Free radical scavenging activity for infusions of 30 medicinal plants detected with Electron Paramagnetic Resonance

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S u m m a r y

DPPH radical scavenging activity for thirty medicinal plant infusions was investigated with a use of Electron Paramagnetic Resonance. Infusions were prepared in similar way in which herbs or herbal teas are prepared directly for human consumption. Samples were obtained mostly from popular and easy available medicinal plants, used as single herbs or in herbal mixtures. The total flavonoid content for fifteen selected plants was measured using modified Christ-Müller method.
The best DPPH radical scavenging activity (more than 50% of DPPH radical reduced in examined sample after 1 h) was obtained for *Melissae folium* (87%), *Sambuci flos* (81%), *Ulmariae flos (79*%), *Hyperici herba* (67%) *and Arnicae anthodium* (66%). Green tea confirmed to be strong DPPH scavenger among examined plants (65%). The highest flavonoid amounts were discovered for *Ulmariae flos* (1.5%), *Hyperici herba* (1.2%), *Betulae folium* (0.9%). There was no correlation between DPPH scavenging activity and the total content of flavonoids in examined plan material.

Key words: DPPH, free radicals, flavonoids, medicinal plants, plant infusions

INTRODUCTION

Balance disorder with dominating uncontrolled pro-oxidative reactions in the human body is called an oxidative stress and there are many diseases and health disorders connected with this state: atherosclerosis, cancerous diseases, diabetes, glaucoma, pulmonary emphysema, Alzheimer's disease, intoxication (with methanol, heavy metals) and inflammatory cases [1-3]. Reactive oxygen species (ROS) and other free radicals, due to their strong oxidative character, can cause severe damage of important cellular structures, such as DNA, proteins and lipids [4-7]. They occur in the human body in natural physiology processes, created as accidental products of tissue metabolism or having their special destination for inactivating viruses and bacteria, prostanoid synthesis or cytochrome activation [8]. Free radicals can be formatted as a result of ionising radiation and are received from surrounding environment: inhaled with cigarette smoke, smog or in taken with food [4, 5, 7]. Neutralized by specialized enzymes and natural antioxidants are not dangerous as soon as the balance between their appearance and protective defense mechanisms is reached. The proper diet provides antioxidants (including radical scavengers) such as vitamins C, E, B-carotene and poliphenolic compounds. The role of radical scavengers is to prevent damage of important cellular components.

Polyphenols, such as flavonoids, antocyjans, fenolic acids, tannins, katechins are widely spread in fruits, vegetables, medicinal plants and beverages (tea, wine) [9-11]. Flavonoids show various antioxidant properties, concerning also free radical scavenging ability [6, 12-15]. Many researchers are concentrated on comparing chemical structure and antioxidant activity of flavonoids [16-18]. A lot of medicinal plants are defined as flavonoid sources and used in therapy as diuretics, spasmolytics or anti-inflammatory agents [19].

Concerning paramagnetic character of free radicals they can be studied directly with use of Electron Paramagnetic Resonance. Using proper standard samples we can investigate the range of free radical scavenging activity for chosen antioxidant agents.

The aim of this study was to examine DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity for thirty infusions of medicinal plants, using Electron Paramagnetic Resonance (EPR) method. The content of flavonoids for selected fifteen herbal infusions was also determined to compare the results and free radical scavenging activity.

Examined plants were selected because of their widespread use in traditional herbal medicine or containing flavonoid compounds. Green tea, known as a strong antioxidant among other teas (also as DPPH scavenger), was included in the study [20].

MATERIALS AND METHODS

Plant material

Examined herbal samples were supplied by Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Warsaw. Green tea „yer-vita", imported by Argentyna Ltd. (produced: Cabrales, Argentina).

Thirty medicinal plants were selected for examining radical scavenging activity. Most of them are described in monographies of FP VI or Ph. Eur. 5,0 (tab. 1). A lot of them is commonly used as a single or mixed herbal infusions. To compare with the others, green tea, known as a popular beverage having free radical scavenging ability, was also investigated [20, 21].

Table 1

DPPH scavenging activity after 1 h $(I_0–I)/I_0$ [%] and the content of flavonoids in examined plant material

	material	DPPH scavenging activity after 1 h $(I_0–I)/I_0$ [%]	content of flavonoids [%]
1	*Melissae folium* (Ph. Eur. 5,0; FPVI)	87	0.07
2	*Sambuci flos* (Ph. Eur. 5,0; FPVI)	81	0.78
3	*Ulmariae flos*	79	1.54
4	*Hyperici herba* (Ph. Eur. 5,0; FPVI)	67	1.24
5	*Arnicae anthodium* (FPVI)	66	0.36
6	*Green tea*	65	*
7	*Lamii albi flos*	53	0.53
8	*Lavandulae flos* (Ph. Eur. 5,0; FPVI)	29	*
9	*Althaeae folium* (Ph. Eur. 5,0; FPVI)	25	0.24
10	*Plantaginis lanceolate folium* (Ph. Eur. 5,0; FPVI)	25	*
11	*Helianthii flos*	24	*
12	*Menthae piperitae folium* (Ph. Eur. 5,0; FPVI)	22	0.16
13	*Betulae folium* (Ph. Eur. 5,0; FPVI)	21	0.90
14	*Crataegi inflorescentia* (FPVI)	20	0.40
15	*Convallariae herba* (FPVI)	19	*
16	*Malvae sylvestris flos* (Ph. Eur. 5,0)	17	*
17	*Frangulae cortex* (Ph. Eur. 5,0; FPVI)	17	*
18	*Violae tricoloris herba* (FPVI)	15	*
19	*Absinthii herba* (Ph. Eur. 5,8; FPVI)	13	*
20	*Sambuci fructus* (FP IV)	12	*
21	*Herniariae herba* (FP IV)	11	0.39
22	*Polygoni avicularis herba* (Ph. Eur. 5,0; FPVI)	11	*
23	*Euphrasiae herba*	11	*
24	*Calendulae flos* (Ph. Eur. 5,0)	10	*
25	*Chamomillae anthodium* (FPVI)	8	0.36
26	*Tiliae inflorescentia* (Ph. Eur. 5,0; FPVI)	8	0.38
27	*Helichrysi inflorescentia* (FPVI)	6	0.61
28	*Sennae folium* (Ph. Eur. 5,0; FPVI)	6	*
29	*Urticae folium* (Ph. Eur. 5,6; FPVI)	3	*
30	*Equiseti herba* (Ph. Eur. 5,0; FPVI)	2	0.30

* not examined

EPR measurements

The weighted amount (1 g) of plant material was poured with 100 ml of boiling distilled water and left under cover for 1 h, shook several times. The filtered infusion (0.2 ml) was added to 1 ml of standard solution (DPPH in acetone, concentration 2.5 mM/dm^3). Mixed sample was placed in an EPR tube. As a reference sample (external standard), 0.2 ml of distilled water added to the DPPH solution was used.

The EPR spectra in the X-band were measured on a Radiopan SE/X 2542 spectrometer equipped with a magnetometer. The spectrometer was interfaced with computer for data acquisition. The signal was integrated twice to determine its area, and thus the concentration of the radical. The sweeping for the EPR spectrum started 2 min. after the addition of plant infusion. Reaction was followed for 60 min or to the disappearance of DPPH radical signal. The radical scavenging activity was expressed by the ratio $(I_0–I)/ I_0$ [%] (I – integral intensity of the signal after addition of the extract, I_0 – the intensity of DPPH signal in control solution). The EPR spectrum for reference solution of DPPH radical and upon addition of *Melissae folium* infusion is shown in figure 1.

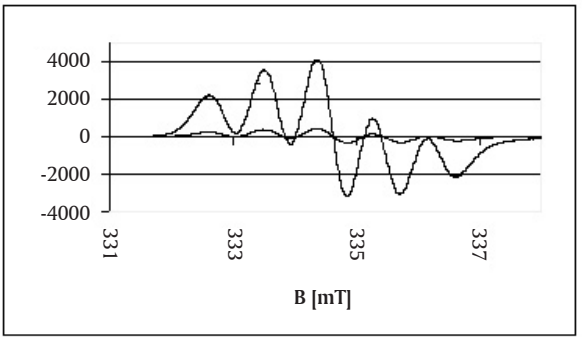

Figure 1.
EPR spectra of DPPH radical: a) reference solution, b) upon addition of Melisae folium infusion

Determination of flavonoids

Fifteen selected plants (previously examined for scavenging ability) were investigated for total content of flavonoids, using modified Christ-Müller method, calculated for quercetin [22].

Absorbance (A) was measured at 425 nm on a Shimadzu spectrophotometer. The content of flavonoids (x) was calculated from the equation:

$$X = \frac{8{,}75 \cdot A}{m}$$ where m [g] was the amount of dry material.

RESULTS AND DISCUSSION

The DPPH radical scavenging activity – $(I_0–I)/I_0$ [%] – in the examined sample after 1 h was the measure of antioxidant ability of plant infusion (tab 1, fig. 2). Therefore, the most active appeared to be: *Melissae folium* – 87%, *Sambuci flos* – 81%, *Ulmariae flos* – 79%, *Hyperici herba* – 67%, *Arnicae anthodium* – 66%. The green tea infusion investigated for comparison came to 65%. The weakest DPPH scavenging ability showed *Equseti herba* (2%). The hignest amount of flavonoids for selected 15 investigated plants (concerning 5 of the highest scavenging activity) showed: *Ulmariae flos* – 1.54%, *Hyperici herba* – 1.24%, *Betulae folium* – 0.90%. The comparison of both examinations did not show any correlation between DPPH scavenging activity and the amount of flavonoid compounds in examined material (fig. 3). In other studies the correlation between DPPH scavenging and the content of flavonoids was also not confirmed [15, 23, 24]. Investigated plants contain other compounds, ex. phenolic acids and tannins [19] which also show antioxidant activity. There is still a matter of investigation if total poliphenolic amount shows better correlation with scavenging activity [21, 25].

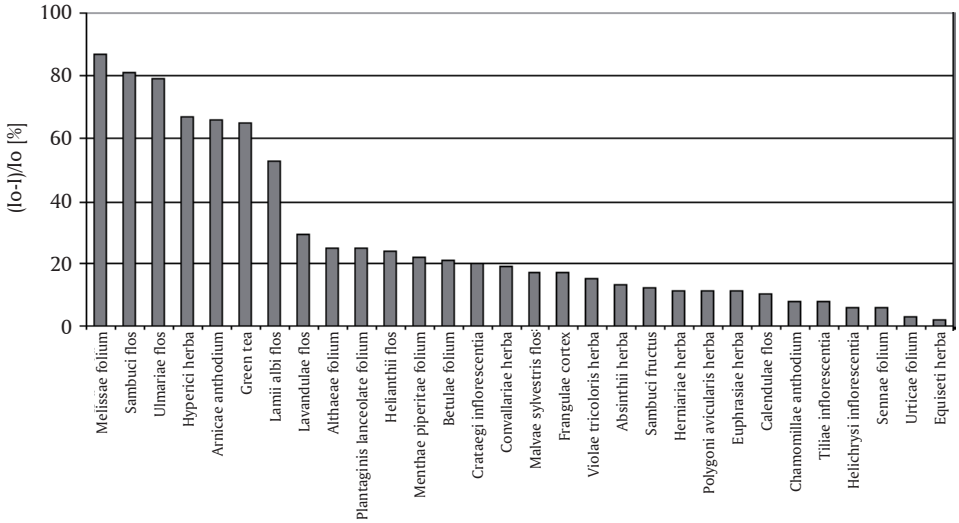

Figure 2.
Scavenging activity of DPPH radical (I0–I)/I0 [%] in examined samples after 1 h

Three of investigated herbs showed significant free radical scavenging ability: scavenged over 70% of DPPH radical after 1 h (*Melissae folium, Sambuci flos, Ulmariae flos*). *Melissae folium* infusion confirmed significant antioxidant properties and high phenolic concentration among other herbal infusions [25]. *Melissae folium* and *Sambuci flos*, both described in FP VI and FE 5.0, possess low toxicity for

human, are easy available and commonly used as single herbs or in herbal compositions. They could be considered for recommendation as helping in free radicals elimination.

Numerous researchers investigated and compared antioxidant properties of medicinal plants and teas using various extracts and methods [23-27]. Usage of herbs as sources of antioxidants in food or pharmaceutical industry and health benefits resulting from drinking herbal infusions as preventing oxidative stress-related diseases, require however further experimental work.

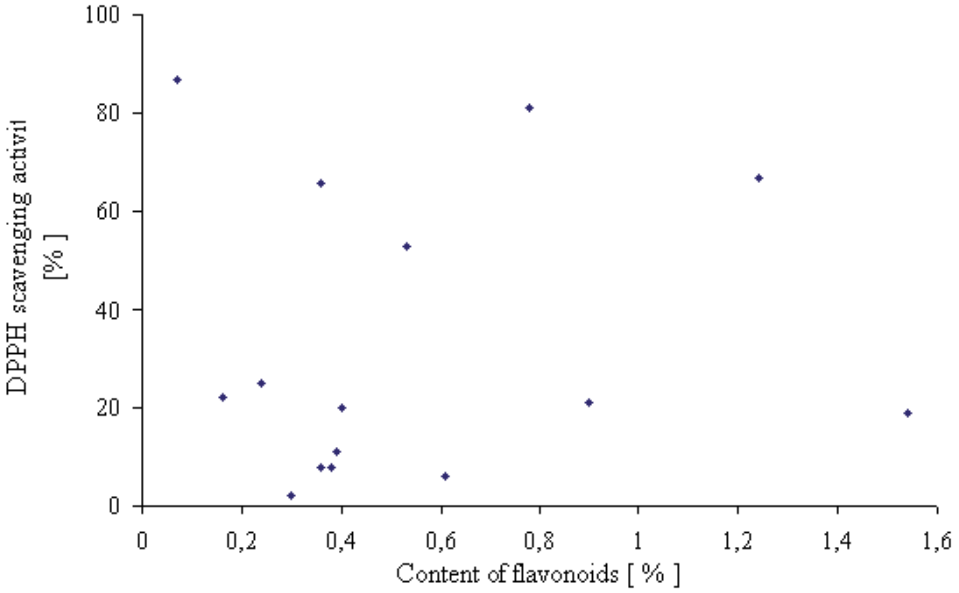

Figure 3.
Relation between DPPH scavenging activity and the content of flavonoid compounds

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BADANIE ZDOLNOŚCI WYMIATANIA WOLNYCH RODNIKÓW PRZEZ NAPARY WODNE 30 ROŚLINNYCH SUROWCÓW LECZNICZYCH METODĄ ELEKTRONOWEGO REZONANSU PARAMAGNETYCZNEGO

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Streszczenie

Celem podjętych badań było określenie zdolności wymiatania rodnika DPPH przez napary wodne 30 wybranych surowców roślinnych metodą elektronowego rezonansu paramagnetycznego (EPR). Napary zostały przygotowane w sposób podobny do tego, w jaki przygotowuje się do spożycia zioła lub herbatki ziołowe. Najsilniejsze właściwości przeciwrodnikowe (ponad 50% redukcji wzorcowego rodnika DPPH w badanej próbce po 1 h) wykazały: liść melisy (87%), kwiat bzu czarnego (81%), kwiat wiązówki (79%), ziele dziurawca (67%) i koszyczek arniki (66%). W celach porównawczych zbadano napar z zielonej herbaty, co potwierdziło jej względnie silne działanie przeciwrodnikowe. Dla 15 surowców zbadano dodatkowo całkowitą zawartość flawonoidów zmodyfikowaną metodą Christa-Müllera w celu porównania zdolności wymiatania rodnika DPPH z zawartością tych związków. Najwyższą zawartość flawonoidów wykazały: kwiat wiązówki (1,5%), ziele dziurawca (1,2%) i liść brzozy (0,9%). Nie wystąpiła korelacja pomiędzy zdolnością wymiatania rodnika DPPH a zawartością flawonoidów w badanych surowcach.

Słowa kluczowe: DPPH, wolne rodniki, flawonoidy, napary roślin leczniczych