

The measurement of antiradical activity of some plant raw materials and extracts with use of TAU_{734} (Total Antiradical Unit)

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Summary

The aim of this work was to measure the antiradical activity of plant extracts and plant raw materials with use of TAU_{734} (total antiradical unit). For the study, three raw materials were used: oak bark (*Quercus cortex*) rich in tannins, inflorescence of hawthorn (*Crataegi inflorescentia*) rich in flavonoids and seeds of coffee (*Coffeae semen*) rich in phenolic acids (caffeic, chlorogenic acids).

The methanol-water (1:1) extract was obtained from each raw material. A part of the extract was evaporated under reduced pressure to obtain dry extract **A**. The remaining part of methanol-water extract was submitted for further extraction and evaporation in order to obtain final dry extracts **B** (precipitate), **C** (ethyl acetate) and **D** (remaining aqueous).

The antiradical activity was measured using ABTS^{•+} radical (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt). Antiradical potential was demonstrated as a number of antiradical units per mg of extract ($TAU_{734/mg}$) and g of raw materials ($TAU_{734/g}$). The highest number of antiradical units per g of raw material ($TAU_{734/g} = 13837 \pm 1726$) was noticed for bark of oak. The weakest antiradical properties were observed for seeds of

coffee ($TAU_{734/g} - 5929 \pm 856$). The largest number of antiradical units ($TAU_{734/mg} - 99.6 \pm 15.9$) was noticed for mg of extract B_o from bark of oak. The weakest antiradical properties ($TAU_{734/mg} - 16.0 \pm 2.0$) exhibited extract B_c from seeds of coffee.

Key words: *plant extracts, antiradical potential, antiradical activity units*

INTRODUCTION

Many extracts of natural raw materials [1, 2] and isolated compounds, especially phenols [3], show antiradical and antioxidant activity.

Antiradical activity of substances is measured with use of various methods, based on different physicochemical mechanisms. These methods are: a) Hydrogen Atom Transfer (HAT) methods such as fluorimetric, spectrophotometric, lumino-metric [4, 5] as well as the techniques based on lipid peroxidation; b) Single Electron Transfer (SET) methods using cations of metals such as Cu^+ and Fe^{2+} ; c) methods using DPPH \cdot and ABTS $^{+\cdot}$ radicals based on both HAT and SET mechanisms [6].

The antioxidant and antiradical activities of substances one can present as values EC_{50} or T_{EC50} [7, 8]. The results can also be presented as equivalents of popular antioxidants such as trolox [9] or ascorbic acid [10]. This activity could be demonstrated as equivalents (units) calculated per g of substance (trolox equivalents antioxidant capacity - TEAC/g, ascorbic acid equivalents - AAE/g).

Sroka and Franciczek 2008 [11] defined antiradical unit as so called *TAU* (total antioxidant unit) which let to demonstrate the antiradical features as units per mg of extract or g of raw material. This activity do not refer directly to popular antioxidants such as trolox, ascorbic acid and others.

In this paper, the number of TAU_{734} antiradical activity units per mg of extracts ($TAU_{734/mg}$) and per g of raw materials ($TAU_{734/g}$) rich in different classes of phenolic compounds were measured.

MATERIALS AND METHODS

Characteristics of raw material

Three kinds of raw materials rich in different groups of polyphenols were used for investigation. The bark of oak (*Quercus cortex*) especially rich in hydrolysable and condensed tannins, inflorescence of hawthorn (*Crataegi inflorescentia*) rich in flavonoids, seeds of coffee (*Coffeae semen*) rich in phenolic acids especially caffeic and chlorogenic acids. The bark of oak and inflorescence of hawthorn were bought in Herb Factory "Kawon-Hurt", Gostyń, Poland; seeds of coffee (*Coffea arabica* L.) was produced in Astra coffee-roasting factory in Poznań, Poland.

Reagents

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and potassium persulphate ($K_2S_2O_8$) were purchased from Sigma-Aldrich, methanol was obtained from Merck.

Preparation of extracts

The 50 g of raw material was extracted with 900 ml methanol-water solution (1:1) for 7 days at 50°C. The 180 ml of methanol-water extract (20% of the whole amount) was separated and condensed to dryness under reduced pressure to obtain dry extract **A**. The methanol was evaporated from the rest of methanol-water extract (720 ml) under reduced pressure at 40°C and the remaining aqueous solution was stored at 4°C for 24 hours. The precipitate was separated by decanting and dried under reduced pressure to obtain extract **B**. The remaining aqueous solution, after precipitate separation, was exhaustively extracted with ethyl acetate (7 x 100 ml). Then the ethyl acetate extract and water remainder was condensed to dryness under reduced pressure to obtain dry extracts **C** and **D**, respectively.

The extracts obtained from bark of oak were marked additionally with letter "o" (A_o , B_o , C_o and D_o), extracts from inflorescence of hawthorn - letter "h" (A_h , B_h , C_h and D_h), extracts from seeds of coffee letter "c" (A_c , B_c , C_c and D_c).

The measurement of antiradical activity and calculation of the number of TAU_{734} units per mg of extracts and per g of raw material

The antiradical activity of extracts was measured using $ABTS^{\bullet+}$ radical according to the method described by Re *et al.* 1999 [12].

Preparation of reagents

The ABTS solution was prepared by resolving of 0.096 g of ABTS in 25 ml 50% methanol in water (7 mmole/l).

The potassium persulphate ($K_2S_2O_8$) solution was prepared by dissolving 0.016 g of substance in 25 ml 50% methanol in water (2.45 mmole/l).

The $ABTS^{\bullet+}$ (cation radical) was formed after joining of two above described solutions in the volume ratio (1:1) and stored for 16 h at room temperature in the dark place. Before use, the absorbance of $ABTS^{\bullet+}$ solution was adjusted with 50% methanol to the value of 1.0 at 734 nm.

Measurement of antiradical activity

The dry extracts were dissolved in 50% methanol in water. The concentration of extracts taken for the test was individually chosen depending on the antiradical activity of the extract.

The 1.5 ml ABTS^{•+} in 50% methanol in water was placed in the glass cuvette with the optical path length 1 cm, then 15 μ l of extract solution in 50% methanol in water was added in order to start the reaction. The intensity of ABTS^{•+} scavenging was determined by the assay of absorbance of sample at 734 nm. The absorbance was measured at the beginning of reaction (A_0) (time of addition of extract) and after 1 minute from extract addition (A_1). The intensity of radical scavenging is the subtraction $A_0 - A_1$.

The control sample was prepared by addition of 15 μ l of 50% methanol in water to the ABTS^{•+} solution. The control sample was not taken to further calculations because the absorbance of control sample was not changed in the time of 1 minute. The blank was obtained by addition of 15 μ l of extract solution to 1.5 ml of 50% methanol. All measurements was sixfold repeated.

CALCULATIONS

The antiradical unit (TAU_{734}) was defined by Sroka and Franiczek in 2008 [11]. One unit of TAU_{734} corresponds with the mass of sample in 1 ml of reaction mixture which causes the decrease of absorbance of ABTS of 1 in the first minute of reaction at 25°C.

The number of antiradical activity units was calculated per mg of extracts ($TAU_{734/mg}$) according to the equation (1):

$$TAU_{734/mg} = \frac{A_0 - A_1}{C_e} \quad (1)$$

where A_0 is the absorbance of sample at the beginning of reaction; A_1 is the absorbance after one minute from the addition of the extract solution; C_e is the concentration of extract [mg/ml] in reaction mixture.

The maximal error $\Delta TAU_{734/mg}$ was estimated on basis of the total differential method.

Then, the number of units per g of raw material ($TAU_{734/g}$) was calculated, basing on the data obtained for extracts A ($TAU_{734/g}$) according to the equation (2):

$$TAU_{734/g} = \frac{TAU_{734/mg} \times m_A}{0.2 \cdot W_r} \quad (2)$$

where $TAU_{734/g}$ is number of antiradical units in 1 g of raw material; m_A is mass of A extract [mg]; W_r mass of raw material taken for extraction (50 g); $TAU_{734/mg}$ number

of antiradical units calculated per 1 mg of extract A; (m_A) mass of extract A [mg] obtained from bark of oak, hawthorn inflorescence or coffee seeds was respectively 1451 mg, 2269 mg and 2188 mg, respectively.

The maximal error ($\Delta TAU_{734/g}$) was estimated on the basis of the total differential method.

Statistical analysis

Each measurement was repeated for 5 times and standard deviation was calculated. Then maximal error of the final results was determined using the total differential method.

RESULTS AND DISCUSSION

The number of antiradical units calculated per 1 mg of extract ($TAU_{734/mg}$) is presented in table 1 and figure 1. Extracts isolated from oak bark exhibited the strongest antiradical properties. Among them the higher antiradical activity was noted for dried precipitate (B_o) 99.6 ± 15.9 units per mg ($TAU_{734/mg}$), lowest for ethyl acetate extracts 71 ± 11.6 units per mg of extracts C_o .

Table 1.

The number of antiradical units calculated per 1 mg of extract ($TAU_{734/mg}$) and per 1 g of raw material ($TAU_{734/g}$). The extracts obtained from oak bark were marked additionally with "o" (A_o , B_o , C_o and D_o), extracts from inflorescence of hawthorn - "h" (A_h , B_h , C_h and D_h), extracts from seeds of coffee "c" (A_c , B_c , C_c and D_c).

Raw material	Extract	$Tau_{734/mg}$	$Tau_{734/g}$
Oak bark	A_o	$95,3 \pm 11.5$	13837 ± 1726
	B_o	99.6 ± 15.9	
	C_o	71 ± 11.6	
	D_o	91.4 ± 10.3	
Inflorescence of Hawthorn	A_h	26.5 ± 3.7	6122 ± 876
	B_h	41.9 ± 5.2	
	C_h	53.8 ± 7.9	
	D_h	25.3 ± 3.0	
Coffee seeds	A_c	27.1 ± 3.8	5929 ± 856
	B_c	16.0 ± 2.0	
	C_c	35.6 ± 4.3	
	D_c	18 ± 2.1	

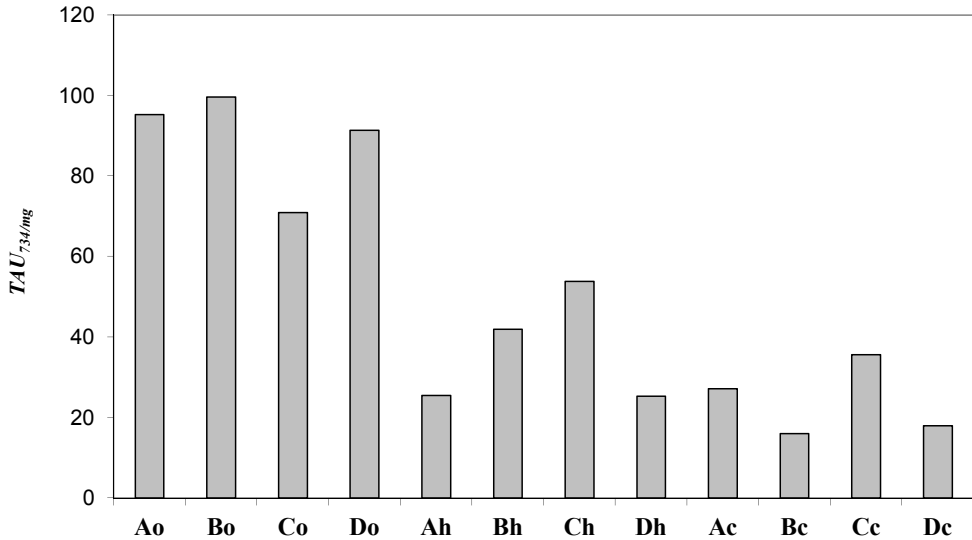


Figure 1.

The number of antiradical units ($TAU_{734/mg}$) calculated per 1 mg of extract. Extracts Ao, Bo, Co, Do, were obtained from oak bark, extracts Ah, Bh, Ch and Dh from inflorescence of hawthorn and extracts Ac, Bc, Cc and Dc from coffee beans. $TAU_{734/mg}$ unit definition and preparation of extracts is described in section Materials and Methods.

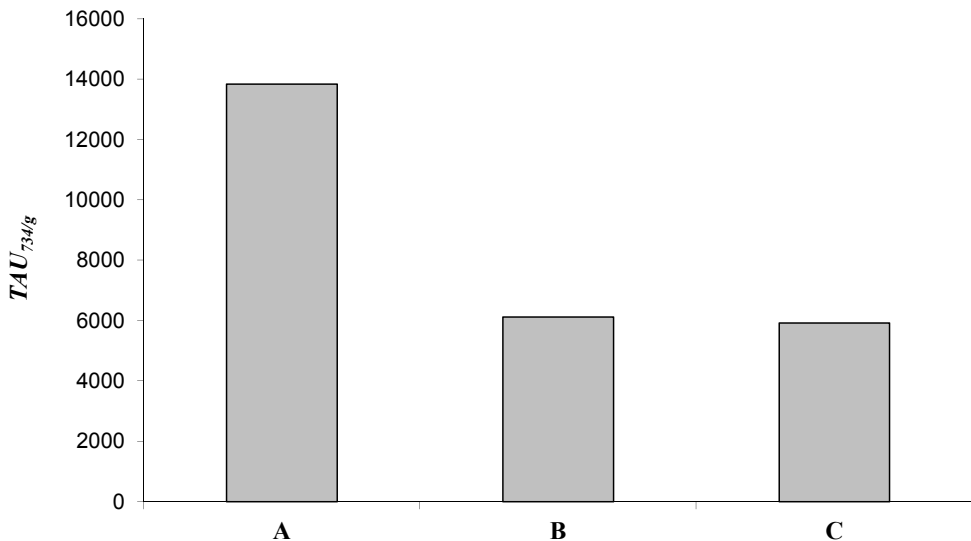


Figure 2.

The number of antiradical units calculated per 1 g of raw material ($TAU_{734/g}$). A stands for the oak bark, B for the inflorescence of hawthorn, C for seeds of coffee. $TAU_{734/g}$ unit definition is described in the section Materials and Methods.

Lower antiradical properties was measured for extracts obtained from inflorescence of hawthorn. Among them the highest number of antiradical units was calculated per mg of C_h extract ($53.8 \pm 7.9 \text{ TAU}_{734/\text{mg}}$) and the lowest for water remaining D_h ($25.3 \pm 3.0 \text{ TAU}_{734/\text{mg}}$).

The lowest antiradical activity exhibited extracts obtained from seeds of coffee. The highest activity was observed for ethyl acetate C_c extract ($35.6 \pm 4.3 \text{ TAU}_{734/\text{mg}}$) and the lowest for precipitate B_c ($16.0 \pm 2.0 \text{ TAU}_{734/\text{mg}}$).

When the antiradical properties of raw materials were studied the highest activity was demonstrated for oak bark: $13837 \pm 1726 \text{ (TAU}_{734/\text{g}})$ (tab. 1, fig. 2), lower for inflorescence of hawthorn $6122 \pm 876 \text{ (TAU}_{734/\text{g}})$ and the lowest for seeds of coffee $5929 \pm 856 \text{ (TAU}_{734/\text{g}})$.

There are many methods of the measurement of antiradical features of extracts or substances. Rice-Evans et al. 1996 [13] proposed the demonstration of antioxidant activity as so called Trolox equivalents (TEAC). Sanchez-Moreno 1998 [14] compared antioxidant and antiradical activity of substances with EC_{50} value. Compounds with the small EC_{50} value is characterized by high antioxidant or antiradical features.

The method proposed in this work: the use of TAU_{734} unit does not need the reference substance such as trolox or ascorbic acid. TAU_{734} unit let to demonstrate of antiradical properties of substances, extracts and raw materials.

CONCLUSION

1. The strongest antiradical activity was noticed for oak bark and extracts obtained from this raw material;
2. The TAU_{734} unit demonstrated the antiradical activity of substances, extracts and raw materials. It also could be a very convenient tool for monitoring the yield of isolated of substances with antiradical activity.

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WPOMIAR AKTYWNOŚCI PRZECIWWOLNORODNIKOWEJ WYBRANYCH SUROWCÓW I WYCIĄGÓW ROŚLINNYCH ZA POMOCĄ TAU_{734} (TOTAL ANTIRADICAL UNIT)

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Streszczenie

Celem pracy był pomiar właściwości przeciwrodnorodnikowych wyciągów oraz surowców roślinnych za pomocą jednostki TAU_{734} (total antiradical unit). Przebadano trzy surowce: korę dębu (*Quercus cortex*) bogatą w garbniki, kwiatostan głogu (*Crataegi inflorescentia*) bogaty w flawonoidy i ziarna kawy (*Coffeae semen*) bogate w kwasy fenolowe.

Z każdego surowca otrzymano wyciąg metanolowo-wodny. Część wyciągu odparowano pod zmniejszonym ciśnieniem, otrzymując suchy wyciąg **A**. Pozostałą część wyciągu metanolowo-wodnego poddano dalszej ekstrakcji i odparowaniu, otrzymując ostatecznie suche wyciągi **B** (osad), **C** (octan etylu) i **D** (pozostałość wodna).

Aktywność przeciwrodnikową mierzono za pomocą rodnika ABTS^{•+} (sól amonowa kwasu 2,2'-azyno-bis(3-etylobenzotiazolino-6-sulfonowego). Potencjał przeciwrodnikowy przedstawiono jako liczbę jednostek przeciwrodnikowych na mg wyciągu ($TAU_{734/mg}$) i g surowca ($TAU_{734/g}$). Największą liczbę jednostek aktywności przeciwrodnikowej na g surowca ($TAU_{734/g} - 13837 \pm 1726$) obserwowano w korze dębu. Najśłabsze właściwości przeciwrodnikowe obserwowano w nasionach kawy ($TAU_{734/g} - 5929 \pm 856$). Najwyższą liczbę jednostek ($TAU_{734/mg} - 99.6 \pm 15.9$) zaobserwowano w 1 mg wyciągu **B_o** z kory dębu. Najśłabsze właściwości przeciwrodnikowe ($TAU_{734/mg} - 16.0 \pm 2.0$) wykazywał wyciąg **B_c** z nasion kawy.

Słowa kluczowe: wyciągi roślinne, potencjał przeciwrodnikowy, jednostki aktywności przeciwrodnikowej