	B_5A	11989	6±0.1	47	3.76±0.42	3051±53
	B_5E	2132.4	37.9±0.7	53		
P_1	P_1A	9212.7	6.1±0.1	57	3.69±0.15	1984±28
	P_1E	1156.8	36.8±0.4	43		
P_2	P_2A	10479.3	4.5±0.1	70	2.64±0.23	1358±17
	P_2E	919.1	22.4±0.1	30		
P_3	P_3A	6290	5.3±0.3	64	1.26±0.12	1038±39
	P_3E	717.8	26.0±0.4	36		
P_4	P_4A	6362.3	3.0±0.1	56	1.49±0.12	684±30
	P_4E	632.9	23.5±0.7	44		
P_5	P_5A	11005.8	5.5±0.2	68	3.97±0.21	1789.0±55
	P_5E	957.5	30.0±0.9	32		

in the same way as shown above for the test with the DPPH radical. The maximal error ($\Delta TAU_{734/mg}$ and $\Delta TAU_{734/g}$) was calculated on the basis of the total differential method.

Results and Discussion

The weight of extracts [mg], number of antiradical units per mg of extracts ($TAU_{515/mg}$), distribution of the entire number of antiradical units between ethyl acetate and aqueous extracts [%], amount of phenolic compounds [%] calculated per dry weight of raw material, and number of antiradical units per g of raw material ($TAU_{515/g}$) are shown in Table 1. The diagram demonstrating the number of antiradical units per g of raw material ($TAU_{515/g}$) is shown in Figure 1.

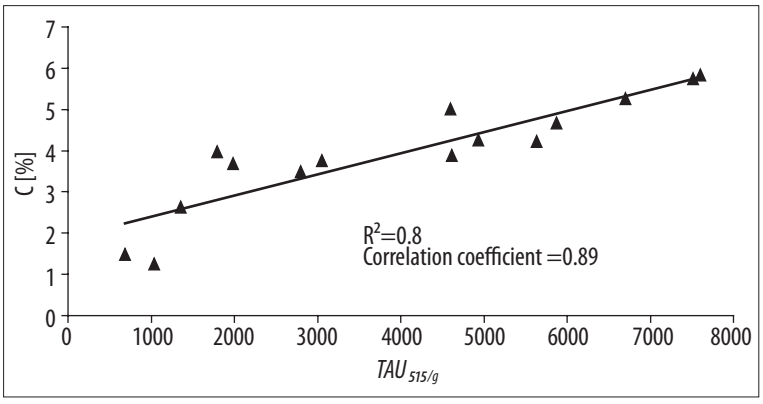

Fig. 2. The correlation between number of antiradical units $TAU_{515/g}$ and amounts of phenolic compounds C [%]

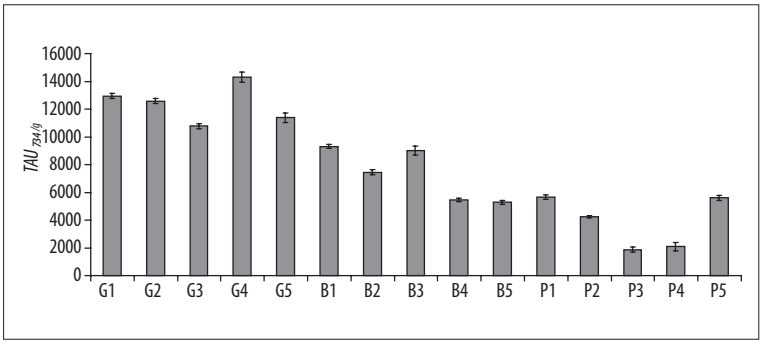

Fig. 3. The number of antiradical units $TAU_{734/g}$ per g of raw material. G1-G5 – green tea, B1-B5 – black tea, P1-P5 – pu-erh

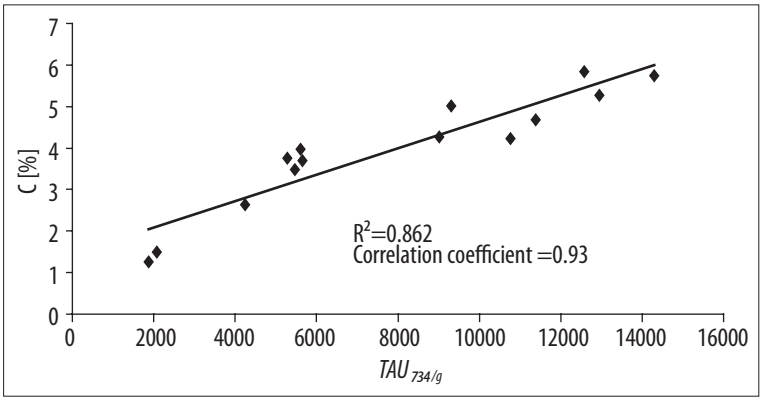

Fig. 4. The correlation between number of antiradical units $TAU_{734/g}$ and amounts of phenolic compounds C [%]

The highest number of antiradical activity units per mg of extract ($TAU_{515/mg}$) was noted for green tea extract **G_4E** (57.7±0.8), the lowest for pu-erh tea extract **P_4A** (3.0±0.1). The number of units ($TAU_{515/mg}$) per mg of Trolox was equal to 51.5±0.9. The highest number of antiradical units calculated per g of raw material ($TAU_{515/g}$) was calculated for green tea classic English leaf from India **G_2** (7601±92), the lowest for pu-erh Bastek Coffee & Tea (China) tea **P_4** (684±30).

The green teas exhibited the strongest antiradical properties, black teas lower and pu-erh the lowest.

The correlation coefficient „*r*" between the number of $TAU_{515/g}$ units and phenolic concentration expressed as a percentage is 0.89 (Figure 2).

The number of antiradical units calculated per mg of extract ($TAU_{734/mg}$), per g of raw material ($TAU_{734/g}$), and the distribution of units between ethyl acetate and aqueous extracts, for the test with $ABTS^{\bullet+}$ radical, are shown in Table 2. A diagram showing the number of antiradical units per g of raw material ($TAU_{734/g}$) is presented in Figure 3.

The highest number of antiradical units ($TAU_{734/mg}$) was calculated for extract **G_4E** (106±2) and the lowest for extract **P_3A** (11.3±1.3). The value $TAU_{734/mg}$ for Trolox is 81.2±1.5. The $TAU_{734/g}$ value was highest for **G_4** (14 303±354) raw material, and the lowest number of $TAU_{734/g}$ units was calculated for **P_3** (1870±180). The correlation coefficient „*r*" between the number of $TAU_{734/g}$ units and the amount of phenolic compounds in the raw material is 0.93 (Figure 4).

The largest amount of phenolic compounds expressed in percentage per mass of raw material was found in **G_2** (5.84±0.36) and the lowest in **P_3** (1.26±0.12).

The distribution of antiradical units between ethyl acetate and aqueous extract for each of the raw materials is shown in Table 1 for tests with the DPPH radical and Table 2 for tests with the $ABTS^{\bullet+}$ radical. For green tea leaves, 60–78% of $TAU_{515/mg}$ and 58–74% of $TAU_{734/mg}$ units were calculated

Table 2. The number of antiradical units per mg of extract ($TAU_{734/mg}$), number of antiradical units per g of dry raw material ($TAU_{734/g}$), antiradical units distribution in percentage between ethyl acetate and aqueous extracts. Value of $TAU_{734/mg}$ for Trolox was 81.2±1.5

Raw material	Extract	$TAU_{734/mg}$	Antiradical unit distribution [%]	$TAU_{734/g}$
G_1	G_1A	28.4±0.6	40	12942±193
	G_1E	80.3±0.9	60	
G_2	G_2A	12.1±0.1	26	12573±176
	G_2E	78.0±1.1	74	
G_3	G_3A	22.0±0.4	44	10771±184
	G_3E	90.9±1.5	56	
G_4	G_4A	19.7±0.8	30	14303±354
	G_4E	106±2	70	
G_5	G_5A	20.3±0.3	42	11379±351
	G_5E	95.9±4.1	58	
B_1	B_1A	23.7±0.3	67	9302±137
	B_1E	76.9±1.5	33	
B_2	B_2A	21.5±0.5	71	7444±173
	B_2E	49±1.2	29	
B_3	B_3A	25.2±1	68	9022±317
	B_3E	53.5±1.6	32	
B_4	B_4A	18.6±0.4	74	5461±121
	B_4E	42.8±1.2	26	
B_5	B_5A	13.9±0.4	63	5283±141
	B_5E	45.4±1.2	37	
P_1	P_1A	23.3±0.7	76	5655±165
	P_1E	58.8±1.2	24	
P_2	P_2A	15.9±0.2	79	4245±67
	P_2E	49.4±1.2	21	
P_3	P_3A	11.3±1.3	76	1870±180
	P_3E	31.4±1.2	24	
P_4	P_4A	12.0±1.3	74	2081±293
	P_4E	43.4±1.2	26	
P_5	P_5A	20.3±0.7	80	5608±188
	P_5E	59.5±1.2	20	

for ethyl acetate extracts. For black tea, the respective values were 32–53% of $TAU_{515/mg}$ and 26–37% of $TAU_{734/mg}$ units. For pu-erh tea, the amount of $TAU_{515/mg}$ units in ethyl acetate extracts was 30–44%, and the amount of $TAU_{734/mg}$ units was 20–26%.

Our results show that the strongest antiradical properties are exhibited by green tea leaves and, in decreasing order, black tea and pu-erh tea. Similar results were described in the literature [1]. The ethyl acetate extracts of green tea leaves showed stronger antiradical activity than extracts obtained from leaves of black or pu-erh teas. The antiradical activity correlated well with the amount of phenolic compounds determined with the colorimetric method.

Analysis of the distribution among extracts of antiradical activity units $TAU_{515/mg}$ and $TAU_{734/mg}$ led to the following observations. For green tea leaf extracts a greater number of antiradical units was present in ethyl acetate extracts (Tables 1 and 2). When black and pu-erh teas were investigated, a greater number of units was found in the aqueous extracts (Tables 1 and 2).

An explanation for this observation is that phenolic compounds present in green tea leaves such as epicatechin gallate and epigallocatechin gallate (strong antioxidants) are more soluble in ethyl acetate than water.

During the fermentation process, green tea phenols are oxidized and turn into more polymerized structures, thearubigins and theaflavins. These macromolecular substances have a better affinity for water than for ethyl acetate.

We can conclude that if we take the green tea leaves for the preparation of extracts with antioxidant activity, ethyl acetate is a better solvent, while in the case of black tea and for pu-erh the better solvent is water.

One can draw the following conclusions from the investigation:

a) The leaves of green tea appeared to have the strongest antioxidant properties but those of pu-erh tea the lowest.
b) For green tea, a higher total number of antiradical units TAU_{515} and TAU_{734} was distributed to ethyl acetate extracts than to aqueous extract, but for black and pu-erh teas, more units were calculated for aqueous extracts.
c) There is a strong positive correlation between the contents of phenolic compounds in raw materials and their antiradical activity.

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