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The antiradical activity of some plant raw materials and extracts obtained from these raw materials*

Aktywność przeciwwolnorodnikowa wybranych surowców roślinnych i wyciągów otrzymanych z tych surowców

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: Free radicals and reactive oxygen species are compounds usually present in healthy organisms as natural products of many metabolic pathways, and they are important in cell signaling and homeostasis. As a source of reactive oxygen species one can mention phagocytic cells and enzymes such as xanthine oxidase. Sometimes the level of reactive oxygen species strongly increases. This may lead to damage of very important cell structures such as nucleic acids, proteins or lipids. In this situation one should provide the organism with powerful antioxidants as a medicine or in the diet. A rich source of strong antioxidants such as phenolic compounds is plant raw materials, which are the subject of our study.

Material/Methods: Antiradical potential of extracts was measured with DPPH radical (2,2-diphenyl-1-picrylhydrazyl) and was expressed as the number of units per mg of extracts ($TAU_{515/mg}$) and per g of raw material ($TAU_{515/g}$). The amount of phenolic compounds was determined colorimetrically using Folin-Ciocalteu phenol reagent ($3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O$).

Results: The strongest antiradical activity was noted for extracts obtained from *Cinnamomi cortex*; the number of antiradical units per mg of extract ($TAU_{515/mg}$) was 10.31±1.052. The lowest antiradical features were exhibited by extract from *Zingiberis rhizoma* (0.28±0.174) and extract from *Cichorii radix* (0.38±0.669). The highest amount of phenolic compounds was measured for extracts from *Bistortae rhizoma*, with a value (in percentage) of 78.6±13.5. The correlation coefficient between the number of antiradical units in extracts and amount of phenolic compounds in these extracts was 0.7273. When the number of antiradical units was calculated per g of raw material ($TAU_{515/g}$) the strongest antiradical properties were noted for *Bistortae rhizoma* (1406±274.9), the weakest for *Cichorii radix* (122±158.3).

Key words: **Plant raw materials • Plant extracts • Phenolic compounds • Antiradical properties**

Streszczenie

Wprowadzenie: Wolne rodniki i reaktywne formy tlenu są połączeniami zwykle obecnymi w zdrowym organizmie, będąc naturalnymi produktami wielu szlaków metabolicznych, są one istotne dla procesów

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sygnałowych w komórce oraz utrzymania homeostazy. Jako źródła reaktywnych form tlenu można wymienić komórki fagocytujące lub enzymy, takie jak oksydaza ksantynowa. Czasem poziom reaktywnych form tlenu znacznie wzrasta. Taki stan może prowadzić do niszczenia bardzo ważnych struktur komórkowych, takich jak kwasy nukleinowe, białka lub lipidy. W takich sytuacjach można dostarczać organizmowi silnych przeciwutleniaczy w postaci leków lub składników diety. Bogatym źródłem przeciwutleniaczy np. związków fenolowych są surowce roślinne, które są przedmiotem naszych badań.

Materiał/Metody: Potencjał przeciwwolnorodnikowy wyciągów mierzono za pomocą rodnika DPPH (rodnik 2,2-difenylo-1-pikrylohydrazylowy) i wyrażono jako liczbę jednostek przeciwwolnorodnikowych na mg wyciągu ($TAU_{515/mg}$) i na g surowca ($TAU_{515/g}$). Ilość związków fenolowych oznaczono kolorymetrycznie za pomocą odczynnika fenolowego Folin-Ciocalteu ($3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O$).

Wyniki: Największą aktywność przeciwwolnorodnikową zaobserwowano dla wyciągów otrzymanych z *Cinnamomi cortex*; liczba jednostek aktywności przeciwwolnorodnikowej na mg wyciągu ($TAU_{515/mg}$) była równa 10,31±1,052. Najsłabsze właściwości przeciwwolnorodnikowe wykazywał wyciąg z *Zingiberis rhizoma* (0,28±0,174) i wyciąg z *Cichorii radix* (0,38±0,669). Największą liczbę związków fenolowych zmierzono dla wyciągów z *Bistortae rhizoma* z wartością (w procentach) 78,6±13,5. Współczynnik korelacji pomiędzy liczbą jednostek aktywności przeciwwolnorodnikowej w wyciągach i liczbą związków fenolowych wynosił 0,7273. Gdy liczbę jednostek aktywności przeciwwolnorodnikowej obliczono na g surowca ($TAU_{515/g}$) najsilniejsze właściwości przeciwwolnorodnikowe wykazywał *Bistortae rhizoma* (1406±274,9), najsłabsze *Cichorii radix* (122±158,3).

Słowa kluczowe: **surowce roślinne • wyciągi roślinne • związki fenolowe • właściwości przeciwwolnorodnikowe**

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Abbreviations: **$TAU_{515/mg}$ – the number of total antiradical units calculated per mg of extract using DPPH; $TAU_{515/g}$ – the number of total antiradical units calculated per g of raw material using DPPH.**

Introduction

Plant raw materials are known to be a very rich source of natural antioxidants such as phenolic compounds [11,28], carotenoids [22] and tocopherols [14]. The most effective are polyphenolic compounds. Among them one can mention, in decreasing order of antiradical activity: green tea phenols such as epigallocatechin gallate (very strong antioxidants) [7]; tannins (also strong antioxidants) [27]; phenolic acids (the antioxidant activity strongly depends on the number of hydroxyl groups in the molecule and the position of these groups) [13]; and flavonoids (flavonols seems to be the strongest among them, and their activity strongly depends on the number and position of the hydroxyl group in the B ring) [17,23]. Green and black tea polyphenols, as well as tannins, always exhibit strong antioxidative features [26]. The activity of flavonoids and phenolic acids varies from strong, such as myricetin [9], gallic [18] and caffeic acids [12], to the almost completely inactive diosmin [19] and salicylic acids [18].

Free radicals are formed in animal and human organisms as a result of normal metabolic processes, for example as a result of action on xanthine oxidase [1], or action of cells of the immunological system, for example stimulated phagocytes produce bulks of superoxide radical anion and hydrogen peroxide [5]. However, sometimes the overproduction of free radicals takes place, for instance during chronic or acute inflammatory states [16]. Then the natural antioxidant strength of the organism may not be enough, and providing the organism with antioxidants in the form of medicine or dietary components could be advantageous [4].

Such a source of antioxidants could be plant extracts rich in phenolic compounds, especially those with strong antioxidant and antiradical activity.

In this study, different extracts were obtained from 6 raw materials: *Bistortae radix* (root of bistort), *Dioscoreae rhizoma* (root of wild yam), *Curcumae radix* (root of temulawak), *Cichorii radix* (root of common chicory), *Zingiberis rhizoma* (rhizomes of ginger), and *Cinnamomi*

cortex (bark of *Ceylon cinnamon*). The antiradical activity of each extract was measured and the general antiradical potential of raw materials was evaluated.

Material and Methods

Preparation of extracts

Each raw material, i.e. *Bistortae rhizoma* (50 g), *Dioscoreae rhizoma* (50 g), *Curcumae radix* (50 g), *Cichorii radix* (49.9 g), *Zingiberis rhizoma* (49.4 g), and *Cinnamomi cortex* (50 g), was extracted with 900 ml of methanol for 4 days at 50°C. 180 ml of methanol solution was poured off and condensed to dryness under reduced pressure to obtain extract **WA**. The remaining 780 ml of methanol solution was condensed to dryness under reduced pressure and the dry extract was dissolved in hot water. After cooling, the aqueous solution was stored at 4°C for 24 hours for the precipitate to form. Then the precipitate was separated (extract **WD**). After precipitate separation, the aqueous solution was extracted with ethyl acetate (1000 ml). The ethyl acetate and remaining aqueous solution were condensed to dryness to obtain extracts **WB** and **WC** respectively.

The extracts of each raw material were marked with the following additional letters: b – *Bistortae rhizoma*, d – *Dioscoreae rhizoma*, cu – *Curcumae radix*, cy – *Cichorii radix*, i – *Zingiberis rhizoma*, ci – *Cinnamomi cortex*. For example, extracts from *Bistortae rhizoma* were marked as **WAb**, **WBb**, **WCb** and **WDb**.

Colorimetric measurement of total phenols

Total phenolic compounds in extracts were measured according to Singleton and Rossi et al. [15].

A 20% aqueous solution of sodium carbonate (Na_2CO_3) was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent ($3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O$) was added to 7 ml of water. 0.5 ml of methanol solution of extract (2.9 mg/ml) was added to the reaction mixture. After 3 min, 2 ml of aqueous solution of sodium carbonate was added and the sample was heated in a boiling water bath for 1 min. The absorbance was measured at 685 nm in a glass cuvette with 1-cm optical path against blank (without extract). The amount of phenolic compounds was expressed in percentage per mg of extract.

Measurement of antiradical activity with DPPH radical

Antiradical activity of extracts was measured according to the method of Brand-Williams et al. [2].

50 µl of methanol solution of extract at the concentration 2.9 mg/ml was added to 2 ml of methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl radical) (0.037 mg/ml) in a cuvette, mixed and absorbance was measured at 515 nm in a glass cuvette with optical path 1 cm at the time of 0 min (start time) and after 1 minute. The control sample was prepared by the addition of 50 µl of methanol instead of extract solution and the absorbance was measured at 0 and after 1 minute. The measurement was repeated five times and the standard deviation was calculated.

Antiradical unit definition and calculation of number of units per mg of extract and g of raw material

Antiradical unit definition and number of units per mg of extracts and g of raw material were calculated according to Sroka et al. [20]. One unit of antiradical activity is the amount of substance which causes a decrease of absorbance of the reaction mixture of 1 after 1 minute at 20°C.

The amount of antiradical units per mg of extract was measured according to the equation:

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

where $TAU_{515/mg}$ is the number of antiradical units per mg of extract; A_0 is the absorbance of the sample at 0 min; A_1 is the absorbance of the sample after 1 minute of reaction; m is the amount of extract [mg] in 1 ml of reaction mixture.

The maximal error $\Delta TA_{U515/mg}$ was calculated according to the total differential method.

The number of antiradical units per g of raw material was calculated according to the equation:

$$TAU_{515/g} = \frac{TAU_{515/mg/met} \cdot m_e}{0.2 \cdot m_R}$$

where $TAU_{515/g}$ is the number of antiradical units per g of raw material (approximate value); $TAU_{515/mg/met}$ is the number of antiradical units per mg of methanol extract; m_ε is the weight of extract [mg]; m_R is the weight of raw material [g] taken for extraction.

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

Results and Discussion

Antiradical activity of investigated extracts expressed as the number of antiradical units per mg of extract is demonstrated in Figure 1 and Table 1. The strongest antiradical features were noted for extracts **WBci** and **WDci** from *Cinnamomi cortex*; the number of antiradical units ($TAU_{515/mg}$) was 10.3±0.868 and 10.31±1.052 respectively. The lowest number of antiradical units was calculated for extract **WCi** (0.28±0.174) obtained from *Zingiberis rhizoma* and extract **WCcy** (0.39±0.669) from *Cichorii radix*.

Amounts of phenolic compounds measured by the colorimetric method of Singleton et al. [15] are shown in Figure 2 and Table 1. The highest amount of phenols was noted for extracts **WBb**, **WAb** and **WDb**, with the value of 78.6±13.5, 45.2±7.68 and 45.1±7.24 respectively.

Pearson's correlation coefficient between the number of antiradical units in extracts and the amount of phenolic compounds was 0.7273 (Fig. 3).

The antiradical features of raw materials ($TAU_{515/g}$) are demonstrated in Figure 4 and Table 1. When the number of antiradical units was calculated per g of raw materials,

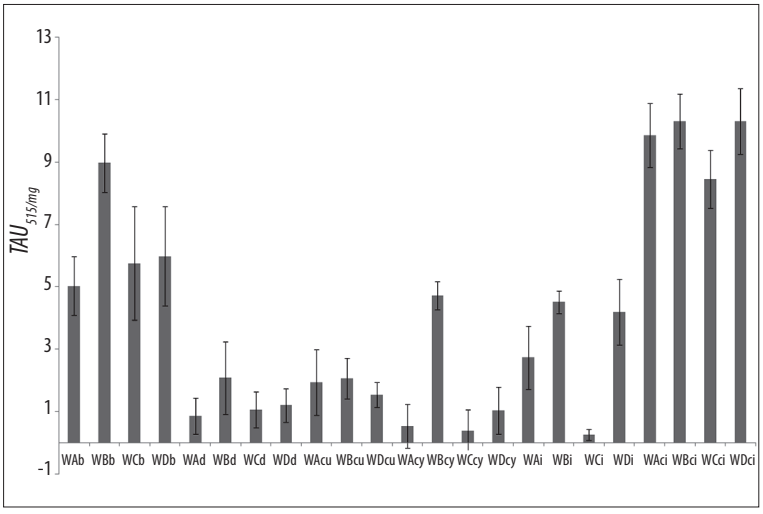

Fig. 1. The number of antiradical units per mg of extracts ($TAU_{515/mg}$). **WAb**, **WBb**, **WCb** and **WDb** are methanol, ethyl acetate, aqueous extracts and precipitate (see preparation of extracts) from *Bistortae rhizoma*, **WAd**, **WBd**, **WCd**, **WDd** are respective extracts from *Dioscoreae rhizoma*, **WAcu**, **WBcu**, **WCcu**, **WDcu** are extracts from *Curcumae radix*, **WAcy**, **WBcy**, **WCcy**, **WDcy** – extracts from *Cichorii radix*, **WAi**, **WBi**, **WCi**, **WDi** – extracts from *Zingiberis rhizom*a, **WAci**, **WBci**, **WCci**, **WDci** – extracts from *Cinnamomi cortex*.

Table 1. The weight of extracts [mg], number of antiradical units per mg of extracts ($TAU_{515/mg}$), number of antiradical units per g of raw material ($TAU_{515/g}$), amount of phenolic compounds [%]. **WAb**, **WBb**, **WCb** and **WDb** are methanol, ethyl acetate, aqueous extracts and precipitate (see preparation of extracts) from *Bistortae rhizoma*, **WAd**, **WBd**, **WCd**, **WDd** are respective extracts from *Dioscoreae rhizoma*, **WAcu**, **WBcu**, **WCcu**, **WDcu** are extracts from *Curcumae radix*, **WAcy**, **WBcy**, **WCcy**, **WDcy** – extracts from *Cichorii radix*, **WAi**, **WBi**, **WCi**, **WDi** – extracts from *Zingiberis rhizoma*, **WAci**, **WBci**, **WCci**, **WDci** – extracts from *Cinnamomi cortex*

Raw material	$TAU_{515/g}$	Extract	Weight of extract [mg]	$TAU_{515/mg}$	Amount of phenolic compounds [%]
Bistortae rhizoma	1406±274.0	**WAb**	2793.4	5.03±0.933	45.2±7.68
		WBb	2219.3	8.97±0.942	78.6±13.5
		WCb	8164.0	5.76±1.815	33.4±8.65
		WDb	815.7	5.98±1.589	45.1±7.24
Dioscoreae rhizoma	186±123.9	**WAd**	2122.3	0.87±0.575	7.6±1.0
		WBd	517.3	2.09±1.165	29.2±3.72
		WCd	5127.5	1.07±0.584	6.01±0.69
		WDd	2398.7	1.22±0.539	4.7±0.82
Curcumae radix	170±94.1	**WAcu**	877.5	1.94±1.052	21.8±2.53
		WBcu	176.1	2.07±0.660	24.7±2.49
		WDcu	2660.0	1.53±0.404	22.4±3.34
Cichorii radix	122±158.3	**WAcy**	2229.7	0.55±0.704	2.36±0.146
		WBcy	427.2	4.72±0.454	16.3±1.058
		WCcy	8471.2	0.39±0.669	1.47±0.118
		WDcy	445.6	1.05±0.747	3.39±0.568
Zingiberis rhizoma	399±153.5	**WAi**	1461.1	2.73±1.023	8.23±0.471
		WBi	337.6	4.51±0.367	17.4±0.142
		WCi	201.6	0.28±0.174	2.07±0.801
		WDi	1668.7	4.19±1.054	14.1±0.668
Cinnamomi cortex	795±91.1	**WAci**	807.1	9.84±1.029	30.6±1.07
		WBci	518.0	10.3±0.868	33±1.022
		WCci	1588.0	8.46±0.924	30.2±1.051
		WDci	856.5	10.31±1.052	36.6±1.200

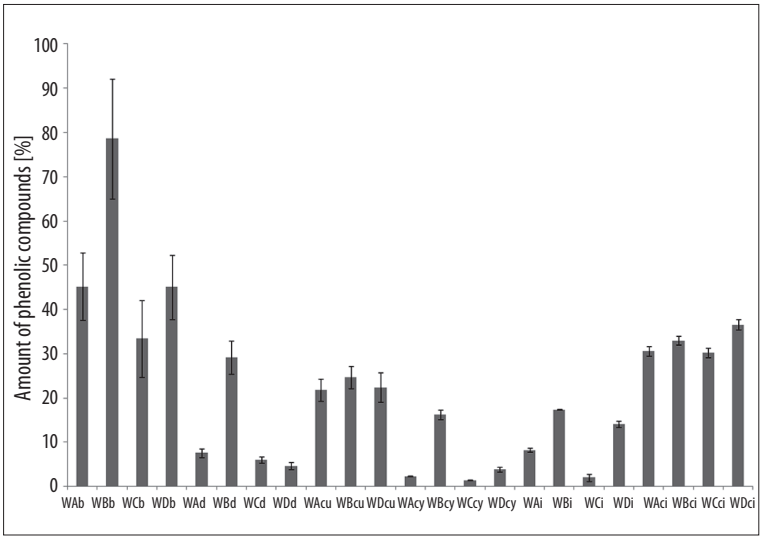

Fig. 2. Amount of phenolic compounds in extracts expressed in percentage. **WAb**, **WBb**, **WCb** and **WDb** are methanol, ethyl acetate, aqueous extracts and precipitate (see preparation of extracts) from *Bistortae rhizoma*, **WAd**, **WBd**, **WCd**, **WDd** are respective extracts from *Dioscoreae rhizoma*, **WAcu**, **WBcu**, **WCcu**, **WDcu** are extracts from *Curcumae radix*, **WAcy**, **WBcy**, **WCcy**, **WDcy** – extracts from *Cichorii radix*, **WAi**, **WBi**, **WCi**, **WDi** – extracts from *Zingiberis rhizoma*, **WAci**, **WBci**, **WCci**, **WDci** – extracts from *Cinnamomi cortex*.

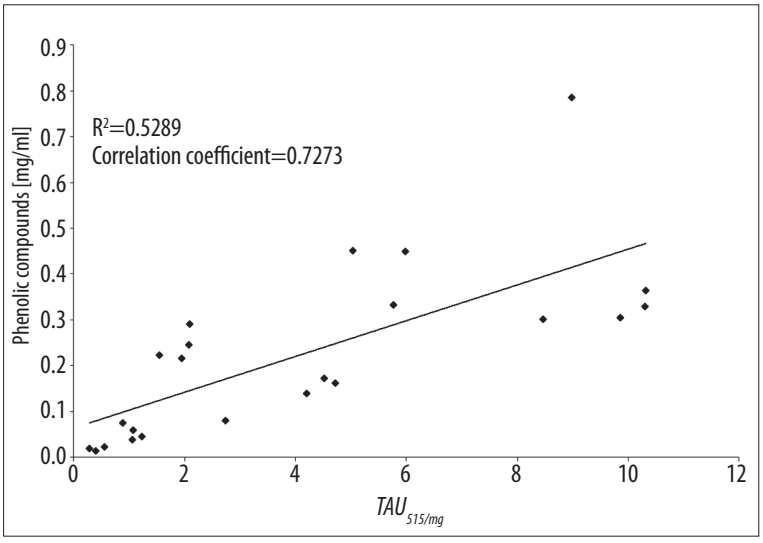

Fig. 3. Correlation coefficient between amount of phenolic compounds in extracts and number of antiradical units ($TAU_{515/mg}$) per mg of extracts.

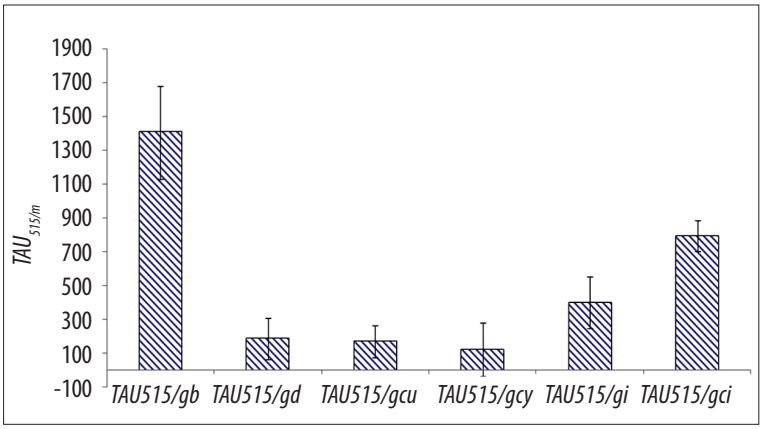

Fig. 4. Number of antiradical units in g of raw materials ($TAU_{515/g}$). $TAU_{515/gb}$ is the number of antiradical units per g of *Bistortae rhizoma*, $TAU_{515/gd}$ – number of antiradical units per g of *Dioscoreae rhizoma*, $TAU_{515/gcu}$ – per g of *Curcumae radix*, $TAU_{515/gcy}$ – per g of *Cichorii radix*, $TAU_{515/gi}$ – per g of *Zingiberis rhizoma*, $TAU_{515/gci}$ – per g of *Cinnamomi cortex*.

the highest value was calculated for Bistortae rhizoma (1406±274.9), the lowest for *Cichorii radix* (122±158.3).

We chose for the study roots, bark and rhizomes because these parts of plants are always rich in antioxidant phenolic compounds, first of all tannins.

Root of common bistort exhibited the highest antiradical features (1406±274.9) among raw material investigated in this study. This result is much lower in comparison to the antiradical potential of green tea leaves (7601±92) which we demonstrated in our previous study [26]. The relatively strong antiradical activity well correlated positively with the highest phenolic compounds in bistort extracts. According to the literature, root of bistort contains 15–20%

tannins [3], which are known to be powerful antiradical compounds [27].

The second in terms of antiradical activity appeared to be bark of cinnamon. Our investigation showed that the amount of phenolic compounds was lower than in root of bistort. According to the literature [24], among phenolic compounds, bark of cinnamon contains phenolic carboxylic acids (hydroxycinnamic acid derivatives, dihydroxybenzoic acid), tannins, and especially oligomeric proanthocyanidins, in amounts of no more than 2%. We demonstrated higher amounts of phenols than reported in the literature for this raw material. Our results could be caused by the presence of additive of primary bark. The cinnamon bark antiradical activity was lower than root of common bistort when calculated per g of raw material ($TAU_{515/g}$) but the extracts exhibited stronger antioxidant features ($TAU_{515/mg}$).

Antiradical potential of rhizome of ginger was average in comparison to raw materials investigated in this study but low in comparison to the above-cited tea leaves. The main therapeutic component of rhizome of ginger is essential oil, but some phenolic compounds were identified in this raw material, such as quercetin, rutin, catechin, and phenolic acids (gallic, vanillic ferulic, tannic acid) [6]. The gallic and tannic acids are known to have strong antiradical properties [10,27].

Rhizomes of yams did not show strong antiradical activity in our investigation. There is some information in the literature showing certain antioxidant properties of yam rhizome [21].

Root of temulawak and root of common chicory appeared to exhibit the lowest antiradical properties. Root of temulawak contains 1–2% dicinnamoyl methane derivatives (curcumin), which are not very effective antiradical compounds [25].

Root of common chicory is mainly used as a bitter medicine. Among the phenolics, there were identified monocaffeoyl tartaric acid, chicoric acid, chlorogenic acid, some cyanidin and delphinidin derivatives, flavonoids such as quercetin and luteolin derivatives [8]. The amount of total phenols appeared to be very small, which correlated with low antiradical features.

One can conclude that:

a) Common bistort appeared to have the strongest antiradical features, probably due to the presence of high amounts of total phenols and especially tannins – strong antiradical agents;
b) Antiradical activity positively correlated well with the total phenol amounts.

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